SEGREGATION WITHIN AFFERENT PATHWAYS IN PRIMATE VISION

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Abstract

The current knowledge of the visual pathways in primates includes the patterns of projection from the retina through the dorsal lateral geniculate nucleus (dLGN) to the striate cortex (V1) and the extra-striate projections towards the dorsal and ventral streams. Cells with short wavelength sensitive cone (S-cone) inputs in the dLGN have been studied extensively in New World marmosets but not in Old World macaques. This thesis presents results from studies in the macaque monkey which are more relevant to humans since humans are closer in evolution to Old World than New World monkeys.

The spatial, temporal, chromatic and orientation preferences of neurons in the dLGN of the macaque were investigated by electrophysiological methods. The physiological findings of cells with S-cone inputs were compared to cells with opponent inputs from the long and medium wavelength sensitive cones (L-cones & M-cones, respectively). The cells receiving S-cone inputs (blue-yellow or B-Y cells) preferred lower spatial frequencies than the cells with opponent L-cone and M-cone inputs (red-green or R-G cells). Orthodromic latencies from optic chiasm stimulation were measured where possible to distinguish differences in conduction velocity between the cell groups. Although the B-Y cells usually had longer latencies than R-G cells, there was considerable overlap between the cell groups.

The recorded cells were localised through histological reconstruction of dLGN sections stained for Nissl substance. The distribution of B-Y cells within the dLGN was compared to the distribution of R-G cells. The majority of B-Y cells were located within the intercalated koniocellular layers as well as the koniocellular bridges (extensions of the koniocellular layers into the adjacent parvocellular layers). The B-Y cells were also largely segregated within the middle dLGN layers (K3, P3, K4 & P4). The R-G cells were
mainly concentrated within the parvocellular layers (P3, P4, P5 & P6) and were evenly distributed throughout the middle and outer layers of the dLGN.

The study also included recordings from the extra-striate middle temporal area (MT) to determine whether a fast S-cone input exists from the dLGN to area MT which bypasses V1. The pattern of cone inputs to area MT neurons was investigated before and during inactivation of V1. The inactivation was done through reversible cooling with a Peltier thermocouple device or focal inactivation with \(\gamma\)-amino butyric acid (GABA) iontophoresis. Precise inactivation of V1 to the topographically matching visual fields of the recording sites in area MT revealed a preservation of all three cone inputs in many cells. The subcortical sources of these preserved inputs are discussed with their relevance to blindsight, which is the limited retention of visual perception after V1 damage. Analysis of the latencies of area MT cells revealed a rough segregation into latencies faster or slower than 70 ms. Cells both with and without a significant change in response during V1 inactivation were present in each group. The findings reported in this thesis indicate that some of the preserved inputs in area MT during V1 inactivation may be carried by a direct input from the dLGN which bypasses V1.
DECLARATION

This is to certify that:

1. The thesis comprises only my original work toward the PhD except where indicated in the Preface;

2. Due acknowledgment has been made in the text to all other material used;

3. The thesis is less than 100,000 words in length, exclusive of words in tables, figures, bibliographies and appendices.

Sujata Roy
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Some of the results described in this thesis have appeared in the following publications and abstracts:

Publications:


Abstracts:


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<table>
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<th>Description</th>
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<tbody>
<tr>
<td>dLGN</td>
<td>Dorsal lateral geniculate nucleus</td>
</tr>
<tr>
<td>V1</td>
<td>Primary visual cortex (striate cortex)</td>
</tr>
<tr>
<td>MT</td>
<td>Middle temporal area</td>
</tr>
<tr>
<td>M</td>
<td>Magnocellular</td>
</tr>
<tr>
<td>P</td>
<td>Parvocellular</td>
</tr>
<tr>
<td>K</td>
<td>Koniocellular</td>
</tr>
<tr>
<td>L – cone</td>
<td>Long wavelength sensitive cone</td>
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<tr>
<td>M – cone</td>
<td>Medium wavelength sensitive cone</td>
</tr>
<tr>
<td>S – cone</td>
<td>Short wavelength sensitive cone</td>
</tr>
<tr>
<td>R – G</td>
<td>Red-green opponent cells</td>
</tr>
<tr>
<td>B – Y</td>
<td>Blue-yellow opponent cells</td>
</tr>
<tr>
<td>PSTH</td>
<td>Peri-stimulus time histogram</td>
</tr>
<tr>
<td>F1</td>
<td>First Fourier component</td>
</tr>
<tr>
<td>F2</td>
<td>Second Fourier component</td>
</tr>
<tr>
<td>EEG</td>
<td>Electro-encephalogram</td>
</tr>
<tr>
<td>ECG</td>
<td>Electro-cardiogram</td>
</tr>
<tr>
<td>CIE</td>
<td>Commission Internationale de l’Eclairage</td>
</tr>
<tr>
<td>DKL</td>
<td>Derrington, Krauskopf and Lennie (refers to colour space)</td>
</tr>
<tr>
<td>CMF</td>
<td>Colour matching functions</td>
</tr>
<tr>
<td>DOG</td>
<td>Difference of Gaussians</td>
</tr>
<tr>
<td>LFR</td>
<td>Low frequency ratio</td>
</tr>
<tr>
<td>TFR</td>
<td>Temporal frequency ratio</td>
</tr>
<tr>
<td>OI</td>
<td>Orientation index</td>
</tr>
<tr>
<td>DI</td>
<td>Direction index</td>
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<tr>
<td>R_m/b</td>
<td>Contrast gain</td>
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<tr>
<td>GABA</td>
<td>γ-amino butyric acid</td>
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Chapter 1

Introduction

1.1 Background

The visual system of the primate consists of three distinct pathways known as the parvocellular, magnocellular and koniocellular channels of vision. The pathways start at the retina with the stimulation of the photoreceptors from changes in incident light. This initiates a phototransduction process to send signals from the retina via the optic nerve to the dorsal lateral geniculate nucleus (dLGN) of the thalamus and on to other subcortical and higher visual cortical areas.

The photoreceptors include three types of cones, named for the wavelength of light they are most sensitive to. These are the long wavelength sensitive cones (L-cones) which respond best to wavelengths of 565 nm, the medium wavelength sensitive cones (M-cones) which respond best to 535 nm and the short wavelength sensitive cones (S-cones) which respond best to 440 nm. The signals from these cone receptors combine additively (in phase) or subtractively (out of phase) to provide input to the three pathways of vision.

Parvocellular neurons in the dLGN receive antagonistic inputs from the L-cones and M-cones of the retina through the midget ganglion cells, re-
sulting in red-green opponent (R-G) signals (Perry et al., 1984; Creutzfeldt et al., 1986). The magnocellular neurons receive additive inputs from the L-cones and M-cones through parasol ganglion cells in the retina to convey luminance information (Wiesel and Hubel, 1966; Schiller and Malpeli, 1978). The parvocellular neurons are arranged into four main dorsal layers in the macaque dLGN, while the magnocellular cells form the two main ventral layers (Malpeli and Baker, 1975; Kaas et al., 1978). The parvocellular pathway is involved in transmitting information about form and colour to mainly the ventral stream, while the magnocellular pathway transfers signals about motion and depth to mainly the dorsal stream (De Monasterio and Gouras, 1975). High spatial frequency achromatic vision and chromatic opponency are conveyed by the parvocellular pathway (Derrington et al., 1984; Derrington and Lennie, 1984). In contrast, the magnocellular system primarily conveys low spatial frequency achromatic vision and sensitivity to movement (Schiller and Malpeli, 1978). There is minimal S-cone input transmitted through the magnocellular stream (Sun et al., 2006a) although claims had been made to the contrary (Chatterjee and Callaway, 2003). The details about the inter-mixing of signals from these pathways will be described in Chapter 2.

Most of the knowledge about S-cone inputs to date has come from studies of the retina and dLGN of New World monkeys, such as the common marmoset (*Callithrix jacchus*). Studies of S-cone inputs have been conducted only in the retina of the Old World monkey, such as the macaque (*Macaca fascicularis* and *Macaca nemestrina*). The data from the New World marmoset indicates that the retinal ganglion cells with S-cone inputs responding to both increments and decrements of light send signals predominantly into the koniocellular pathway. The koniocellular pathway had previously eluded a distinct function until studies in the diurnal New World marmoset monkey implicated this pathway in containing the blue-yellow opponent (B-Y) cells
which receive S-cone inputs (Martin et al., 1997; White et al., 1998; Solomon et al., 1999; White et al., 2001; Szmajda et al., 2006). The intercalated koniocellular layers lie ventral to each of the main parvocellular and magnocellular layers in the dLGN (Hendry and Yoshioka, 1994).

The S-cone On signals are transmitted to the dLGN through the small bistratified ganglion cells (Dacey and Lee, 1994). These cells have been shown to terminate in the middle koniocellular layer K3 of the marmoset dLGN (Szmajda et al., 2008). Preliminary studies in the macaque dLGN have indicated these cells may also send inputs to the middle koniocellular regions (Calkins and Hendry, 1996). The S-cone Off inputs have been proposed to be carried by an Off midget pathway (Klug et al., 2003), although this has been disputed (Lee et al., 2005; Lee and Grunert, 2007). The “giant” melanopsin-expressing sparse ganglion cells of the retina may also carry S-cone Off signals (Dacey et al., 2005).

Studies to correlate the anatomical location with the physiological properties of koniocellular neurons within the dLGN have not been attempted in the Old World monkey due to the relative thinness and unclear demarcation of these intercalated layers. The thinness makes it difficult to target the koniocellular layers during electrophysiological recordings. Hence, the physiological properties of cells located within the koniocellular layers in the Old World macaque monkey remain unknown. However, investigations in the Old World monkey would be much more relevant to humans since the evolutionary branch between the two species split only 12 million years ago, as opposed to at least 30 million years of separation between Old World and New World monkeys (McKenna and Bell, 1997; Goodman et al., 1998). Therefore, it needs to be determined whether the koniocellular layers in Old World monkeys contain a majority of S-cone input cells with distinct physiological properties similar to findings in New World marmosets (Martin et al., 1997; White et al., 1998; Solomon et al., 1999).
The dLGN, a major relay centre for signals from the retinal projections, transmits these signals to predominantly the primary visual cortex (striate cortex or V1). The dLGN also sends direct inputs to other subcortical areas (such as the superior colliculus) and to a higher order visual extrastriate area known as the middle temporal area (MT). An anatomical study using a retrograde tracer injected into area MT of the macaque monkey revealed a direct koniocellular projection from the dLGN to area MT (Sincich et al., 2004). Furthermore, there is evidence from psychophysical studies in humans of a distinct fast latency S-cone input to area MT which may bypass V1 (Morand et al., 2000) while the inputs with slow latencies may arrive in area MT via V1. Hence, it is possible that the direct koniocellular input from the dLGN may contain fast S-cone inputs to area MT. This suggestion could be investigated by determining the physiological nature of cone inputs with short latencies arriving in area MT. Such a study could be supplemented by recordings in area MT before and during reversible inactivation of V1. The responses and latencies of area MT cells in these conditions may be compared to distinguish which cone inputs from subcortical areas either bypass or propagate through V1 to area MT. The nature of direct subcortical inputs to area MT would help to explain the phenomenon of blindsight (Weiskrantz et al., 1974), where there is a limited retention of visual perception with V1 damage.

1.2 Aims of the thesis

In the course of this study, the following aims have been investigated:

1. Determine the physiological properties of B-Y cells compared to R-G cells in the Old World macaque monkey and record the orthodromic latencies to optic chiasm stimulation for each cell type.
2. Investigate whether the B-Y cells are segregated within the koniocellular layers of the dLGN in the Old World macaque monkey.

3. Record responses in area MT with V1 inactivation to reveal whether S-cone inputs are carried directly to area MT by a fast conduction pathway.

1.3 Chapter outline

Chapter 2 : Literature Review - The Visual Pathways

This chapter summarises the current state of knowledge of the three main parallel pathways of the primate visual system, namely the magnocellular, parvocellular and koniocellular streams. The properties of the magnocellular and parvocellular pathways have been extensively studied and documented in the Old World macaque monkey (Wiesel and Hubel, 1966; De Monasterio and Gouras, 1975; Dreher et al., 1976; Norden and Kaas, 1978; Schiller and Malpeli, 1978; Hicks et al., 1983; Derrington et al., 1984). However, the koniocellular pathway has not been well investigated in Old World monkeys since the anatomical segregation at the level of the dLGN is less distinct for this stream than in New World monkeys such as the marmoset.

The neurons in the dLGN of the macaque monkey are clustered into two ventral magnocellular layers (M1 & M2) and four dorsal parvocellular layers (P3, P4, P5 & P6), while the intercalated koniocellular layers (K1- K6) lie ventral to each of the main magnocellular and parvocellular layers (Hendry and Reid, 2000).

An earlier physiological study (Schiller and Malpeli, 1978) demonstrated the presence of a higher proportion of Off-centre cells (cells responding better to decrements of light) in the middle layers of the dLGN. Before the classification of koniocellular layers at that time, there were also more B-Y cells reported in the ventral parvocellular layers P3 and P4 compared to the dorsal
layers P5 and P6 (Schiller and Malpeli, 1978). Recent studies in the New World diurnal marmoset (Martin et al., 1997; White et al., 1998; Solomon et al., 1999; Szmajda et al., 2006) have shown that B-Y cells tend to be located in the intercalated koniocellular layers.

The visual signals from the various dLGN laminae are transmitted to V1 and further on to various extrastriate areas. One of these is area MT which predominantly processes information about the motion and depth of objects by speed and binocular disparity analysis.

A recent anatomical study has shown that the koniocellular layers of the dLGN provide a direct input bypassing V1 to visual area MT (Sincich et al., 2004). This is also consistent with earlier psychophysical studies in humans (Morand et al., 2000) showing fast S-cone inputs to area MT. However, it is still not known whether this direct anatomical pathway contains S-cone signals. Blindsight (Sanders et al., 1974; Weiskrantz et al., 1974) could be explained by these direct subcortical inputs to area MT which bypass V1.

Chapter 3 : Experimental Methods

Recordings were made using lacquer-coated tungsten electrodes from 13 macaque monkeys under anaesthesia and muscular paralysis. The physiological properties of cells within the dLGN of the thalamus were investigated to categorise neurons according to the type of cone inputs they received from the retinal L-cones, M-cones or S-cones. Spatial modulation transfer functions were obtained for cell responses from presentation of stimuli that were calibrated to modulate selective cone types individually or in combination. The latency to orthodromic stimulation of the optic chiasm was measured where possible.

The recorded cells were localised in the dLGN layers through histological reconstruction of electrode tracks in alternate sections stained with Cresyl Violet for Nissl substance. A normalised ratio was used to quantify the distance
of each cell from the border of the koniocellular and overlying parvocellular layer to the cell’s actual location within the overlying parvocellular layer.

Recordings were also made from corresponding visual fields of area MT cells while reversibly inactivating V1 by cortical cooling using a Peltier thermocouple device, as done in previous studies (Girard et al., 1991, 1992). An additional method of precise focal inactivation using γ-amino butyric acid (GABA) iontophoresis for inactivating V1 was employed to investigate responses of topographically corresponding area MT cells.

The responses were recorded during presentation of stimuli which additively or subtractively modulated L-cones, M-cones and S-cones in different combinations. The responses revealed whether there was a preservation of signals in area MT cells during reversible V1 inactivation due to inputs from subcortical areas which bypass V1. The latencies of the cone inputs to area MT were obtained from the responses before and during V1 inactivation.

Chapter 4: Physiological Properties of S-cone input cells in the dLGN

Electrophysiological recordings were made from cells in the dLGN. This study provided a comparison for the findings already established for B-Y cells in the dLGN of the New World marmoset (Martin et al., 1997; White et al., 1998; Solomon et al., 1999; White et al., 2001; Szmajda et al., 2006). In the present study, the S-cone modulating stimuli evoked strong responses from the Blue On and Blue Off (B-Y) cells. These cells gave minimal responses to L-cone and M-cone opponent stimuli modulated in opposite phase and often also to achromatic stimuli.

The R-G cells were categorised as Red On, Red Off, Green On and Green Off cells. These cells yielded a moderate response to the luminance contrast in the achromatic stimuli but a vigorous response to stimuli where L-cones and M-cones were modulated in opposite phase.
It was found that B-Y cells overwhelmingly preferred low spatial frequencies compared to the R-G cells. Temporal frequency preferences, contrast sensitivities, orientation selectivities and direction preferences were largely similar for the R-G and B-Y cells.

The receptive field properties suggested that the B-Y cells tended to have larger centre radii than R-G cells at any given eccentricity in the visual field. The integrated sensitivity of the centre mechanisms were independent of the centre radius for both cell types. There was considerable overlap in the orthodromic latencies to optic chiasm stimulation between the two cell groups although the B-Y cells usually tended to have longer latencies than the R-G cells.

Chapter 5 : Laminar distribution of S-cone input cells in the dLGN

This chapter introduces the concept of koniocellular bridges, which are extensions of the koniocellular layers spanning into the adjacent parvocellular layers of the dLGN (Hendry and Yoshioka, 1994; Hendry and Reid, 2000). A novel way of identifying the koniocellular bridges using low-pass spatial filtering is described. The koniocellular bridges were included in the analysis as being part of the koniocellular regions along with the intercalated koniocellular layers (K3, K4, K5 & K6). The majority of B-Y cells were segregated within the koniocellular layers or the koniocellular bridges. In contrast, very few R-G cells were found within these koniocellular regions.

The B-Y cells also had the tendency to be mainly contained within the middle layers (K3, P3, K4, P4) of the dLGN while the R-G cells were evenly distributed throughout the middle layers and the outer layers (K5, P5, K6, P6). For the whole cell population, there was no segregation of On centre cells to the outer layers and Off centre cells to the middle layers of the dLGN, as had been reported previously in an early study (Schiller and Malpeli, 1978).
A mild tendency for such a segregation was noticed when the R-G cells alone were analysed.

Chapter 6 : Responses in area MT with cortical inactivation

This chapter presents results for the cone inputs of neurons in area MT before and during reversible inactivation of V1 for visuotopically corresponding area MT receptive fields. The responses recorded during inactivation of V1 indicated that the inputs from all three cone types in the majority of area MT neurons were preserved. This was particularly true of the additive L-cone and M-cone inputs highly prevalent in most responses from area MT cells. About half the area MT cells recorded also showed a sustained S-cone input during V1 inactivation. The findings suggest a direct pathway from the subcortical areas bypassing V1 to area MT. This has direct implications for the involvement of area MT in blindsight.

Response latencies for the neurons in area MT were recorded for the different stimulus presentations. There was a rough segregation of cells into two groups of fast and slow response latencies. The fast latency group contained both cells with and without a significant change in response during V1 inactivation. The slow latency group mainly contained cells with a significant change in response during inactivation. This may indicate that cells with slow response latencies are likely to project to area MT via V1 but cells with fast latencies may either bypass or propagate through V1 to area MT.

Chapter 7 : Summary & Conclusions

The correlation of the physiological properties with the anatomical locations of neurons within the macaque dLGN revealed that the majority of B-Y cells were contained within the koniocellular regions, similar to findings in the New World marmoset (Martin et al., 1997; White et al., 1998; Solomon et al., 1999). Further correlation using neurochemical tracers for the konio-
cellular bridges with their identification using low-pass spatial filtering may validate the bridges as being functionally and morphologically similar to the koniocellular layers. The location of the koniocellular bridges may suggest the basis of an evolutionary process within the macaque dLGN.

Inactivation of V1 for recordings in topographically corresponding visual fields of area MT revealed a preservation of all cone inputs in many area MT cells. The S-cone inputs may be relayed to area MT through the B-Y cells within the koniocellular layers of the dLGN, which have been anatomically shown to send direct inputs to area MT in the macaque (Sincich et al., 2004). However, the sustained L-cone and M-cone inputs to area MT may come from the pulvinar, regions of the dLGN and projections from V2. Inter-hemispheric transfer through the corpus callosum may also have a contribution. The findings are supportive of a direct subcortical input to area MT which bypasses V1 and may result in the limited visual perception reported in blindsight.
Chapter 2

The Visual Pathways

The primate afferent visual system is comprised of distinct classes of relay cells which transmit information from the visual environment to the brain. Starting at the retina and advancing through certain landmarks of the brain, signals are relayed on to V1 and to the extrastriate areas. The visual system is connected in a complex way so as to provide feedforward, lateral and feedback signals. These signals help to direct attention to specific objects or features in order to initiate an appropriate behavioural response. This study will address some of the functions of, and interaction between, the components of the visual pathways.

2.1 Old World and New World Primates

Most of our knowledge of the afferent channels of vision in the human brain has been derived from studying animal models, predominantly non-human primates. Primates share a common evolutionary branch to humans (McKenna and Bell, 1997; Goodman et al., 1998). The common characteristics that categorise them as a distinct group include their large, convoluted brains which allow complex social behavior (McKenna and Bell, 1997; Goodman et al., 1998). They have frontally directed eyes which give them binocular vision.
Depth perception (or stereopsis) arising due to the slightly different views from their two eyes is advantageous to their ability to carry out complex tasks.

Primates had branched out from the early prosimians into the two subcategories of Prosimians (Prosimii) and Anthropoids (Anthropoidea). The Prosimians (from “primitive primates”) are the smaller of the two mammals and are all nocturnal (Kay et al., 1997). They include the galagos or bushbabies, which have been well-studied in vision research. The Anthropoids are the “human-like” suborder of primates with usually excellent colour-enabled vision (Goodman et al., 1998). Along with humans, the widely-studied monkey is included in this group. Anthropoids can be further subdivided into “Old World” and “New World” monkeys. Old World monkeys are found in parts of Asia and Africa. In contrast, the New World monkeys are native to Central and South America (McKenna and Bell, 1997; Goodman et al., 1998).

Old World monkeys (Cercopithecidae) are somewhat more closely related to humans (separated by 25 million years out of the 60 million years of primate existence) than the New World monkeys (Ceboidea; Goodman et al., 1998), who have at least 30 million years of evolutionary gap with humans (McKenna and Bell, 1997; Goodman et al., 1998). However, Old World monkeys include the macaque, which have been used very extensively in studies of the visual system due to the similarities they have with humans with regard to binocular vision, highly advanced trichromatic vision and the scaling of cortical connectivity in relation to brain sizes (Ringo, 1991).
2.2 Overview of the Visual Pathways

Light rays reflected from objects travel through the media of the eye and are focused on the retina. Individual photoreceptors at the back of the retina, consisting of rods and cones, maintain a steady state of depolarization in the dark through a constant influx of sodium. The light reaching the retina starts a transduction process in the photoreceptors. The photoreceptors synapse on to bipolar cells, which in turn synapse onto ganglion cells (Rodieck, 1965; Enroth-Cugell and Robson, 1966; Leventhal et al., 1981; Perry et al., 1984). The ganglion cells are known to relay specific attributes of vision depending on their functional types and receptive fields. The receptive field corresponds to that part of the visual field in which a stimulus would evoke a response from the cell. The axons of the ganglion cells become myelinated as they pass into the optic nerve. At the chiasm, the nasal ganglion cell fibres from each eye cross over to the contralateral optic nerve. This results in a hemianopic segregation of the ganglion cells which represent the visual field. The post-chiasmal fibres, known as the optic tract, relay the segregated hemifields primarily onto the dorsal lateral geniculate nucleus (dLGN) of the thalamus (Kaas et al., 1978; Malpeli et al., 1981; Kaplan and Shapley, 1982; Lee et al., 1983).

The cells of the dLGN have been extensively studied and found to be systematically arranged into distinct layers depending on the anatomical, physiological and visual functions of the relay cells (Malpeli and Baker, 1975; Norton and Casagrande, 1982; Derrington et al., 1984; Derrington and Lennie, 1984). The signals from the dLGN layers are transmitted in their segregated forms to V1. Beyond this region, the information is forwarded on to the various extrastriate areas (Maunsell et al., 1990; Yoshioka et al., 1992; Nakamura et al., 1993; Levitt et al., 1994a,b; Felleman et al., 1997b; Gegenfurtner et al., 1997; Roe and Ts’o, 1999; Sincich and Horton, 2002; Xiao and Felle-
man, 2004; Anderson and Martin, 2005; Crowder et al., 2005). Depending on the layer of origin of the cell bodies within the dLGN, these distinct visual channels either remain largely segregated, or converge on to the same cortical area (Maunsell et al., 1990; Nakamura et al., 1993; Ferrera et al., 1994; Felleman et al., 1997a; Roe and Ts’o, 1999; Sincich and Horton, 2002; Xiao and Felleman, 2004).

The main role of the visual system is to encode attributes such as form, colour and motion, so that behaviourally meaningful information can be extracted from the surrounding environment (Pollen, 1999; Juan and Walsh, 2003). This process of encoding information involves many factors. The form of an object is encoded based on interactions between receptive fields. Perception of colour comes from the opponent responses of the three types of retinal cone photoreceptors. The detection of motion comes from the differentiation of visual information over time.

2.3 Retinal ganglion cells

The neural responses of individual photoreceptors are initially processed within the retina. Signals from one or more photoreceptors may converge onto a single ganglion cell through the horizontal, amacrine and bipolar cells. The number of photoreceptors sending inputs to a ganglion cell is dependent on the retinal eccentricity of the cell. Peripheral ganglion cells receive inputs from numerous photoreceptors while foveal ganglion cells usually receive inputs from a single photoreceptor.

Ganglion cell receptive fields are modeled using the “Difference of Gaussians” method to reveal two overlapping concentric regions showing unique sensitivity profiles (Rodieck, 1965). The central region is narrower and more sensitive than the surround. The surround is larger, less sensitive and antagonistic compared to the centre (Hubel and Wiesel, 1962). The response at
any point of the cell’s receptive field is often assumed to be the linear sum of the Gaussian centre and surround sensitivity profiles. Receptive fields, whether in the retina or at higher stages of visual processing, can be defined in terms of spatial and temporal response properties of a cell (Movshon et al., 1978b), as well as wavelength, orientation and direction selectivities (Hubel and Wiesel, 1962, 1968; Dreher et al., 1976; Levick and Thibos, 1980).

Classically, receptive fields are described as having a circular, centre-surround antagonistic organization. An increased response of a ganglion cell with stimulation from a spot of light at the centre depicts an “On” response (Figure 2.1A). This type of cell is termed an “On-centre” (On) cell. An annulus of light outside the centre of an On cell results in inhibition from the surround, followed by a burst of response at the removal of the surround annulus (Figure 2.1B).
General illumination of both the centre and surround regions results in a response which is not as strong as the response from stimulation of the central excitatory region alone (Figure 2.1C). Should the central response increase when the spot of light disappears, this is categorized as an “Off” response. The cell in Figure 2.1 would be termed an “Off-centre” (Off) cell (Wiesel, 1959). Stimulation of the surrounding peripheral zone of an Off-centre cell produces the opposite response to that from stimulation of the central region.

Apart from being categorised as On-Centre and Off-centre cells, ganglion cells may also be described by their morphology and response properties. Retinal ganglion cells may be classified as midget and parasol ganglion cells (Polyack, 1941). These give rise to the axons of the parvocellular and magnocellular pathways, respectively, which synapse onto cells in specific layers of the dLGN (Kaas et al., 1978; Norden and Kaas, 1978; Schiller and Malpeli, 1978; Leventhal et al., 1981; Derrington et al., 1984; Perry et al., 1984; Rodieck et al., 1985; Valberg et al., 1987; Callaway, 2005). They are distinct from each other in terms of their cell sizes, receptive field properties, morphology, and conduction velocities of their axons.

The midget ganglion cells are connected to cone photoreceptors on a one-to-one basis from the fovea up to 50° eccentricity (Milam et al., 1993; Wassle et al., 1994). The cell bodies are small and have compact dendritic arbors (Watanabe and Rodieck, 1989; Dacey, 1999a). They are responsible for encoding L-cone and M-cone opponent signals through input into the central region of the receptive field by one type of cone and to the surround region by the other type (Wiesel and Hubel, 1966). This leads to the red-green colour opponency of the parvocellular stream (Wiesel and Hubel, 1966). The red-green colour opponency is found mainly in the foveal retina and changes to a non-opponent luminance response in the retinal periphery due to input from all cone types onto the midget ganglion cell (Dacey and Lee,
The midget ganglion cells project to the parvocellular layers of the dLGN (Leventhal et al., 1981; Perry et al., 1984). Parasol ganglion cells have larger cell bodies at any given eccentricity compared to the midget ganglion cells and project to the magnocellular layers of the dLGN (Perry et al., 1984; Watanabe and Rodieck, 1989; Dacey and Petersen, 1992). These cells encode additive inputs from the L-cones and M-cones both in the centre and surround regions of the receptive field, leading to luminance perception and broad spectral sensitivities (Lee et al., 1988). They are less numerous in both the central and peripheral retina than the midget ganglion cells, although are encountered more frequently in the retinal periphery (Lee et al., 1988).

Signals from the S-cones are encoded by the small bistratified ganglion cells (Dacey, 1999a; Dacey and Lee, 1994) which are distinct from the ganglion cells of the midget red-green colour-opponent pathway (Figure 2.2). The small bistratified ganglion cells’ receptive field contains spatially co-extensive On signals from the S-cones and opponent Off signals additively from the L-cones and M-cones (Dacey, 1999a; Dacey and Lee, 1994; Calkins et al., 1998; Chichilnisky and Baylor, 1999).

These ganglion cells receive inputs from the axons of the distinct S-cone (blue-cone) bipolar cells (Kouyama and Marshak, 1992) situated deep in the inner plexiform layer onto which the large inner segments of the S-cone photoreceptors synapse (Mariani, 1984). The blue-cone bipolar cells transmit the On signals from the S-cones to the inner dendritic tier of the small bistratified ganglion cell (Dacey and Lee, 1994; Dacey, 1999b).

In addition, the additive L-cone and M-cone inputs from diffuse bipolar cells to the outer dendritic tier produce the colour opponentcy of the small bistratified ganglion cell (Dacey and Lee, 1994; Dacey, 1999b). The blue-cone bipolar opponentcy originates from the H2 horizontal cell which contacts all three cone types, but particularly S-cones, to provide input to the blue-
Figure 2.2: The S-cone On pathway involves the blue-cone bipolar cells receiving signals exclusively from the S-cones. These signals are then forwarded to the distinct small bistratified ganglion cell. The spatially mutual colour-opponent signals are a result of additive L-cone and M-cone inputs; adapted from Dacey, 2000.

cone bipolar surround (Ahnelt and Kolb, 1994; Dacey et al., 1996). The H1 horizontal cell contacts solely L-cones and M-cones and produces opponency in the diffuse bipolar surround (Dacey et al., 1996).

With their role in the encoding of blue-yellow vision (Dacey and Lee, 1994; Calkins et al., 1998; Cottaris and De Valois, 1998) the small bistratified ganglion cells were initially thought to project to the parvocellular layers of the dLGN (Kaplan and Shapley, 1986; Rodieck and Watanabe, 1993). However, the likely projection of the small bistratified cells is now known to be to the intercalated koniocellular layer K3 of the dLGN in the New World marmoset (Martin et al., 1997; White et al., 1998). Preliminary work in the
macaque suggests the same pattern of projection to the middle koniocellular layers of the dLGN (Calkins and Hendry, 1996).

The S-cone Off pathway has been suggested to involve Off midget bipolar and Off midget ganglion cells (Klug et al., 2003) from the finding that each S-cone in the central retina was connected to a single midget Off bipolar cell (Klug et al., 1992, 1993). The opponency originated from the midget bipolar cells through inputs from H2 horizontal cells in the surround. However, this finding has been recently disputed where investigations revealed no involvement by the midget ganglion cells in the transmission of S-cone off signals (Lee et al., 2005; Lee and Grunert, 2007).

The “giant” melanopsin-expressing ganglion cells have recently been related to the processing of S-cone Off signals (Dacey et al., 2005). They are intrinsically photosensitive and with opponent inputs from rods against the additive L-cone and M-cone signals, lead to encoding of irradiance.

The concentric, spatially antagonistic arrangement of retinal ganglion cells is found in both primates (Wiesel and Hubel, 1966) and cats (Kuffler, 1952). Retinal ganglion cell receptive fields in the rhesus monkey were first classified into three main types (De Monasterio and Gouras, 1975). They were labelled colour-opponent, broadband and non-concentric (De Monasterio and Gouras, 1975). The colour-opponent cells usually had concentric, centre-surround chromatic opponency. The centre received inputs from one particular cone type and the surround from up to two cone types. The colour-opponent cells were found mainly in the foveal regions with little difference in responses to various stimuli.

The broadband cells, still of a concentric centre-surround arrangement, received inputs from two cone types in the centre and demonstrated transient responses with either chromatic or non-chromatic opponency in the surround. These cells were widely found in the peripheral retina and usually had transient responses.
The non-concentric cells usually had no spontaneous activity but did have phasic On, Off or On-Off responses. A few of these cells were responsive to moving stimuli only. Their responses to stimuli could vary markedly. They usually had the largest centre sizes and were found all over the retina.

With the more extensive classification of the target cells in the dLGN (see Section 2.4) the afferent retinal ganglion cells have taken on the names of the geniculate layers they project to. Most parvocellular cells receive inputs from colour-opponent ganglion cells, while most magnocellular cells receive inputs from broadband cells (Wiesel and Hubel, 1966; Schiller and Malpeli, 1978; Reid and Shapley, 2002).

Another classification of ganglion cells into X-cells or Y-cells according to their properties was first described in the retina of the cat using sinusoidally modulated achromatic gratings and the criteria of linearity of spatial summation (Enroth-Cugell and Robson, 1966). X-cells responded linearly and in a sustained manner while Y-cells exhibited non-linear and transient responses.

2.4 The dLGN

The macaque dLGN is organized into six distinct layers (Malpeli and Baker, 1975; von Noorden and Middleditch, 1975; Kaas et al., 1978; Connolly and Van Essen, 1984). The two most ventral layers serve the magnocellular stream of vision, and the four dorsal layers accommodate the parvocellular stream (Figure 2.3). The layers are labelled one to six starting from the ventral layer. The main cell type of the visual stream contained in the layer is used as a prefix to the layer number. Hence, the most ventral magnocellular layer would be M1, the next M2, and the parvocellular layers are P3, P4, P5 and P6 as they extend dorsally (Malpeli and Baker, 1975; von Noorden and Middleditch, 1975; Kaas et al., 1978; Connolly and Van Essen, 1984).

The intercalated spaces between the six main layers contain the koniocel-
Figure 2.3: The organization of the dLGN into 6 layers of varying functional and eye dominant inputs in the macaque monkey. The ventral two magnocellular layers are M1 and M2. The dorsal four parvocellular layers are P3, P4, P5 and P6; from www.psych.ucalgary.ca/ PACE/VA-LAB/ Brian/dLGN.jpg

...lular neurons of the visual system. Layers M1, P4, P6 and the intercalated koniocellular layers directly ventral to each of them receive innervation from the contralateral eye. The remaining layers of the dLGN receive innervation from the ipsilateral eye (Malpeli and Baker, 1975; Livingstone and Hubel, 1988b).

The dLGN is organized retinotopically. The posterior dLGN contains the foveal and central visual fields. The visual field representation progresses peripherally as the dLGN extends anteriorly. This is shown schematically in Figure 2.4. The inferior visual field is situated in the medio-superior dLGN and the superior field infero-laterally (Malpeli and Baker, 1975).

Geniculate cells have a concentric receptive field arrangement of spatially exclusive On and Off regions (Wiesel and Hubel, 1966; Hubel and Wiesel, 1972), similar to that of the retinal ganglion cells described earlier. The stimulation of the surround leads to suppression of the centre responses.
The receptive fields of dLGN cells serving central vision are found to be smaller and more sensitive to suppression from stimulation of a spatially antagonistic region than cells in the periphery (Wiesel and Hubel, 1966). Bursts of synaptic potential from fibres of the optic tract precede the responses of geniculate cells, confirming the excitatory feedforward nature of these inputs (Wiesel and Hubel, 1966). Suppressive extra-classical receptive fields (ECRF) can also be found in the surrounding region of the dLGN relay cells (Solomon et al., 2002). Visually evoked responses are reduced by the stimulation of the ECRF. This reduction is referred to as extra-classical inhibition (ECI).

Four types of cells were distinguished by their receptive field properties to spatially and spectrally distinct stimuli within the dLGN (Wiesel and Hubel, 1966). Two of these types of cells, Type I and Type II, were found to encode colour information (Wiesel and Hubel, 1966; Conway, 2001). The more common Type I cell was both spatially and chromatically antagonistic (Wiesel and Hubel, 1966). It had a circular central region responding most vigorously to the colour of a restricted range of wavelengths. The opponent
surround region responded well to a different spectral sensitivity. Type I cells had a neutral point where increments or decrements of a wavelength caused neither an On or an Off response. The neutral point was usually a wavelength intermediate to those that the centre and surround regions were sensitive to.

The less common Type II cell was only spectrally antagonistic (Wiesel and Hubel, 1966) without a centre-surround antagonistic arrangement. Instead of having two regions of different preferences, the cell would respond well to an increment of light of one colour on the entire receptive field and be suppressed by an increment of light of another colour on the entire field area. Type II cells were more often found to have a neutral point at 500nm and were chromatically antagonistic without spatial antagonism (Wiesel and Hubel, 1966).

Another cell type was the Type III cell which was not spectrally opponent but did have a spatially antagonistic centre-surround arrangement. Type III cells showed similar chromatic preferences in both the centre and the surround. In fact, they usually had approximately equal additive inputs the L-cones and M-cones in both the centre and surround regions (Wiesel and Hubel, 1966).

A further cell type was the Type IV cell which was also spatially antagonistic, having concentric centre-surround regions, but with overwhelming peripheral suppression from large spots of light (Wiesel and Hubel, 1966). In the ventral pair of magnocellular layers, the cells were either of Type III or Type IV. The latter were spatially antagonistic but with a far larger surround region. The chromatic preference of the surround of Type IV cells seemed to be displaced towards long wavelengths (Wiesel and Hubel, 1966).

The four types of cells above can also be classified as X-cells and Y-cells, as has been described in the cat retina and dLGN, based on linearity of spatial summation (Dreher et al., 1976; Kaplan and Shapley, 1982). Type
I, II and certain Type III cells were reported to show X-like properties and were found in the parvocellular layers of the dLGN. Parvocellular neurons of the dLGN were originally likened to cat X cells (Dreher et al., 1976) but this was later disputed (Benardete et al., 1992).

Parvocellular cells show linear properties and sustained responses to standing contrast. Parvocellular cells also have slower axonal conduction velocities, most likely originating from the tonic ganglion cells of the retina (Gouras, 1968, 1969). They have smaller receptive fields than magnocellular cells at the same eccentricity and smaller cell bodies. Parvocellular cells, by virtue of their smaller receptive field sizes and higher sampling density (nearly ten times that of magnocellular cells) can potentially subserve high resolution spatial vision to encode for object recognition and form detection (Kaplan and Shapley, 1986; Kaplan et al., 1982). They often exhibit colour-opponent properties, low contrast sensitivity and low contrast gains (Purpura et al., 1988).

Primate magnocellular cells were reported to be in many ways similar to cat Y cells (Dreher et al., 1976) although this was later questioned (Benardete et al., 1992). Type IV cells and certain type III cells, also likened to cat Y cells (Dreher et al., 1976), are mostly contained within the magnocellular layers of the dLGN. Some magnocellular cells do not demonstrate linear spatial summation and most yield only transient responses (Kaplan and Shapley, 1986). They have faster axonal conduction velocities, possibly due to input from phasic ganglion cells, larger cell bodies and larger receptive fields at any given eccentricity compared to parvocellular cells. The magnocellular pathway is concerned with visual attributes related to space, such as movement, depth and positional relationships (Kaplan and Shapley, 1986; Livingstone and Hubel, 1988a; Kaplan et al., 1982). The defining properties of magnocellular cells are that of short response latencies to optic chiasm stimulation (Dreher et al., 1976), poor response to isoluminant chromatically opponent
gratings, greater sensitivity to luminance contrast and fast phase detection from better temporal resolution for the sensitivity to rapidly moving stimuli (Hicks et al., 1983).

Studies in New World monkeys, such as the squirrel monkey, owl monkey and the marmoset (Norton and Casagrande, 1982; Norton et al., 1988; Irvin et al., 1993; Yamada et al., 1998; Allison et al., 2000; Usrey and Reid, 2000; Ichida and Casagrande, 2002; Xu et al., 2002) also yielded similar distinguishing properties between parvocellular and magnocellular cells as is found in Old World monkeys. To understand the origin and combination of colour and high spatial acuity signals, parvocellular responses in dichromatic and trichromatic marmosets have been studied (Blessing et al., 2004). The sensitivity of parvocellular cells to red-green chromatic modulation was found to depend on the spectral separation between the medium and long wavelength sensitive cone pigments in trichromats. The means by which chromatic sensitivity depended on temporal frequency was also consistent with center-surround interactions of the receptive field. Some evidence for chromatic selectivity was seen in peripheral parvocellular cells. Receptive field dimensions were the same in dichromatic and trichromatic marmosets, but the achromatic contrast sensitivity of the cells were higher in dichromats. It was concluded that the primary role of the parvocellular stream was to transmit high acuity spatial signals. The red-green opponent signals of the parvocellular pathway were an additional response dimension in the trichromatic group of marmosets (Blessing et al., 2004).

2.5 The Koniocellular Pathway

The third functional pathway, namely the koniocellular pathway, has been found to be neurochemically, anatomically and physiologically distinct from the magnocellular and parvocellular pathways (Fitzpatrick et al., 1983; Tootell
et al., 1988; Jones and Hendry, 1989; Diamond et al., 1993; Hendry and Yoshioka, 1994; Hendry and Casagrande, 1996; Hendry et al., 1997; Martin et al., 1997; Reid et al., 1997; Hendry and Calkins, 1998; White et al., 1998; Solomon et al., 1999; Yoshioka and Hendry, 1999; White et al., 2001; Calkins et al., 2005). It has been named the koniocellular pathway because of the cells’ small, lightly-stained, “grain-like” appearance within the intercalated layers of the dLGN.

There are three main proteins which allow the koniocellular (K) cells to be neurochemically distinguished from the magnocellular (M) or parvocellular (P) cells in primates. First, there is the 28-Kda calcium binding protein “calbindin”. Second, there is the alpha sub-unit of Type II calmodulin-dependent protein kinase (α-CaM II kinase). The third is the gamma (γ) subunit of protein kinase C. A fourth protein, the calcium binding protein “calretinin”, is found only in the koniocellular neurons of fetuses of mid-late gestation (Diamond et al., 1993; Johnson and Casagrande, 1995; Hendry and Reid, 2000). Neurons considered as koniocellular cells are usually calbindin immunoreactive. Homologous neurochemical properties exist in many species including prosimians, nocturnal and diurnal New World monkeys, macaques, apes and humans (Diamond et al., 1993; Johnson and Casagrande, 1995; Hendry and Reid, 2000).

The process of using α-CaM II kinase to distinguish koniocellular cells has been applied to macaque retinal ganglion cells (Calkins et al., 2005). The aim of the Calkins et al., study (2005) was to reveal which axons at the retinal level provided input to the koniocellular retinogeniculocortical stream. Two groups of cells were differentiated which both had broad, sparsely branching dendritic trees and cell bodies intermediate in size between magnocellular and parvocellular ganglion cells. The first group usually had four primary dendrites, thick axons and rounded cell bodies of multiple types. The second group had only two primary dendrites, thinner axons and smaller, elliptical
cell bodies with highly regular morphology. These resembled the “large-sparse” cells which make up 2% of all ganglion cells retrogradely labelled from the dLGN.

In the macaque, the koniocellular cells form comparatively thin “intercalated” layers between the magnocellular and parvocellular divisions of the dLGN (Kaas et al., 1978; Fitzpatrick et al., 1983; Hendry and Yoshioka, 1994). The first layer is located between the optic tract axons and the ventral-most magnocellular layer; the second between the two ventral magnocellular layers; the third between the dorsal magnocellular layer and the ventral-most parvocellular layer and the following three koniocellular layers between each of the four parvocellular layers (Hendry and Yoshioka, 1994; Calkins et al., 2005). The koniocellular layers are labelled according to the main dLGN layer they are directly ventral to. Hence, koniocellular layers K1, K2, K3, K4, K5 and K6 progress from ventral to dorsal dLGN (Yoshioka and Hendry, 1999). The density of koniocellular cells decreases going from the ventral koniocellular regions of the dLGN to the dorsal parts.

The organization and size of the koniocellular layers in the macaque dLGN has been determined by cells that were stained immunocytochemically for α-CaM II kinase (Hendry and Yoshioka, 1994; Rodman et al., 2001). K1 is the largest out of the six layers, with K2, K3, and K4 being quite substantial. Layers K5 and K6 is much thinner and contains relatively fewer cells (Figure 2.5).

In conjunction with forming the distinct layers, koniocellular cells have also been identified neurochemically within all the magnocellular and parvocellular layers (Hendry and Yoshioka, 1994). They have been anecdotally reported to be dense enough to form “bridges” through the magnocellular and parvocellular layers between adjacent koniocellular layers, predominantly in the middle layers P3 and P4 of the macaque dLGN (Hendry and Yoshioka, 1994; Hendry and Reid, 2000). The largest of these bridges lies across layer
Figure 2.5: Koniocellular neurons stained with calbindin (black dots) in the macaque dLGN are most dense in the ventral layers close to the magnocellular (M1 & M2) regions and become thinner as they progress dorsally. Note that the stained cells are not limited to the intercalated layers; adapted from Rodman et al., 2001.

M1 and it has been suggested that the bridges may be in a part of the visual field which represents the optic disc (Hendry, 1991; Hendry and Yoshioka, 1994; Hendry and Reid, 2000).

Regions within the koniocellular layers which do not include cells that can be immunostained with the proteins Calbindin or α-CaM II kinase are mainly found close to the magnocellular layers. Ipsilateral neurons from magnocellular layer M2 have been found to be situated within K1 (Hubel et al., 1977; Kaas et al., 1978; Fitzpatrick et al., 1983; Yoshioka and Hendry, 1999). There is also a contralaterally innervated group of cells which is displaced from parvocellular layer P4 to koniocellular layer K3 (Hubel et al., 1977; Kaas et al., 1978; Yoshioka and Hendry, 1999).

The development of koniocellular neurons had been established by neuronal birthdating in the dLGN of the rhesus monkey. It was found that they were generated at the same time as magnocellular and parvocellular cells. The neurons first appeared ventrally and then extended dorsally layer by
layer (Rakic, 1977).

Functionally, the koniocellular cells may form three pairs of layers in the macaque dLGN (Hendry and Yoshioka, 1994; Yoshioka and Hendry, 1995, 1999; Hendry and Reid, 2000). The ventral pair may be tied to the function of the superior colliculus (such as in the reflexive control of eye movements) since they receive most of the collicular afferents to the koniocellular layers and the only afferents to the dLGN (Harting et al., 1973, 1978, 1980, 1991). The ventral pair also receives inputs from the parabigeminal nucleus of the midbrain, usually seen as a supporting structure for the function of the superior colliculus (Harting et al., 1991).

The middle koniocellular pair may aid in the relay of S-cone inputs to the cytochrome-oxidase (CO) blobs of layer 2/3 in area V1 (Hendry and Yoshioka, 1994; Hendry and Casagrande, 1996; Hendry et al., 1997; Yoshioka and Hendry, 1999). The source of these inputs is most likely from the axons of the small bistratified retinal ganglion cells carrying opponent blue-yellow inputs to the middle dLGN layers (Calkins and Hendry, 1996; Calkins et al., 1997; Martin et al., 1997; White et al., 1998; Hendry and Calkins, 1999; Solomon et al., 1999; White et al., 2001). In the macaque, blue-yellow opponency has been reported at the ventral borders of P3 and P4 (Wiesel and Hubel, 1966; Schiller and Malpeli, 1978; Reid et al., 1997) and may well be from the koniocellular layers.

The dorsal koniocellular pair had been reported in early studies to relay low-acuity visual information to layer 1 of the striate cortex (Hendry and Yoshioka, 1994; Hendry and Casagrande, 1996; Ding and Casagrande, 1998; Hendry and Calkins, 1999; Yoshioka and Hendry, 1999). Hence, in parallel with the magnocellular and parvocellular divisions, the koniocellular pathway transmits different attributes of the visual scene to V1 (Hendry and Reid, 2000).

Much of the knowledge of koniocellular properties comes from studies in
the Prosimian bushbaby Galagos (Kaas et al., 1978; Casagrande and De-Bruyn, 1982; Fitzpatrick et al., 1983; Irvin et al., 1986; Diamond et al., 1993; Irvin et al., 1993; Johnson and Casagrande, 1995) and New World primates (Martin et al., 1997; White et al., 2001; Solomon et al., 2002). The marmoset has been used to study the variability in the properties and physiology of dLGN cells between dichromats and trichromats. This is due to the fact that the marmoset displays a polymorphism of cone opsins (molecules sensitive to light) of medium to long wavelength range in the retina (White et al., 1998). The marmoset dLGN has a prominent koniocellular division between the parvocellular and magnocellular layers and hence it has been an useful model for koniocellular studies (Ghosh et al., 1997; Martin et al., 1997; White et al., 1998; Solomon et al., 1999; White et al., 2001).

Many koniocellular cells in marmosets respond briskly to stimuli which modulate S-cones and exhibit a classical center-surround blue-yellow colour opponent organization (Martin et al., 1997; White et al., 1998; Solomon et al., 1999; Szmajda et al., 2006). The colour opponent cells arise due to inputs from S-cones in both dichromatic and trichromatic marmosets. This suggests the dLGN organization in the species, whether dichromatic or trichromatic, is essentially the same (White et al., 1998). The koniocellular neurons in the marmoset also have larger receptive fields at any eccentricity, slower conduction velocities and longer latencies (White et al., 2001) than cells of the magnocellular and parvocellular streams. Low spatial frequencies were preferred by these cells, while the temporal frequency preference, orientation selectivity and contrast sensitivity were comparable to those of magnocellular and parvocellular neurons (White et al., 2001).

The koniocellular cells in the dLGN of marmosets respond briskly to grating stimuli, unlike the W cells of cats (Hirsch, 2003) although koniocellular cells are similar in connectivity and physiology to the W cells. Koniocellular cells have previously been shown to represent a functionally heterogeneous
group where the spatial properties of some koniocellular cells are between those of magnocellular and parvocellular cells (Norton and Casagrande, 1982; Norton et al., 1988). Their temporal contrast sensitivity at any given eccentricity also lies between that of magnocellular and parvocellular cells. However, the overall response properties of koniocellular cells more closely resemble that of parvocellular rather than magnocellular cells (Casagrande, 1999; Solomon et al., 1999).

To investigate the physiology of koniocellular cells in the owl monkey, extracellular recordings were made in the dLGN after inactivation of the primary visual cortex (Xu et al., 2001). It was found that a third of koniocellular cells responded poorly to drifting gratings. Although all cells showed an increase in size with eccentricity, koniocellular cells showed more scatter. Koniocellular cells in the owl monkey also had lower spatial and intermediate temporal resolution, with contrast thresholds and gains more similar to magnocellular cells than parvocellular cells (contrary to findings in the marmoset). Koniocellular cells in different koniocellular layers differed in spatial, temporal and contrast sensitivity characteristics. There was found to be higher spatial resolution and lower temporal resolution in K3 than in K1 or K2 cells (Xu et al., 2001). This would suggest that koniocellular cells may be divided into several classes of cells, which may contribute to conventional aspects of spatial and temporal resolution.

There are about 1 million parvocellular cells and 100,000 magnocellular cells in the dLGN of the rhesus monkey. A quarter of the parvocellular cells and a third of the magnocellular cells are GABAergic interneurons. The number of koniocellular cells appeared to equal the number of magnocellular cells when immunoreactivity for α-CaM II kinase was used to mark the koniocellular cells (Montero and Zempel, 1986; Peters et al., 1994). The divergence ratio describes the number of cells in the striate cortex that a dLGN cell would project to. Koniocellular cells matched the 1:50 divergence ratio.
of parvocellular cells, so they can be expected to play an equally important role in spatial vision. However, koniocellular cells in the retina are closer in number to the magnocellular cells projecting to V1. They do not dominate cortical circuits with the high divergence ratio (1:300) or larger axons that magnocellular cells display, so may also be more limited in their scope to serve vision in comparison to magnocellular cells.

A recent study in the Old world macaque monkey involved injections into physiologically identified koniocellular layers of the dLGN in order to track terminations of these neurons within the striate cortex (Casagrande et al., 2007). In contrast to early studies (see Chapter 2: Introduction), the koniocellular layers K1 and K2 were found to project to layers 1 and 3A of V1 in a simple synaptic fashion with few boutons. Layers K3 to K6 seemed to terminate in layer 2/3 and also within layer 3Ba of V1. The neurons in these layers had dense arbors with complex projection patterns.

Layer 2/3 of the striate cortex displays regions of metabolically active output zones due to a regular pattern of dense staining areas rich in the mitochondrial enzyme cytochrome oxidase (CO) (Horton and Hubel, 1981; Livingstone and Hubel, 1982; Horton, 1984; Wong-Riley and Norton, 1988; Ding and Casagrande, 1998). These region have been termed CO “blobs” and the surrounding areas without the dense staining are termed CO “interblobs”. Layer 2/3 of V1 receives direct signals from the dLGN koniocellular pathway (Hendry and Yoshioka, 1994; Hendry and Calkins, 1999) and indirect magnocellular and parvocellular inputs from within the cortex. The ipsilateral and contralateral segregation from the koniocellular layers to the CO blobs is maintained, with about 30 koniocellular cells innervating each blob. While retrograde labeling of blobs with blue-yellow opponency reveals cells in layers K3 and K4, the red-green opponent blobs reveal cells in P3 and P4 of the dLGN which show immunostaining for α-CaM II kinase (Ts’o and Gilbert, 1988; Hendry et al., 1997).
The koniocellular pathway projections to the CO blobs of layer 2/3 of V1 may indicate chromatic processing within these layers (Bullier and Henry, 1980; Livingstone and Hubel, 1988a; Ts'o and Gilbert, 1988; Hendry and Yoshioka, 1994; Hendry and Calkins, 1998; Yabuta and Callaway, 1998; Shostak et al., 2003). A greater proportion of the koniocellular axons also synapse with larger glutamatergic shafts in diurnal primates than in nocturnal primates (Ding and Casagrande, 1998; Shostak et al., 2002). This may indicate a facilitation within the koniocellular pathway of diurnals due to the presence of colour signals. The absence of colour in nocturnal primates may have led to smaller glutamatergic shafts in the koniocellular pathway (Ding and Casagrande, 1998; Shostak et al., 2002). Moreover, the absence of layer K4 in owl monkeys, the only nocturnal simian species without S-cones, indicates that K4 may be an S-cone dominated pathway (Shostak et al., 2002). However, the similarities between axon circuitry within the CO blobs of the diurnal and nocturnal primates suggests that this pathway plays a common role across the species, whether they are diurnal or nocturnal (Ding and Casagrande, 1998; Shostak et al., 2002).

The spatial density of blobs, blob diameter, proportion of cortical area within blobs, and pattern of geniculate projection to V1 from the koniocellular layers is not significantly different between dichromatic and trichromatic marmosets (Solomon, 2002). The koniocellular layers of the dLGN contribute 11% of all relay cells to V1. They form the only dLGN input to V1 within layer 2/3 but about half of all relay cells in the koniocellular layers express calbindin. Hence, although the CO blobs in diurnal primates contain colour-selective cells, the koniocellular layers and CO blobs are also well developed in colour-blind nocturnal primates (Shostak et al., 2002, 2003). Koniocellular cells may therefore contribute to some “conventional” aspects of visual processing, or they may have a number of other modulatory roles. Anatomical connections between koniocellular cells suggest that this pathway serves some
common function across species that is not only limited to the processing of short wavelengths (Casagrande, 1999).

Direct extrastriate projections from the koniocellular layers is primarily from a small proportion of neurons along the medial and caudal borders of the dLGN which have a larger cell body size than the majority of the cells in the koniocellular layers (Hendry, 1995; Yoshioka and Hendry, 1999). They have nevertheless been labelled as koniocellular neurons due to their ability for immunostaining with either Calbindin or α-CaM II kinase. A large number of these neurons situated along the caudal and medial borders of the dLGN seem to send direct inputs to extra-striate area V2 in the representation of the foveal visual field (Hendry, 1995; Yoshioka and Hendry, 1999). These larger cell bodies in the koniocellular layers also send signals to other extrastriate areas, although not nearly as densely as to V2.

An anatomical study using a retrograde tracer injected into the motion-selective middle temporal area (MT) of the macaque monkeys has revealed direct inputs from the intercalated koniocellular divisions of the dLGN to area MT (Sincich et al., 2004). These inputs bypassed V1 completely to project to area MT which is not considered as a primary visual area. The physiological nature of these koniocellular cells are yet to be determined. They may be central to the transfer of information in blindsight (Rosa et al., 2000), which includes the persistence of motion sensitivity following V1 injury (Weiskrantz et al., 1974).

Reports of large, intensely Calbindin immunoreactive neurons (a marker for koniocellular cells) scattered throughout the otherwise degenerated dLGN of a macaque which had sustained a lesion of V1 at infancy (Rodman et al., 2001) supports the view that koniocellular neurons may be responsible for preserving vision after damage to V1. The Calbindin immunoreactive neurons in the injured macaque constituted about 95% of the remaining neurons identified in the dLGN.
A study using visual evoked potentials (VEPs) was used to record spatiotemporal properties in the human brain from S-cone isolating tritan and luminance stimuli. Two times intervals were reported in which significant VEPs were evoked specific to the stimuli. The faster response latencies were between 40 to 75 ms and the slower latencies between 175 to 240 ms (Morand et al., 2000). The VEPs evoked by the tritan-motion stimuli appeared even earlier than the motion specific fields, although the location of the fields for both stimuli were identical. This indicates a very fast activation of cortical areas with a putative S-cone input for motion processing in the same cortical regions as the magnocellular pathway for encoding luminance motion (Morand et al., 2000). The fast S-cone inputs may well be arriving from the koniocellular pathway and this hypothesis is strengthened by the evidence of direct anatomical projections of koniocellular cells in the macaque (Stepniewska et al., 1999; Sincich et al., 2004).

### 2.6 The Visual Cortex

The local connections of neurons within the visual cortex has been studied to unravel the functional and organizational interconnections that lead to early visual processing (Movshon et al., 1978a,c; Blasdel and Fitzpatrick, 1984; Lachica et al., 1992; Yoshioka et al., 1992; Casagrande and Kaas, 1994; Callaway, 1998; Sincich and Horton, 2003). The lateral connections within the subcortical and extrastriate areas show the patterns of neural activity of the individual cortical neurons, and also their visual response properties (Yoshioka et al., 1994).

Cells in the striate cortex had been classified (Hubel and Wiesel, 1962, 1968) as simplex or complex according to whether they (a) had spatially segregated On and Off subregions and (b) exhibited summation within each region and (c) had On and Off subregions that were antagonistic and (d)
if it was possible to predict the neuron’s response to any stimulus from the arrangement of excitatory and inhibitory subregions. Fulfillment of all four criteria indicated that the recorded cell was a simple cell, while a complex cell was one that did not meet at least one of the criteria. A quantitative ratio between the response amplitude of the first harmonic and the overall increase in activity of the cell (0th harmonic) to drifting sine wave gratings was later employed as a means of classification of simple and complex cells (Maffei and Fiorentini, 1973; Movshon et al., 1978b; De Valois et al., 1982; Skottun et al., 1991).

The cortical receptive fields in the macaque were discovered to be arranged within cortical columns (Mountcastle, 1957). Cortical columns were defined by their horizontal transition of ocular dominance tangential to the cortical surface along one axis and their transition in orientation along the other axis (Hubel and Wiesel, 1969, 1972; Hubel et al., 1977). Cortical columns were seen to extend vertically through the depth of the cortex in V1 and V2. The ocular dominance columns were centered over the CO blobs, found in layers 2/3 of V1 (Horton and Hubel, 1981; Horton, 1984; Livingstone and Hubel, 1984). These areas were claimed to display greater contrast sensitivity and lower spatial frequency than the so-called inter-blob areas (Livingstone and Hubel, 1982, 1984).

The cortical columns contained simple, complex and hypercomplex cells arranged in the macaque visual cortex (Hubel and Wiesel, 1968, 1969). Cells termed as hypercomplex were defined as those with increasing responses up to a preferential stage, such as up to a specific length of a line grating, followed by a decrease in response past the optimum length. This behaviour was termed end-stopping. There was a segregation of complex and hypercomplex cells in V1 upper layer 2 and the top of layer 3, as well as the lower layers 4 and 6. The simple cells, in contrast, were found mainly in the geniculo-recipient layers 4Co and 4Cβ. These layer, along with layer 4A, did not seem
to demonstrate orientation selectivity and was mostly monocular (Hubel and Wiesel, 1968, 1969).

The laminar organisation of the intracortical circuits in the visual cortex has important implications for transmission of neural signals to higher areas of visual processing and for feedback influences (Felleman and Van Essen, 1991; Salin and Bullier, 1995; Angelucci et al., 2002; Muller et al., 2003; Yabuta et al., 2001). The cortex shows multiple neuronal types and many different types of connectivity. The arbors of dendrites often extend beyond one layer, as shown in Figure 2.6.

Figure 2.6: The input streams of the magnocellular, parvocellular and koniocellular pathways from the dLGN to the various levels of the geniculo-recipient layer 4 of V1; adapted from Callaway, 1998.

Magnocellular neurons from the macaque dLGN project mainly to layer 4Cα (Hubel and Wiesel, 1972; Blasdel and Lund, 1983) and to a lesser degree to lower layer 6 of V1. The parvocellular pathway from the dLGN projects mainly to layer 4Cβ and also to layers 4A and the upper part of layer 6 of V1 (Hubel and Wiesel, 1972; Blasdel and Lund, 1983). Layer 1 and the CO blobs of 2 /3 are innervated by the koniocellular pathway (Hendry and
Layer 4Cα has two types of magnocellular axons from the dLGN terminating within it, as shown in Figure 2.7 (Bauer et al., 1999). One set consists of axons (M2) dispersed throughout the extent of layer 4Cα and sends a few collaterals to other layers. The other set (M1) is mainly limited to the upper portion of layer 4Cα and sends a large percentage of collaterals to layer 4B (Blasdel et al., 1985). The differences in the characteristics of these two subsets of magnocellular cells may account for the differences in receptive field size and contrast sensitivities with depth within layer 4Cα (Boyd et al., 2000).

Figure 2.7: Distribution of M1 and M2 axons within layer 4Cα, adapted from Bauer et al., 1999.

Layer 4B receives strong unbranched magnocellular collaterals from 4Cα onto its spiny stellate and pyramidal cells (Nassi and Callaway, 2007). The cells in layer 4B are direction tuned (Livingstone and Hubel, 1984) but also respond to isoluminant stimuli (Johnson et al., 2001). The pyramidal cells project to the CO thick stripes in V2, which in turn project to area MT.
Spiny stellate cells in contrast project directly to area MT (Shipp and Zeki, 1985, 1989; Nassi and Callaway, 2007). Layer 2/3 neurons also provide a small input to layer 4B (Blasdel et al., 1985; Sawatari and Callaway, 1996). There is evidence that parvocellular inputs from 4Cβ may synapse onto layer 4B pyramidal neurons (Yabuta et al., 2001) along with magnocellular inputs from 4Cα (Shipp and Zeki, 1989).

The pyramidal neurons conveying parvocellular information are co-localised with those receiving magnocellular inputs for conveying directly to area MT or indirectly through the V2 thick stripes and V3 (Maunsell and Van Essen, 1983a; Burkhalter and Van Essen, 1986; Shipp and Zeki, 1989; Zeki and Shipp, 1989; Sincich and Horton, 2002, 2003; Xiao and Felleman, 2004). Hence, layer 4B may be involved in mixing parvocellular and magnocellular inputs rather than simply conveying magnocellular inputs (Sincich and Horton, 2002, 2003).

Layer 4A is a narrow strip where the neurons have dendrites that extend into layers 3B or 4B (Lachica et al., 1992). 4Cβ provides parvocellular inputs to layer 4A (Figure 2.7).

The superficial cortical layers 2/3 usually relay signals from the geniculo-recipient layers onto extra-striate areas. However, there is evidence for direct koniocellular inputs from the dLGN to layers 1 through to 3B (Casagrande et al., 2007). These layers also receive intracortical magnocellular inputs from 4Cα and 4B along with parvocellular inputs from layers 4Cβ and 4A (Sawatari and Callaway, 2000). Neurons within the CO blobs of layer 2/3 were reported to have spatially and chromatically opponent responses (double opponency) of both the red-green and blue-yellow categories (Livingstone and Hubel, 1982, 1984). Livingstone and Hubel (1982) also reported that the cells within the CO blobs have poor orientation selectivity and cells in the CO blobs project to adjacent blobs or to the thin stripes of V2. In contrast, the cells located within the interblobs were highly orientation selective but not
chromatically selective and projected to the pale interstripe regions of V2 (Livingstone and Hubel, 1982, 1984).

The visual pathways were originally believed to remain well segregated up to and beyond the primary visual cortex. However, the presence of some degree of convergence in V1 has been reported in the macaque (Hubel and Livingstone, 1990; Levitt et al., 1994a; Yoshioka et al., 1994; Sawatari and Callaway, 1996; Lund and Wu, 1997; Vidyasagar et al., 2002). To ascertain whether extrastriate regions beyond V1 receive specifically one or the other type of input, the layers of the dLGN providing magnocellular or parvocellular inputs were selectively inactivated with small injections of lidocaine or GABA while the activity in the extrastriate areas was measured (Malpeli et al., 1981; Ferrera et al., 1994). It was found that the cortical responses of area MT were greatly reduced when the magnocellular pathway was blocked, but was almost unaffected by parvocellular blockage (Ferrera et al., 1994).

During inactivation of the parvocellular regions of the dLGN, area V4 of the ventral stream which is thought to receive solely parvocellular inputs, remained largely responsive (Ferrera et al., 1994). This suggests that there may be more widespread magnocellular inputs to extrastriate areas than was previously believed. However, inactivation of the magnocellular dLGN layers did not result in equally strong contributions of the parvocellular pathway to the magnocellular-dominated dorsal stream areas (Nealey and Maunsell, 1994).

Responses to achromatic contrast and isoluminant chromatic stimuli, coupled with orthodromic stimulation latencies, were used in a study to find signatures of magnocellular and parvocellular inputs to single cortical cells (Vidyasagar et al., 2002). A population of V1 cells were found to respond well to high achromatic contrast sensitivity and also to isoluminant chromatic stimuli. Such a finding may be a result of convergence of magnocellular and parvocellular signals in V1. However, this result may also be due to a con-
vergence of magnocellular and koniocellular layers or simply the responses from a distinct class of koniocellular neurons.

The major population of neurons that form extra-striate projections arise from layer 2/3 and 4B of V1 (Figure 2.8). The dorsal (parietal) stream from the magno-recipient zones of V1 primarily conveys signals related to movement, depth and spatial localisation (DeYoe and Van Essen, 1988; Livingstone and Hubel, 1988a; Zeki and Shipp, 1988; Merigan and Maunsell, 1993). This pathway is also known as the “where” pathway. The cells within this pathway are often orientation selective and can potentially convey form information (Gegenfurtner et al., 1996; Kiper et al., 1997; Yabuta et al., 2001).

Layer 2/3 blobs and interblobs project via the V2 thin and pale stripes, respectively (Gegenfurtner et al., 1996; Johnson et al., 2001; Sincich and Horton, 2003) to area V4 in the ventral (temporal) stream. This pathway is often known as the “what” pathway (Livingstone and Hubel, 1988a; Zeki and Shipp, 1989). The koniocellular dominated CO blobs of V1 convey colour information to the V2 thin stripes and the interblobs to the V2 pale stripes (Callaway, 1998; Sincich and Horton, 2005). These patterns describe the tri-partite arrangement of projections in the visual system (Livingstone and Hubel, 1988a). The magnocellular cells were proposed to project to layer 4B and onward to the thick stripes of V2, while the parvocellular and later koniocellular cells were thought to project to the layers 2/3 blobs and interblobs on their way to the V2 thin and pale stripes, respectively.

However, another pattern of projections from V1 to V2 segregated only on the basis of the CO blobs and interblobs has been described recently. It has been reported that the interblob (interpatch) areas of layers 2/3 and 4B of V1 project to both the pale and thick stripes of V2 (Sincich and Horton, 2002, 2005). In contrast, the blobs (patches) of these layers project only to the thin stripes of V2. The thin and pale stripes send inputs to V4 (DeYoe and Van Essen, 1985) and the thick stripes project to area MT (Shipp and
While the thin and pale stripes of V2 contain colour selective cells, the thin stripes display cells with orientation selectivity while the pale stripes contain these cells organised within orientation maps (Livingstone and Hubel, 1988a; Ts’o and Gilbert, 1988). The thick stripes receive mixed magnocellular and parvocellular inputs from the layer 4B pyramidal cells of V1 (Yabuta et al., 2001), as well as inputs from layers 2/3 and 4A which may be disparity tuned (DeYoe and Van Essen, 1985). Hence, this may provide a substrate for mixing of magnocellular, parvocellular and koniocellular inputs which may be transferred via their projections to the dorsal and ventral streams, respectively.

Most primate species, including the macaque, appear to have similar arrangements of synaptic connections from V1 to V2 (Ding and Casagrande, 1998). The processing of signals is possibly advanced at each level as well as partially maintaining the segregation of the magnocellular, parvocellular and koniocellular pathways in their projection to the extrastriate areas (Maunsell and Newsome, 1987).

### 2.7 The Middle Temporal area (MT)

Area MT in the primate is located in the dorsal third of the posterior bank of the superior temporal sulcus (Campbell, 1908; Allman and Kaas, 1971; Zeki, 1974; Gattass and Gross, 1981; Van Essen et al., 1981) named after its location in the middle temporal gyrus of the owl monkey (Allman and Kaas, 1971). Area MT is especially sensitive to the direction and speed of stimulus motion and is regarded to have a primary role in motion processing (Allman and Kaas, 1971; Zeki, 1974; Maunsell and Van Essen, 1983a,b,c; Albright, 1984; Albright et al., 1984). The preferred direction for moving edges remains unchanged at various speeds (Rodman and Albright, 1987).
Figure 2.8: Projection of the magnocellular, parvocellular & koniocellular layers from the dLGN through the termination layers in V1 and onto the dorsal (parietal or “where” for motion) and ventral (temporal or “what” for perception) streams in the extrastriate areas. The koniocellular projections are thought to send signals to the CO blobs of layer 2/3 in V1; adapted from www.skidmore.edu/~hfoley/images/Visual-Paths.jpeg
The cells in area MT have been classified as Speed Class 1 (S1) or Speed Class 2 (S2). S1 cells show no inhibition of response to preferred speeds at the non-optimal direction of motion and have a speed tuning curve which is similar in all directions of motion (Rodman and Albright, 1987). The S2 cells respond optimally to various speeds in the preferred direction of motion, but the optimum speed changes with direction.

The majority of cells in area MT are binocular (Maunsell and Van Essen, 1983a). Approximately two-thirds of the cells have a preference for binocular disparity. This is the judgment of depth from the difference in perceived object distance between the two eyes.

Area MT is organized in such a way that attributes such as the preferred speed of the moving stimuli and amount of binocular disparity change tangentially to the surface of the cortex. Vertically traversing through the cortex with a recording electrode reveals preservation in the selectivity of such attributes (Albright, 1984; Maunsell and Van Essen, 1983a,b). Hence, in the macaque monkey area MT is thought to be organized into columns of preference for direction (Albright, 1984), as well as orientation, speed and binocularity (Maunsell and Van Essen, 1983a,b). Similar findings have recently been reported in the marmoset monkey (Lui et al., 2007). This columnar organization is also seen with orientation, ocular dominance, colour and even spatial frequency in the V1 cells that project to area MT (Hubel and Wiesel, 1968; Schiller et al., 1976; De Valois et al., 1982; Orban et al., 1986).

The pyramidal and spiny stellate cells of layer 4B cells that project to area MT have longer dendrites and larger cell bodies (Nassi and Callaway, 2007), with the cell bodies usually located under the CO blobs. This would indicate a fast axonal conduction velocity for the transmission of magnocellular inputs from their location deep within layer 4B.

Although motion processing was traditionally thought to be exclusive of colour signals in area MT (Zeki, 1974; Maunsell and Van Essen, 1983a,c;
Albright, 1984), recent reports contradict this conclusion. Evidence of colour-based motion processing has been shown in area MT (Saito et al., 1989; Dobkins and Albright, 1994; Gegenfurtner et al., 1994; Dobkins and Albright, 1998; Seidemann et al., 1999; Thiele et al., 1999, 2001). This may be due to the mixing of parvocellular inputs within layer 4B cells of V1 (Yabuta et al., 2001) which project to area MT via the thick stripes of V2 (Sincich and Horton, 2002, 2003) while 4B cells projecting directly to area MT receive a segregated magnocellular input (Nassi and Callaway, 2007). There were also larger and clearer responses reported in area MT neurons when edge segmentation was aided by colours (Croner and Albright, 1999). Memory may also play a major role in area MT, as has been shown with integrating recollections of stimuli locations for motion processing (Bisley et al., 2004).

The larger receptive fields of area MT compared to V1 allow better detection of motion at a faster rate. This results in better sensitivity of motion at higher spatial frequencies (about three times that in V1) and temporal frequencies (Mikami et al., 1986a,b). When shown stimulus bars of different lengths, area MT neurons demonstrated inhibition to bars much shorter than the size of the receptive fields in contrast to neurons in area V4. In V4 the bars had to be much longer than the receptive fields to evoke inhibition (Cheng et al., 1994). V4 and area MT also have similar preferences for speed. Area MT cells are more sensitive to luminance contrast and are found in clusters of cells preferring the same speed.

Area MT is a part of the dorsal stream that receives mainly magnocellular inputs. The inputs to area MT are summarised in Figure 2.9 as detailed in a study of primate hierarchical processing (Felleman and Van Essen, 1991). Recently there has been evidence suggesting that parvocellular inputs are transmitted disynaptically from the dLGN to area MT through the layer 6 Meynert cells in V1 (Nassi et al., 2006).

Area MT has been reported to remain active after inactivation of V1
Figure 2.9: Major pathways, direct and indirect, to area MT. The thickness of the lines is proportional to the quantity of inputs; from Born and Bradley, 2005.

(Rodman et al., 1989; Girard et al., 1992) although this does not hold when inactivated in conjunction with the superior colliculus (Rodman et al., 1990). These response within area MT with V1 inactivation could be a result of direct inputs from the dLGN as described in the macaque (Stepniewska et al., 1999; Sincich et al., 2004) or via the thick stripes of V2 (DeYoe and Van Essen, 1985; Shipp and Zeki, 1985). Anatomical tracers have indicated that V2 may receive inputs directly from the macaque dLGN (Yukie and Iwai, 1981; Bullier and Kennedy, 1983), similar to projections in the prestriate cortex reported in the cat (Dreher and Cottle, 1975).

Responses in the thick and pale bands of V2 have been reported to arrive 20 ms earlier than responses in the thin bands (Munk et al., 1995). The direction selective and non-colour specific neurons in V2 have also been
reported to have shorter response latencies than colour specific and unidirectional neurons. V2 does receive feedback from area MT (Maunsell and Van Essen, 1983c; Kennedy and Bullier, 1985) outside layer 4 of V2 (Maunsell and Van Essen, 1983c). However, inactivation of V1 diminishes responses of all cell types in the thick, pale and thin stripes of V2 (Schiller and Malpeli, 1977; Girard and Bullier, 1989). This loss of response in V2 with V1 inactivation may suggest that the direct subcortical inputs and feedback from area MT to V2 plays more of a role in modulating those inputs from V1 instead of leading to V2 responses.

Besides the major striate input that area MT receives, there may also be inputs through subcortical projections from various sources. Response latencies in certain area MT cells have been found to be less than 40 ms, even shorter than latencies in V1 (Maunsell and Newsome, 1987; Raiguel et al., 1989; Schmolesky et al., 1998; Raiguel et al., 1999). This suggests that selective subcortical inputs may completely bypass V1 to reach area MT. Retrograde tracers injected into area MT of the macaque have revealed a direct projection of cells from the koniocellular layers of the dLGN (Sincich et al., 2004). Selective inactivation of magnocellular layers of the dLGN has resulted in reduced responses in area MT neurons (Maunsell et al., 1990).

Visual signals can also reach extrastriate areas via the superior colliculus and the pulvinar (Benevento and Yoshida, 1981; Fries, 1981; Yukie and Iwai, 1981; Bullier and Kennedy, 1983). The superior colliculus receives direct retinal inputs (Hubel et al., 1975; Pollack and Hickey, 1979) and projects to the lateral and inferior pulvinar (Benevento and Standage, 1983). Along with the signals from the pulvinar, the superior colliculus projects directly to area MT (Maunsell and Van Essen, 1983c; Standage and Benevento, 1983; Ungerleider et al., 1984; Stepniewska et al., 1999). Removal of the superior colliculus alone does not affect the binocularity, orientation, and directional responses of area MT, nor their receptive field dimensions (Rodman et al., 1983).
Apart from the tectopulvinar connections, there has been evidence of reciprocal connections between area MT and the claustrum and pons (Glickstein et al., 1980; Maunsell and Van Essen, 1983c; Ungerleider et al., 1984). These subcortical inputs may be responsible for the phenomenon of blindsight (Weiskrantz et al., 1974). This phenomenon was described as the retention of non-reflexive implicit visual functions in fields of cortical blindness due to the destruction of V1 (Sanders et al., 1974; Weiskrantz et al., 1974). Human patients have been reported to still be able to detect, localize and discriminate the size, motion, orientation, direction, wavelength or shape of visual stimuli within the fields of cortical blindness (Kentridge et al., 1999). The study of blindsight involves quantifying the dissociation between perception and performance. Blindsight has been proposed to be the result of retinal pathways propagating through subcortical regions which completely bypass V1 (Cowey and Stoerig, 1991; Rosa et al., 2000).

The preserved inputs may project to motion processing areas of the dorsal stream since the direction and orientation of stimuli are well discriminated in blindsight without the acknowledgement of visual awareness (Weiskrantz et al., 1995). However, those stimuli which are acknowledged to be seen by human patients tend to be of a different speed. Contrary to earlier reports, shape is well perceived in blindsight (Marcel, 1998). The spatial tuning curves obtained psychophysically for blindsight subjects seem to show preferences to spatial frequencies below 4 cycles per degree (Sahraie et al., 2003). Those patients not able to respond to these stimuli were found to have lesions from the thalamus to the occipital lobe, so the subcortical sources bypassing V1 may also have been affected. Repeated training of blindsight patients with “discriminating grating visual stimuli optimally configured for blindsight from homogenous luminance-matched stimuli” resulted in an improvement in performance to awareness of stimuli in these patients (Sahraie et al., 2006). Studies in the monkey also showed an improvement of discrim-
inination of direction of motion from two to five years after V1 lesions (Moore et al., 2001) although there was a 98% rapid retrograde degeneration of the dLGN fibres immediately after V1 lesions (Cowey and Stoerig, 1991).

About two thirds of area MT neurons were reported to be unaffected by inactivation of V1 in the macaque monkey (Rodman et al., 1989). Inactivation of V1 also results in the retention of some visual responses in area V3a but total abolition of responses in V2, V3 and V4 (Schiller and Malpeli, 1977; Girard and Bullier, 1989; Girard et al., 1991, 1992). Area MT and V3a have been described as part of the dorso-parietal stream (Boussaoud et al., 1990) due to the outputs from here into the middle superior temporal area (MST), the superior temporal polysensory area (STP) and the lateral intra-parietal area (LIP). V3 and V4 in contrast are part of the infero-temporal stream (Morel and Bullier, 1990; Baizer et al., 1991). Inactivation of V1 seems to affect the infero-temporal stream for form and colour processing selectively and leaves the dorso-parietal stream for encoding position and movement of objects relatively intact.

The nucleus suprachiasmaticus of the hypothalamus which is preserved in pathologies of peripheral blindness (Stoerig, 2006), may contribute to blindsight in the peripheral visual fields by projecting directly to higher cortical areas. The presence of responses in cells of the superior colliculus to moving borders is a further indicator for a direct subcortical input to the higher motion areas. However, it is interesting to note that the inactivation of V1 in conjunction with superior colliculus removal does abolish all responses in area MT (Rodman et al., 1990). Hence the subcortical sources of blindsight remain to be further investigated.
Chapter 3

Experimental Methods

3.1 Experimental Animals

Recordings were made from 13 macaque monkeys (*Macaca fascicularis* and *Macaca nemestrina*) weighing between 4.3 and 5.6 kg, of which ten were male and three were female. The animals were obtained from the Australian National Health and Medical Research Council’s (NHMRC) macaque facility. All experimental designs and procedures were approved by The Department of Optometry & Vision Sciences/ The Victorian College of Optometry/ The National Vision Research Institute Animal Experimentation Ethics Committee and The Animal Welfare Committee of the University of Melbourne. The study was conducted in accordance with the guidelines from the Animal Welfare Act 1992, The Animal Welfare Regulations (VIC) 1993, and the National Health & Medical Research Council’s (NHMRC) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (6th Edition, 1997, listed as E Al, Cat. No. 9719628 in the NHMRC Publications Catalogue under “Ethics - Animal”). Each experiment lasted approximately five days, during the course of which the macaque was fully anaesthetised and paralysed.
3.2 Anaesthesia and Surgery

Anaesthesia was induced by intramuscular administration of a mixture of ketamine hydrochloride (Ketamil, Parnell Laboratories, Australia Pty. Ltd.; 15 mg/kg i.m.) and xylazine (Rompun, Bayer Australia Ltd; 2 mg/kg i.m.). Anaesthesia was maintained during the initial surgery by supplementary administration of ketamine and xylazine as required.

The initial surgery involved catheterizing both cephalic veins for intravenous administration during the experiment. The trachea was cannulated and a custom-made metal tube was inserted. This tube also contained an inner metal tube for periodically removing secretions that drained from the trachea during the experiment. The tracheal tube was connected to a respiratory pump (Harvard Apparatus). A thermister was embedded under the right scapula for monitoring body temperature. The animal was suspended on the second thoracic vertebra in order to promote drainage of respiratory secretions from the lungs.

The animal was placed in a stereotaxic frame with the ear bars inserted after application of a local anaesthetic cream (Xylocaine, Astra). The macaques’ head was positioned on the stereotaxic frame such that the infraorbital line was aligned horizontally with the central axes of the ear bars (using Horsley-Clarke co-ordinates). The frame, with the macaque attached, was then anchored to a vibration-free stereotaxic table.

After the induction of skeletal muscle paralysis with vecuronium (Norcuron, Organon; 0.7 mg/kg i.v.), anaesthesia and muscle relaxation were maintained with an intravenous infusion of a mixture of propofol (Propofol, SICOR Pharmaceuticals, Mayne Pharma Pty Ltd; 10 mg/kg/hr), sufentanil (Sufentanil, Jaansen-Cilag; 2 - 6 µg/kg/hr) and vecuronium (100 - 200 µg/kg/hr). In five out of the 13 animals, only sufentanil (2 - 6 µg/kg/hr) and vecuronium (100 - 200 µg/kg/hr) were used.
An infusion of 5% glucose in normal saline through the second venous line maintained a total fluid administration of about 200 ml per day during the course of the experiment. Added with the 96 ml of infusion solution (at 4 ml per hour) given during each 24 hour cycle, this resulted in about 2.5 ml/kg/hr of fluids. The end-tidal $CO_2$ was adjusted to be between 3.6 and 3.8% by regulating the rate and depth of ventilation. The body temperature was maintained around 36°C using a servo-controlled heating blanket. The electrocardiogram (ECG) and electroencephalogram (EEG) were continually monitored to help in ensuring adequate depth of anaesthesia. A fast Fourier transform (FFT) of the EEG was taken for every 10 seconds and displayed on a screen throughout the experiment. The criterion for determining adequate analgesia and anaesthesia was a predominantly low frequency range (5 - 10 Hz) in the EEG spectrum. A steady EEG signal and steady heart rate during potentially painful stimuli was a further criterion to ensure sufficient analgesia.

Initial recordings of this study were made from the dLGN. In the later studies recordings were made from the extra-striate visual area MT. The extra-striate recordings in area MT followed similar procedures to that for recordings in the dLGN. Methods for area MT recordings also included procedures for the reversible inactivation of V1. See Sections 3.6 and 3.7 for details.

### 3.3 dLGN Recordings

A craniotomy was performed over the dLGN of the left hemisphere between the Horsley-Clark stereotaxic co-ordinates of A2 to A12 antero-posteriorly and L7 to L15 medio-laterally, as guided by a brain atlas (Szabo and Cowan, 1984). The dura mater over the exposed brain was peeled off carefully without damaging the cortex. A chamber was formed around the hole of the
craniotomy by attaching a steel ring to the skull with dental cement. Small craniotomies for inserting stimulating electrodes for optic chiasm stimulation were also performed between the Horsley-Clark stereotaxic co-ordinates of A19 antero-posteriorly and L2 medio-laterally.

Lacquer-coated tungsten recording microelectrodes (FHC Metal Microelectrodes Inc., Bowdoinham, ME, USA; impedance 8 - 12 MΩ at 1000 Hz) were used for the dLGN recordings. It was held by a stable electrode carrier (hydraulic microdrive, model 650 and 607C, David Kopf Instruments, Tujunga, California, USA) that was attached to the stereotaxic frame. The electrode was lowered to the point where it made first contact with the pia mater. A 2% agar solution was poured onto the exposed brain surface to preserve moisture in the area. Wax was occasionally applied in order to seal the chamber and reduce brain pulsations as required.

The electrode was lowered into the dLGN by the electrode carrier and data were recorded to determine the chromatic preferences, spatial frequency tuning, temporal frequency tuning, contrast gain, preferred orientation and preferred direction of the dLGN cells. When possible, the orthodromic latencies of the dLGN cells to contralateral and ipsilateral electrical stimulation of the optic chiasm were also recorded.

3.4 Optics and Visual Stimuli

The animals’ pupils were dilated with topical tropicamide (Mydriacyl 1%) applied at the inferior bulbar conjunctiva. The animals’ eyes were fitted with a pair of gas-permeable contact lenses to protect the corneae from drying during the experiments. The position of the optic nerve head and the fovea of each eye were plotted on a wall chart using a back-projecting fundus camera. The eyes were re-plotted periodically to track their position and movement throughout the course of the experiment. The refractive errors of the eyes
were determined using retinoscopy and corrected by placing appropriate trial lenses in front of the animals’ eyes. An artificial pupil of 3 mm diameter was placed in front of each eye. Daily checks were made to ensure the stimuli were still focused on the retina since small eye drifts may have still occurred despite the maintenance of muscle paralysis.

The visual field of the macaque was scanned while the electrode was being lowered into the dLGN. The visually responsive cells were first identified using a hand-held projectoscope with the presentation of a bar grating or circular stimuli on the wall chart. The visual field was searched using visual stimuli of red, blue, yellow or green colour, achieved by placing appropriate filters over the light emitted from the handheld projectoscope. Black and white gratings of various spatial frequencies were also displayed on the wall chart or projected into the eye through an angled mirror.

This procedure was carried out so as to locate a cell and optimally position the electrode at a depth where the cell would produce a maximal neuronal response from particular stimuli. The micro-electrode signal was amplified and reproduced as sound using a loudspeaker. The loudspeaker output was used to judge the strength of the response. A rough estimate of the cell type being a magnocellular, parvocellular or koniocellular cell of the dLGN, the eye dominance of the cell, the optimal orientation, direction and velocity of the cell were all established according to the response given by the cell to the stimuli presented.

Once the approximate location and features of the cell were determined, the response characteristics were used as a guide to set the parameters of the visual stimuli. The stimuli, in the form of drifting sine-wave gratings within circular apertures, were produced by a Visage video signal generator (VSG) or a VSG Series Three stimulus generator (Cambridge Research Systems, Cambridge, UK). The stimuli were presented on a Barco Reference Calibrator Plus monitor (Barco monitor; Barco Industries, Belgium) at a

55
frame refresh rate of 100 Hz. The cell’s receptive field was located approximately at the centre of the monitor. The screen had a mean luminance of 25 to 60 cd/m$^2$ while the maximum Michelson contrast was 98%. The distance from the monitor to the eyes was 114 cm. Lens absorbance was calculated using published values for human eyes (Wyszecki and Stiles, 1982). The effect of retinal cone receptor self-screening was estimated assuming an axial absorbance of 1.5% and an outer segment length of 20 µm. There was no correction imposed for variations in retinal macular pigment. All calibrations were checked by a PR-650 spectrophotometer (Photo Research, Palo Alto, CA, USA).

The stimuli had been calibrated according to specifications of the Commission Internationale de l’Eclairage (CIE) coordinates in the form of x, y and Y, as well as along colour vectors in the “Derrington, Krauskopf and Lennie” (DKL) colour space (Derrington et al., 1984). The basis of the luminance and spectral specifications of the stimulus set was derived from the relative amount of three primaries $\bar{x}$, $\bar{y}$, $\bar{z}$ used in the Judd-Voss modified CIE 1931 colour matching functions (CMF). These three primaries may be used to match any monochromatic light over the visible spectrum by a standard CIE observer (Brainard, 1996). Each CMF is required in a relative amount to match a light stimulus. They are known as the tri-stimulus values X, Y and Z and are calculated as follows:

\[
X = K_m \sum_{\lambda} L_{e,\lambda} \bar{x}(\lambda) d\lambda \\
Y = K_m \sum_{\lambda} L_{e,\lambda} \bar{y}(\lambda) d\lambda \\
Z = K_m \sum_{\lambda} L_{e,\lambda} \bar{z}(\lambda) d\lambda
\] (3.1)

where $K_m$ is a constant that transforms a physical unit of radiance into a psychophysical unit of luminance (cd/m$^2$); $L_e$ is the radiance at each wavelength ($\lambda$) over the spectral distribution of the stimulus; $\bar{x}(\lambda)$, $\bar{y}(\lambda)$ and $\bar{z}(\lambda)$
are the integrated products of the spectral radiance distribution and the
colour matching functions (Kaiser and Boynton, 1996).

For cones with differing peak wavelengths, the activation of each cone
may be predicted as follows:

\[
\begin{bmatrix}
\bar{x}_1 & \bar{y}_1 & \bar{z}_1 \\
\vdots & \vdots & \vdots \\
\bar{x}_{102} & \bar{y}_{102} & \bar{z}_{102}
\end{bmatrix}
= \begin{bmatrix}
w_x \\
w_y \\
w_z
\end{bmatrix}
\times
\begin{bmatrix}
sC_1 \\
\vdots \\
sC_{102}
\end{bmatrix}
\tag{3.2}
\]

where \( sC_1 \) is the sensitivity of cone \( C \) to a cone with peak wavelength
at 423 nm along with its sensitivity to each of 101 wavelengths between 500
and 600 nm; \( w_x, w_y \) and \( w_z \) give the sensitivity of \( C \) in relative terms of
the colour matching functions \( \bar{x}, \bar{y} \) and \( \bar{z} \). The product of the tristimulus
values \( X, Y \) and \( Z \) and the transformation matrix appropriate for that cone
\( (w_x, w_y \) and \( w_z) \) gives the activation of a cone sensitive to a particular peak
wavelength to any stimulus light specified by \( X, Y \) and \( Z \). Hence the CIE
chromaticity coordinates that define the stimulus set consist of derivations
of the tristimulus values where

\[
x = X/(X + Y + Z) \tag{3.3}
\]
\[
y = Y/(X + Y + Z) \tag{3.4}
\]

and luminance \( Y = Z \).

The cells were classified as being On or Off type according to their re-
sponses to increments or decrements of light, respectively, relative to the
phase of the stimulus. A photometric feedback system for colorimetric spec-
ification and gamma correction was incorporated into the VSG system to
allow direct specification of stimuli in CIE \((x, y, Y)\) co-ordinates. Spectral
absorbance templates (nomograms) with peak wavelengths at 560 nm, 530
nm and 430 nm were generated using a polynomial template (Lamb, 1995).
The cone contrast for a given stimulus was calculated for each nomogram.
The macaque cone sensitivities and the spectral power spectrum of the monitor phosphors were convolved to obtain the cone-selective stimuli, as described in a recent study (Tailby et al., 2008a). The cone dominance and colour opponency of the cells were found by stimuli modulating the L-cone, M-cone and S-cone inputs in different combinations of opponent or additive phase (as already preset by the CIE co-ordinates in the VSG system). The S-cone isolating stimuli modulated between CIE co-ordinates [0.294, 0.268] and [0.336, 0.414] through the gray point [0.317, 0.335]. The L-cone and M-cone modulating stimuli were generated using the monitor phosphor guns. Table 3.1 shows the dominant wavelengths used for the three monitor phosphor guns to produce the cone isolating, achromatic (L-cone and M-cone in phase) and opponent L-M chromatic (L-cone and M-cone out of phase) stimuli.

“Silent” cone modulating stimuli refers to the substitution of wavelengths within the confusion loci of the other cones so that they are not responsive. The stimulus “silent L-cone” modulated M-cones along their (deutanopic) confusion line. The stimulus “silent M-cone” modulated L-cones along their (protanopic) confusion line. The presence of S-cone contrast in stimuli other than the S-cone isolating type were of minimal concern since there is little response from parvocellular and magnocellular cells to high amounts (> 10%) of S-cone contrast (Sun et al., 2006a,b). This was also observed in the current experiments.

The aperture size was increased or decreased and moved around on the monitor to position the aperture at the centre of the cells’ receptive field. The presented stimuli established the dimensions of the receptive field, optimal spatial and temporal frequencies, and the preferred orientation and contrast response properties of the cell. When initially characterizing a cell, a sine wave grating in a circular aperture was presented at different spatial frequencies while other parameters were held constant, to discover the cell’s optimal spatial frequency. The aperture size was usually 4°, the contrast was...
Table 3.1: Cone contrasts of the dominant wavelengths for the red (L), green (M) and blue (S) guns for the various stimuli used during the dLGN recordings.

<table>
<thead>
<tr>
<th></th>
<th>L (558 nm)</th>
<th>M (530 nm)</th>
<th>S (423 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silent M-cone</td>
<td>0.4150</td>
<td>-0.0176</td>
<td>0.3156</td>
</tr>
<tr>
<td>Silent L-cone</td>
<td>-0.0554</td>
<td>-0.4736</td>
<td>-0.1688</td>
</tr>
<tr>
<td>S-cone isolating</td>
<td>0.0013</td>
<td>0.0095</td>
<td>0.4621</td>
</tr>
<tr>
<td>Achromatic</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>L-M chromatic</td>
<td>0.1907</td>
<td>-0.2601</td>
<td>0.0780</td>
</tr>
</tbody>
</table>

100% and temporal frequency was 4 Hz. The orientation was usually 90° to the horizontal plane.

The optimal spatial frequency was read from a log spatial tuning curve. The achromatic contrast sensitivity, orientation tuning, optimum temporal frequency and chromatic selectivity were recorded at the peak spatial frequency given by the spatial frequency tuning curve. The orientation tuning was measured using presentations in 22.5° increments over 16 different orientations covering 180°. The temporal frequency was measured at the optimal spatial frequency and orientation.

To obtain information regarding the chromatic features of the cell, a sine wave grating was presented at 4 Hz. The grating alternated between two colours in the DKL space. The hues were along axes passing through a central white point (CIE D65) with a luminance of 55 cd/m² (Figure 3.1).

One set of colours used was on the same isoluminant plane and another set passed through the white point to brighter and darker elevations above and below the isoluminant plane (as in Blessing et al., 2004). The maximum achromatic Michelson contrast was 48% as opposed to the 98% specified for the screen luminance since the equal vector lengths limited the achievable amount for the maximum contrast. The vector arrows shown on Figure 3.2
represented the phase and amplitude of the response for the point in colour space on which the arrow’s tail was located.

Figure 3.1: Location of stimuli along the isoluminant plane of a chromaticity diagram for determination of the azimuth component (A). The CIE D65 point was the central white point. The luminance varied above and below isoluminance for the elevation component (B); adapted from Blessing et al., 2004.

Figure 3.3 shows the data displayed such that the points along the horizontal axis represented the wavelength of the stimuli presented, while the levels on the vertical axis represented stages of luminance modulation. The white lines connected points of identical luminance. The vector responses had a direction and an amplitude. The direction encoded the phase of the response with reference to stimulus onset while the amplitude of the vector gave an indication of the strength of the response. The vector response was most useful as a tool to categorise cells according to their colour and phase preferences. The largest sized vector arrow pointing downwards over an azimuth position meant the cell’s receptive field centre was tuned for that chromatic component of the stimulus. Likewise, a vector pointing upwards
Figure 3.2: The vector size and direction at each point along the colour space determined the amplitude of the response and the chromatic preference of the cell, respectively; adapted from Blessing et al., 2004.

specified the cell’s central receptive field responded suppressively to the chromaticity of the stimulus.

Stimuli with silent substitution were possible by the adjustment of the red and green phosphor guns to produce equal quantal absorption for the cone type being modulated. The Michelson cone contrast in the primate cones were predicted using tristimulus values such that

$$
\begin{bmatrix}
X \\
Y \\
Z
\end{bmatrix} =
\begin{bmatrix}
\frac{x_R}{y_R} & \frac{y_R}{y_R} & \frac{z_R}{y_R} \\
1 & 1 & 1 \\
\frac{x_G}{y_G} & \frac{y_G}{y_G} & \frac{z_G}{y_G}
\end{bmatrix}
\begin{bmatrix}
L_v, R \\
L_v, G \\
L_v, B
\end{bmatrix}
$$

(3.5)

where $x_R$, $y_R$ and $z_R$ are the CIE x, y, z chromaticity co-ordinates of the red phosphor and $L_v, R$ is its maximum luminance. While the blue phosphor luminance was set to zero, similar calculations were made for the green (G) and blue (B) phosphors as in the equation above.
Figure 3.3: Examples of responses for four cell types. The Red-On and Green-On cells had vectors pointing downwards in the regions where the dominant wavelengths were modulating the cone types. The Blue-On and Blue-Off cells had responses which were shifted horizontally to match the phase of the appropriate modulation wavelengths. The Blue-Off cell showed an upward vector for a response to decrements of the stimuli which correspond to the phase of the decrements. The On cells had downward vectors corresponding to the phase of the increments.
3.5 Electrophysiological data

The extracellular electrophysiological data were recorded with lacquer-coated tungsten electrodes. The signal was amplified and filtered (300 to 3000 Hz). Spikes were visualised through an oscilloscope (Philips PM 3352A, USA) and isolated using a window discriminator. The times of occurrence of these spikes were automatically recorded by an online computer. Cell responses to drifting grating stimuli of 2 to 4 seconds duration, usually repeated 3 to 5 times, were recorded in a peri-stimulus time histogram (PSTH). Some analysis was done online to show the responses to the different stimuli, including the $0^{\text{th}}$, $1^{\text{st}}$ and $2^{\text{nd}}$ harmonics of the fast Fourier transform (FFT) of the response to each stimulus sweep. The $0^{\text{th}}$ harmonic referred to the spontaneous activity of the cell, while the $1^{\text{st}}$ harmonic component displayed the cell’s response.

The stimulus condition (chromatic or achromatic) which resulted in the maximum response in the spatial frequency transfer function for a cell was used for further analysis of non-linearity, receptive field dimensions and spatial frequency preferences. For the R-G cells, the responses from the achromatic luminance modulating stimuli were used for consistency since L-cone and M-cone antagonistic modulation transfer functions were not available for R-G cells. Meanwhile, the S-cone isolating stimuli gave the optimum responses for the spatial frequency functions of the B-Y cells and was used in the analysis for the B-Y population.

3.5.1 Receptive field dimensions

The receptive field dimensions of the centre radius, surround radius, centre sensitivity and surround sensitivity were derived from the difference of Gaussians (DOG) model that was fitted to the spatial frequency modulation transfer functions (Rodieck, 1965; White et al., 2001; Croner and Kaplan,
using a computer program (Matlab, Mathworks 2007). The centre and surround DOG fits have differing spatial frequency tuning curves due to the difference in the two regions’ radii. When contrast is fixed although spatial frequency can vary, the centre response is obtained from

\[ R_c = C \cdot \left( K_c \pi r_c^2 e^{-(\pi r_c f)^2} \right) \]  \hspace{1cm} (3.6)

where \( f \) is the spatial frequency of the stimulus in cycles per degree (cyc/deg); \( C \) is the Michelson contrast of the stimulus; \( K_c \) is the peak sensitivity of the DOG fit for the central region and \( r_c \) is the radius of the central DOG fit where sensitivity is \( \frac{1}{e} \) of the peak (Enroth-Cugell and Robson, 1966; Croner and Kaplan, 1995). The response of the surround (\( R_s \)) may be calculated from an identical equation but with the replacement of \( R_c, K_c \) and \( r_c \) with \( R_s, K_s \) and \( r_s \). The response (\( R \)) of the cell when the centre and surround are assumed to be antagonistic is

\[ R = R_0 + R_c - R_s \]  \hspace{1cm} (3.7)

where \( R_0 \) is the response from spontaneous activity. The free parameters of centre radius, surround radius, centre sensitivity and surround sensitivity that are modeled onto these spatial frequency transfer functions are constrained to be positive.

The centre radius was regressed onto the centre sensitivity on a log scale to examine the relationship between the two properties. The centre radius was also displayed as a function of eccentricity to inspect the association between the two variables.

### 3.5.2 Non-linearity Index

The non-linearity index for all cells was found by dividing the average of the 2\(^{nd}\) harmonic of the FFT (\( F2 \)) component by the average of the 1\(^{st}\) harmonic of the FFT (\( F1 \)) component.
3.5.3 Low Frequency Ratio (LFR)

Cells were presented with gratings of spatial frequencies from 0.01 to 100 cyc/deg in 1 octave steps to measure their spatial frequency tuning. A low frequency ratio (LFR) was employed to determine the preference of the cells for low spatial frequencies. The response at the lowest spatial frequency presented (0.01 cyc/deg) was divided by the response at the optimum spatial frequency. A value close to zero would suggest a spatial band pass filter (Figure 3.4 A) while a value close to or equal to unity would suggest that the optimum spatial frequency preference for the cell was in the low range (Figure 3.4B), effectively a low pass filter.

Figure 3.4: Examples of two spatial frequency transfer functions where the fit for the cell in (A) resulted in a LFR of 0.01. The low pass spatial frequency filter in cell (B) resulted in a LFR equal to 1, as the maximum response was the same as the response at the lowest spatial frequency presented.

3.5.4 Temporal Frequency Ratio (TFR)

A parallel measure similar to the LFR was used for temporal frequency analysis, resulting in the temporal frequency ratio (TFR). This ratio was obtained by dividing the response at the lowest temporal frequency presented (0.5 Hz) by the optimum response at any temporal frequency (0.5 to 32 Hz). As with
the LFR, a value close to unity would suggest a low pass temporal frequency filter, while a value close to zero would suggest a temporal band pass filter (Figure 3.5). The cell response could be thought of as sustained when the TFR value was close to unity from better responses at low temporal frequencies. Values close to zero would indicate a transient cell response from preferences to temporal frequencies outside the low range.

Figure 3.5: A temporal frequency function where the cell response was fairly transient since the optimum response was removed from the lowest temporal frequency presented. This resulted in a TFR of 0.4.

3.5.5 Contrast gain \((R_m/b)\)

The patterns of saturation for contrast presentations were recorded for the cell populations. The contrast function was modeled using the Naka-Rushton function

\[
R(C) = \frac{R_mC}{b + C}
\]  

(3.8)

where \(R(C)\) is the response \(R\) at contrast \(C\); \(R_m\) is the maximum response amplitude in impulses per second and \(b\) is the contrast at half the maximum response (Naka and Rushton, 1966). This represents the gradient of the contrast response function or the contrast gain. Contrast gain in impulses
per second per percent contrast is calculated by (Lee et al., 1990; Croner and Kaplan, 1995)

\[
\frac{R_m}{b}
\]  

(3.9)

A low value for \( b \) would indicate contrast saturation of the cell at low contrasts, while a high value for \( b \) would indicate a shallow gain for low contrast presentations (Figure 3.6). A total of ten variable contrast stimuli were presented, ranging from 1.6% to 100%.

![Figure 3.6](image)

Figure 3.6: Contrast transfer function for a cell response that was saturated at low contrast presentations (A; with a contrast gain of 1.2 imp/s/%) and a cell response which remained unsaturated as contrast increased (B; with a contrast gain of 0.54 imp/s/%). The component \( b \), which would be a low contrast value for the cell response that saturated at low contrasts (A), is useful in representing the contrast gain or slope of the contrast functions.

### 3.5.6 Orientation and Direction Selectivity

Orientation tuning was measured at 22.5° steps over 16 orientations between 0 and 180°. The recorded orientation preference was reversed by 180° to account for the mirror used in the experimental setup. Only cells which were within 20° eccentricity of the fovea were included in the analysis to control
for the image shortening which may have resulted from the elevation of a tilted mirror. Measurement of orientation preferences gave an indication of the orientation selectivity of the cell by calculation of an Orientation Index ($OI$)

$$OI = \frac{OriR_{opt} - OriR_{ort}}{OriR_{opt}}$$

(3.10)

where $OriR_{opt}$ is the response at the optimum orientation and $OriR_{ort}$ is the response at the orthogonal orientation to the optimum. The $OI$ would differ according to the orientation selectivity of the cell. A narrow orientation tuning, as in Figure 3.7(A), would result in a high $OI$, while a response to all orientations (Figure 3.7B) would produce a low $OI$.

Figure 3.7: Response from presentation of stimuli at different orientations where an orientation bias was found for one cell (A), while the second cell had no orientation selectivity (B). The OI gave a measure of the orientation preference. Cell A had an OI of 0.5 while cell B had an OI of 0.1.

Direction selectivity was found using the direction index ($DI$) calculated by a similar method to the ($OI$) formula where
\[ DI = \frac{\text{DirR}_{\text{opt}} - \text{DirR}_{\text{ort}}}{\text{DirR}_{\text{opt}}} \] (3.11)

where \( \text{DirR}_{\text{opt}} \) is the response at the optimum direction and \( \text{DirR}_{\text{ort}} \) is the response at the orthogonal direction to the optimum.

The radial bias of dLGN cells were also investigated. The angle between a horizontal line through the fovea and the line joining the fovea to a cell in the visual field was calculated. This was the radial angle. It has been postulated that the radial angle may be similar to the orientation preference angle of the cell, resulting in a radial bias (Passaglia et al., 2002). The difference between the radial angle and the orientation preference angle of the dLGN cells were calculated. A small difference between the two angles indicated the presence a strong radial bias. A large difference indicated a weak radial bias.

### 3.5.7 Orthodromic latencies

Orthodromic latencies were measured for both the R-G and B-Y populations whenever possible. Craniotomies were performed on either side of the optic chiasm using Horsley-Clark stereotaxic co-ordinates of A19 anterior and L2 lateral (Szabo and Cowan, 1984).

Stimulating electrodes were inserted within these craniotomies for measurement of both contralateral and ipsilateral latencies (Figure 3.8). The stimulating electrodes were of a concentric bipolar type (NEX-100, Clark Electromedical Instruments, Reading, UK; shaft diameter 0.5 mm, tip diameter 0.2 mm).

The stimulating electrodes were adjusted so that a maximal response was obtained for diffuse flashing visual stimuli. Optic chiasm stimulation using constant current pulses of 0.5 - 10 µA of duration 100 - 300 µs at 0.25 - 1 Hz were passed through the stimulating electrodes. The latencies were measured as the time taken for these evoked spikes from the optic chiasm to
Figure 3.8: Placement of the contralateral and ipsilateral stimulating electrodes on either side of the optic chiasm to measure response latencies to the dLGN; adapted from http://thalamus.wustl.edu/course/basvis.html

be recorded at the dLGN.

The ipsilateral latencies to the dLGN were expected to be shorter than the contralateral latencies. The magnocellular pathway, by virtue of its faster axonal conduction velocity, has been reported to have latencies less than 1.6 ms, as opposed to latencies more than 2 ms for the parvocellular cells (Dreher et al., 1976; Vidyasagar et al., 2002). The koniocellular cells may possibly have an even longer latency.

3.5.8 Statistical tests

The Wilcoxon rank sum test was used to assess the significance of the difference in results between the R-G and B-Y populations in the dLGN. Statistical analysis was also conducted between the dorsal and ventral cells, as well as the On and Off cells for the whole population and within the R-G and B-Y cell populations individually.
3.6 Area MT recordings

Similar procedures were used for area MT recordings to that of the dLGN recordings. A craniotomy was performed so as to reveal the cortical surface for access to both areas V1 and MT. The opening was made between the Horsley-Clark stereotaxic co-ordinates of P1 to P28 antero-posteriorly and L5 to 25 medio-laterally (Szabo and Cowan, 1984). Attempts were made to collect data from 8 macaque monkeys which were appropriately anaesthetised, paralysed and prepared surgically for recordings from area MT. Since the aim of these experiments was to determine the nature of cone inputs to area MT directly from subcortical areas, V1 was reversibly inactivated using a Peltier cooling device (9 mm by 8 mm) as done for similar studies in V2, V3, V3a and area MT (Girard and Bullier, 1989; Girard et al., 1991, 1992).

3.6.1 Inactivation of the visual cortex

The region of V1 that was inactivated was defined by the placement of the Peltier device posterior to the lunate sulcus along the putative vertical meridian of V1. Hence, during cooling of V1, the visual field up to approximately 6° directly infero-temporal to the fovea was expected to be affected. This was predicted from the visuotopic arrangement of V1 along the posterior border of the lunate sulcus (Van Essen et al., 1984). In one monkey, the region of the visual field was verified by direct recordings from V1 along the borders of the Peltier device placement upon the V1 cortical surface.

Physical constraints of the experimental set-up prevented easy and accurate access to any other V1 region for direct inactivation by cooling. Hence, the criteria for locating cells to record in area MT were those receptive fields between 2° and 6° of visual field eccentricity inferior to the contralateral fovea of the hemisphere in which the V1 region was being cooled. This would result in the topographical matching of the cooled area in V1 to be within the visual...
field regions of cells being recorded in area MT. Any effect from cooling in this central visual field region up to approximately 6° from the fovea could be directly correlated with lack of inputs from V1 due to the inactivation. The size and location of the visual stimulus was matched to the area of V1 being cooled.

The calcarine sulcus directly beneath the V1 surface posterior to the lunate sulcus may have also been affected by the placement of the Peltier device. This region of the calcarine sulcus represents areas of the visual field from approximately 15° up to 25° infero-temporal to the fovea (Van Essen et al., 1984). Hence, area MT cells representing eccentricities much further than the foveal receptive fields may have been successfully cooled. This spread of cooling was verified by measuring the thermal gradient along the depth of the V1 region being cooled perpendicular to the surface. The cooling effect may have also spread to the cortex directly adjacent to the Peltier device and caused inactivation of the surrounding visual fields of eccentricities slightly greater than 6°.

Moreover, the craniotomy performed over V1 for the Peltier device placement also allowed the cooling to spread to the lunate sulcus. Area V2 is located within the lunate sulcus and is known to transmit L-cone and M-cone additive inputs to area MT through the thick stripes (Shipp and Zeki, 1985; Ungerleider and Desimone, 1986b; Bullier and Girard, 1988; Shipp and Zeki, 1989; Levitt et al., 1994a). Tracer injections within the central visual fields in V2 of less than 30° eccentricity have been shown to proceed to the heavily myelinated parts of area MT, while tracer injections outside this eccentricity were medial to the myelinated area MT (Desimone and Ungerleider, 1986). Hence, the cooling may have also affected V1 inputs to area MT by inactivation of the thick stripes of V2 located within the lunate sulcus.

The visual field region of area MT corresponding to the topographically cooled areas of V1 was found with the help of atlases representing area MT.
visual fields within the superior temporal sulcus (Gattass and Gross, 1981; Van Essen et al., 1981; Ungerleider and Desimone, 1986a; Erickson et al., 1989). This was matched with the representation of the V1 visual fields (Van Essen et al., 1984) in the regions of V1 being inactivated. A summary of the topographic organization (Gattass et al., 2005) of area MT relative to V1 and V2 is shown in Figure 3.9. However, due to the inter-animal variations in the folds of the superior temporal sulcus, a number of penetrations had to be made in each animal. The visual field locations of cells within these tracks were used as a guide to find the central visual fields of area MT within 6° of the fovea.

The inactivation of V1 by cooling was confirmed by control recordings in area MT. Recordings from cells in area MT located outside the topographically corresponding region of V1 being cooled showed no reduction in response during cooling. If the effect of Peltier cooling intended for the central receptive fields of V1 was directly affecting area MT, the peripheral visual fields of area MT would have been affected. This would include regions beyond 25° to 30° of foveal eccentricity, since the representation of the calcarine sulcus and V2 within the lunate sulcus reaches these eccentricities (Van Essen et al., 1984). The peripheral area MT cells within the superior temporal sulcus would have been located anatomically closer to the placement of the Peltier device and the cortical region of V1 being cooled (Van Essen et al., 1981). Hence, the effect of V1 inactivation rather than an effect of any direct cooling of area MT was ensured. This was when peripheral visual fields of area MT were not affected (see Figure 6.1) but a reduction in response during cooling was observed within area MT regions topographically corresponding to the area of V1 being cooled.
Figure 3.9: The location and visual topography of area MT relative to V1 and V2. The plus and minus signs represent the superior and inferior visuotopic locations, respectively. Black squares represent the vertical meridian, black circles represent the horizontal meridian, triangles represent the extreme periphery of the visual field and the fovea is represented by a star; adapted from Gattass et al., 2005.
3.6.2 Aperture size

A preliminary study for the best aperture size (with flashing square wave grating stimuli) to use for area MT recordings was conducted by comparing the responses from a standard six degree aperture to apertures of eight and 12 degrees. The eight and 12 degree apertures were also presented with a central mask of four degrees. An eight degree central mask was used for the 12 degree aperture only. The central and surround regions of area MT receptive fields may receive inputs in different combinations from the L-cones, M-cones and S-cones. If a central mask accentuated a specific cone response, for example to S-cone modulating stimuli, it would indicate removal of inhibition from the centrally masked regions or unmasking of the S-cone inputs in the surround region. It was concluded, however, that the stimulus would be tailored for the recording situation since no one aperture size, masked or unmasked, seemed to be optimum for all cells. The aperture sizes were ultimately reduced to cover specifically the region of area MT topographically matching the area of V1 inactivation.

3.6.3 Stimulus characteristics

The five stimulus conditions used for area MT recordings were the modulation of either the L-cones, M-cones or S-cones individually, modulation of all three cones in combination (L+M+S) or modulation of the L-cones and M-cones in phase (L+M). The response of each cell to the five stimulus conditions were recorded in PSTHs as shown in Figure 3.10. However, it must be noted that the cone contrasts for the individual L-cone and M-cone modulating stimuli were only 8%, whereas that for the individual modulation of the S-cones in the S-cone isolating stimuli was 80%. Furthermore, a 100% contrast for all cone types was used for the L+M+S stimuli, whereas only 50% cone contrast was used for modulation of the L-cones and M-cones in the L+M
condition. Such discrepancies, especially the low cone contrasts for the L-cone and M-cone modulating stimuli, were due to the limitations imposed by achieving the appropriate hues given the phosphor characteristics of the monitor. Hence, the responses between these stimuli were compared only qualitatively.

The data were analysed to determine which of the responses from the five stimulus conditions were statistically significant compared to the average spontaneous activity. The responses for each stimulus condition were averaged over 24 bins, each with up to 20 cycles of presentation. The peak response in a PSTH, which was averaged over the 20 ms bin representing one point in the PSTH, was compared to the average spontaneous activity during that presentation. A statistically significant response was identified by using a student’s t-test (p < 0.05).

The peak response for the control condition, once ascertained to be significantly above the average spontaneous activity, was compared to the peak response for the inactivated condition. A student’s t-test was used again with a significance value of p < 0.05. Cells with responses significantly different between the control and inactivated condition were categorized as showing a significant change in response during inactivation.

The extraction of response latencies was obtained from the PSTHs. A criteria for the time from stimulus onset to the first significant response was used to identify the latency of responses (Maunsell and Gibson, 1992). This involved averaging the cell’s spontaneous activity for that presentation and determining the responses which were significantly above the average spontaneous response. The latency of response for the stimulus presentation was found by identifying three consecutive one millisecond bins which gave a response greater than three, three and two standard deviations, respectively, above the average spontaneous activity (Figure 3.11). The latency of the first of these three bins was taken as the latency of the response. The stimuli
Figure 3.10: Responses in PSTHs from the six stimulus conditions (labelled above each PSTH) while electrophysiological recordings were made from area MT; The stimulus condition included a luminance averaged background to record spontaneous activity (A); L-cones, M-cones and S-cones (L+M+S) modulated in phase (B); L-cone modulation (C); M-cone modulation (D); S-cone modulation (E); L-cones and M-cones (L+M) modulated in phase (F). The square wave gratings were flashed at 2 Hz and the optimum orientation was used within a 4° aperture. The stimuli were phase reversed and the responses folded into one cycle where each bin-width was 20 ms.
were phase reversed when flashing so the latencies were found for each half cycle (250 ms for the cell in Figure 3.10) of each stimulus condition for the control and inactivated states.

Due to time constraints, it was not always possible to record the recovery response after V1 inactivation, since in some cases recovery to the original response may take a number of hours to achieve (personal communication, Bogdan Dreher).

Figure 3.11: Determination of response latencies in the 0 phase (starting at 0 ms) and the 180 phase (starting at 250 ms). The criteria for a response latency involved identifying three consecutive millisecond bins with responses greater than 3, 3 and 2 standard deviations respectively above the average spontaneous activity. The first bin to meet this criteria was taken as the response latency.

3.7 Iontophoretic Inactivation using GABA

An alternative method was also employed using GABA iontophoresis for precise focal inactivation of V1 cells whose receptive fields were topographically corresponding with the receptive field of the area MT cell under investigation. Iontophoresis with GABA has been previously shown to effectively inactivate around 300 µm of the area surrounding each iontophoretic micropipette tip.
(outer diameter between 20 and 45 µm and inner diameter between 10 and 25 µm) within the cortex (Hupe et al., 1999). The 500 mM GABA was delivered with a current of up to 200 nA through four micropipettes glued around a recording tungsten electrode penetrating V1 in the current study. The effect of the focally inactivated V1 region was investigated in area MT by first calculating the magnification factor of the inactivated V1 receptive field in minutes/mm as a function of eccentricity (Dow et al., 1981):

$$\log_{10} M^{-1} = a + b \cdot (\log_{10} E - 1.5) + c \cdot (\log_{10} E - 1.5^2) + d \cdot (\log_{10} E - 1.5^3)$$  (3.12)

where \(M\) is the magnification factor in mm/minutes; \(E\) is the foveal eccentricity in minutes of the inactivated V1 receptive field and \(a, b, c,\) and \(d\) are constants of value 0.8124, 0.5324, 0.0648 and 0.0758, respectively. The Field Size (\(FS\)) in minutes of the inactivated V1 receptive field through focal GABA iontophoresis was found by

$$FS = 13.32 + (0.037E)$$  (3.13)

An allowance for the discrepancies in the topographic transition of receptive field locations within V1 was made by calculating the Field Size Scatter (\(FSS\)) in minutes of the inactivated V1 receptive field using

$$FSS = 3.32 + (0.0116E)$$  (3.14)

These formulae were used to determine the aperture size of stimulus to be presented within an area MT cell’s receptive field that overlapped the visual field location of the V1 cells being inactivated. The use of a stimulus aperture not directly overlapping the V1 receptive field or larger in size than the combination of \(FS\) and \(FSS\) calculated for the inactivated V1 receptive field would result in responses being recorded in area MT from a region not topographically corresponding to the focal area of V1 inactivation.
3.8 Histological processing

The depths of the recorded cells were noted from the readings of a hydraulic microelectrode advance (David Kopf Model 640). Selected recording positions were marked with electrolytic lesions (6 - 10 µA for 6 - 10 s, electrode negative) so the site of recordings could be histologically identified.

At the end of the experiment, the macaque was sacrificed with a lethal intramuscular overdose of pentobarbitone sodium (5 ml of Nembutal, 60 mg/ml, Merial Australia Pty Ltd). The animal was then intracardially perfused first with 0.1M phosphate buffer saline-solution (PBS) and next with 4% paraformaldehyde (PFA; BDH Lab Suppliers, England) in 0.1% PBS. The brain was then removed and post-fixed in 4% PFA solution if necessary. It was later immersed in 10% sucrose in 0.1M phosphate buffer (PB) followed by 30% sucrose in 0.1M PB until infiltration with sucrose had caused it to be submerged in these solutions. The brain was blocked stereotaxically. This was done by mapping the curvature of the dorsal surface of the brain with the position and angle of the electrode on to a glass plate during the experiment. The brain was then precisely aligned upon this surface for blocking.

The brain was subsequently prepared for histological sectioning using a Leica freezing microtome (Leica SM 2000R, Leica Microsystems, Nussloch, Germany). Sections were made coronally in 50 µm intervals from the anterior to posterior surface of a block around the dLGN or area MT as appropriate. The sections were collected in 0.1M PB with 0.1% sodium azide (NaAzide, Sigma Chemicals, Germany). Alternate sections were stained with Cresyl Violet for Nissl substance and antibody against Calbindin.

The sections were mounted first using 0.5% gelatin on gelatinized slides and left to air-dry overnight (a minimum of 12 hours). The slides were then stained and dehydrated by dipping into various solutions in the following order: various ethanol concentrations; distilled water; Cresyl violet stain;
ethanol; glacial acetic acid and ethanol again. Ultramount glue was then used to mount the glass coverslips on to the slides.

Staining for antibody against Calbindin involved incubating in the following: PT (5 % BSA, 0.5 % Triton X) solution; primary anti-calbindin antibody made in the reagent IT solution (1 % BSA, 1 % NaAzide); horse anti-mouse biotin antibody, made in the reagent 2T solution; Elite ABC solution on a shaker at room temperature and a solution of 0.02 % DAB and 0.003 % $H_2O_2$ on ice for 5 - 10 minutes. The sections were removed as soon as the cells within the sections appeared darkly stained under a microscope. This was followed by mounting the sections, dehydration and cover-slipping as for the sections stained for Nissl body.

The silver staining for detection of myelinated fibres in area MT first required the sections to be post-fixed in 10% formol for two weeks before being mounted on slides streaked with egg white instead of the gelatinised slides. This preparation may be more effective in maintaining the sections on the slides during the silver staining procedure (personal communication, Jonathan Levitt). The slides were dried overnight and incubated with various concentrations of tungstosilicic acid and silver nitrate in phosphate buffer, before being coverslipped similar to that for the dLGN sections.

A Zeiss Axiocam digital camera was used to make digital micrographs and the stacks of images were analysed using custom software written in Matlab (Image Processing Toolbox, Mathworks, Natick, New Jersey, USA). Tracks were found with the help of electrolytic lesions which were made during physiological recordings. The eye dominance, cell depth from microdrive readings and eccentricity were also taken into account before locating a cell. The LGN sections appropriate to a particular track were aligned from anterior to posterior using an image processing software (Adobe Illustrator Version CS2) before the cells were located within them. The track length was re-sized for the shrinkage (usually 20% to 30%) of the processed sections before being
overlaid on to the dLGN layers within the sections.

Cells were also identified within the koniocellular “bridges” which partially interdigitate or span across the main parvocellular layer between two adjacent koniocellular layers (Hendry and Reid, 2000). The koniocellular bridges were defined by spatial low-pass filtering of the sections using an image manipulation software (Adobe Photoshop or Matlab) and standard Matlab image processing toolbox functions (\texttt{imfilter}, \texttt{imopen}, Mathworks). The filtering enhanced the koniocellular regions by reducing the contrast within them due to the relatively small cell bodies and their relatively low densities (le Gros Clark, 1941; Ahmad and Spear, 1993; Hendry and Yoshiioka, 1994). In this way the koniocellular regions were identified with ease (see Section 5.3.1).
Chapter 4

Physiological properties of S-cone input cells in the dLGN

4.1 Introduction

The functional and morphological differences between the three main streams in primate vision have been well documented (reviewed in Dacey, 1999b; Masland and Martín, 2007; Werblin and Roska, 2007). Two of these streams, the parvocellular and magnocellular divisions, have had their patterns of projections, as well as their response properties to various characteristics of the stimulus, extensively recorded in the Old World (Wiesel and Hubel, 1966; Dreher et al., 1976; Norden and Kaas, 1978; Schiller and Malpeli, 1978; Hicks et al., 1983; Derrington et al., 1984) and New World monkeys (Sherman et al., 1976; Norden and Kaas, 1978; Yeh et al., 1995; Martin et al., 1997; White et al., 1998; Blessing et al., 2004).

The koniocellular stream in Old World monkeys has eluded exhaustive physiological investigation in the past. This is due to the thinness and unclear demarcation of the intercalated layers (Kaas et al., 1978; Hendry and Reid, 2000) situated ventral to the main four parvocellular layers and the two
main magnocellular layers of the dLGN. This physical constraint has made it very difficult for cells in these regions to be targeted and the physiological properties of the population within them to be accurately recorded. Findings in other species have revealed that the functional properties of cells within these layers can show great diversity (Irvin et al., 1986; Xu et al., 2001).

The koniocellular layers in the New World diurnal marmoset Callithrix jacchus are much broader and more distinct than in the Old World monkeys, making it easier for the anatomical reconstruction of recording sites after physiological investigations. Studies of the dLGN in this species have revealed that many of the cells within the koniocellular layers have distinct properties when compared to the parvocellular and magnocellular cells in the surrounding layers (Martin et al., 1997; White et al., 1998; Solomon et al., 1999; White et al., 2001; Szmajda et al., 2006). The koniocellular cells, so called after the layers in which they are located, tended to have larger receptive fields at any eccentricity, slower conduction velocities and longer latencies than magnocellular and parvocellular cells (White et al., 2001). There was a preference for low spatial frequencies in these cells but temporal preferences did not differ overtly from the cells in the main parvocellular and magnocellular layers. Orientation and contrast sensitivities of the koniocellular cells were also comparable to the parvocellular and magnocellular cell types in the marmoset (White et al., 2001).

The chromatic properties of many neurons within the koniocellular layers of the marmoset were found to be preferential for stimuli which modulated the S-cones of the retina (Martin et al., 1997; White et al., 1998; Solomon et al., 1999; Szmajda et al., 2006). These cells may be categorised as B-Y cells since increments of blue and yellow light in opposite phase evoked responses from spatially segregated or overlapping regions of the receptive field. Hence, one of the main functions of the koniocellular pathway in the New World marmoset is thought to be the propagation of S-cone inputs.
through the B-Y cells of the dLGN to V1 (White et al., 1998; Szmajda et al., 2006).

As for Old World primates, the S-cone signals in the macaque retina are known to be carried by the small bistratified ganglion cells (Dacey and Lee, 1994) but their inputs within the dLGN have not yet been documented. Preliminary studies have suggested that they may project to the middle koniocellular regions (Calkins and Hendry, 1996) similar to findings in the marmoset (Szmajda et al., 2008). It has been proposed (Hendry and Reid, 2000) that the S-cone inputs may have a similar segregation within the koniocellular regions of the macaque dLGN as in the marmoset. This insight is yet to be experimentally substantiated.

A pioneering study of the macaque dLGN (Schiller and Malpeli, 1978) localised cells responding to increments and decrements of blue-yellow lights within the middle two of the six main layers of the dLGN. Since a clear description of the koniocellular stream had at that time not yet been defined, these B-Y cells of the middle geniculate layers were not classified as functionally belonging to the koniocellular stream. Similar to other earlier functional studies of the macaque dLGN (Wiesel and Hubel, 1966; Dreher et al., 1976; Hicks et al., 1983; Derrington et al., 1984), these cells were grouped as part of the overlying parvocellular layers P3 & P4. Furthermore, these studies used lights through blue coloured filters instead of S-cone isolating stimuli. A control was also not established for possible luminance artifacts from the blue-yellow stimuli that could cause cells that received only L-cone and M-cone inputs to also respond to apparently S-cone isolating stimuli.

The presence of luminance artifacts is confirmed when responses to high contrasts of S-cone modulating stimuli are comparable to responses in achromatic or L-cone and M-cone opponent stimuli at contrasts less than 10%. This contrast value is estimated from the finding that luminance artifacts from S-cone modulating stimuli are unlikely to cause more than 10% modu-
lation of L-cones and M-cones (Sun et al., 2006b).

The present study involved the investigation of the physiological properties B-Y cells within the dLGN of the macaque monkey. The properties of the B-Y cells were compared to the R-G cells of the parvocellular stream (Perry et al., 1984; Creutzfeldt et al., 1986) that responded to modulation of red-green opponent stimuli. The B-Y cells were encountered far more rarely than the R-G cells. The spatial, temporal, contrast and orientation preferences of the two cell groups were recorded. Presentation of sine-wave gratings were also used to estimate the centre and surround sizes and sensitivities of the receptive fields of recorded cells.

The data were also analysed to ascertain that cells recorded through the same eye with similar eccentricities included two groups of cells. One of these groups would show vigorous responses to achromatic stimuli, but poor responses to S-cone isolating stimuli. The other group would show vigorous responses to S-cone isolating stimuli and lower responses to achromatic stimuli. These cell types would ensure that cells with S-cone inputs were responding to only the S-cone isolating stimuli. This was assumed to be another valid control for luminance artifacts since S-cone inputs to both parvocellular and magnocellular retinal ganglion cells have been reported to be negligible (Sun et al., 2006a). An earlier claim that magnocellular cells received significant inputs from S-cones (Chatterjee and Callaway, 2003) possibly resulted from luminance artifacts in the S-cone isolating stimuli (Sun et al., 2006a,b). Hence, cone isolating stimuli in various combinations were used for the cells recorded in this study to ensure that the responses to S-cone isolating stimuli were not simply due to calibration errors or luminance artifacts.

The response latency of geniculate cell responses to electrical stimulation of the optic chiasm was also used to categorise the cells on the basis of the conduction velocities of their retinal afferents. The orthodromic latencies of the magnocellular pathway are usually less than 1.6 ms to optic chiasm
stimulation, not overlapping with the parvocellular latencies which are usually more than 2 ms (Dreher et al., 1976; Vidyasagar et al., 2002). A recent study of antidromically measured latencies of retinal ganglion cells activated from the optic chiasm of the macaque showed no significant difference between the parvocellular opponent and non-opponent streams (Solomon et al., 2005). The magnocellular stream demonstrated latencies significantly faster than the parvocellular stream (Solomon et al., 2005).

There may be a similar latency difference to orthodromic stimulation of the optic chiasm between parvocellular neurons and the B-Y cells of the dLGN. Measurement of latencies for the B-Y cells may reveal whether they are significantly slower possibly due to the smaller caliber of their fibres.
4.2 Methods

The methods for the electrophysiological recordings within the dLGN have been described in Chapter 3. A brief overview is given here.

The experimental animal was set up in order to view stimuli varying in spatial and temporal frequencies, as well as contrast and orientation increments. The chromatic nature of the stimuli was specified along axes in the isoluminant plane of the 1931 CIE colour space which all passed through a central white point CIE D65 (Figure 3.1). The physiological properties of cells recorded within the LGN were categorized according to their chromatic preferences as being R-G cells when they received antagonistic L-cone and M-cone inputs or B-Y cells when they displayed responses to S-cone isolating stimuli.

A R-G opponent cell with a central receptive field region responsive to increments of light from the dominant wavelength of the red stimulus gun was termed a Red-On cell, while a cell responding to decrements of light in the central region of the receptive field was termed a Red-Off cell. Figure 4.1 shows the response recorded in a peri-stimulus time histogram (PSTH) for such a cell during a presentation of a 0.01 cycle per degree sine wave grating at 4 Hz. A PSTH was created for each of the three presentations of 12 spatial frequencies (ranging from 0.01 to 6 cycles per degree). The responses were combined from all of these presentations for the 1\textsuperscript{st} harmonic component of the FFT modelled with the DOG. The phase of the stimulus (which changed with spatial frequency) in a sine wave form is shown above the recorded response (Figure 4.1).

The gray box beneath the PSTH in Figure 4.1 is divided into ten bins corresponding to 0.25 seconds each (shown by the red divisions). The aggregate responses for each 0.25 second bin throughout the course of the 2.5 second stimulus presentation is shown within the bins. The PSTH displays
the overlap of each of these individual bins to show the average of the total summed response for each 0.25 second period of a particular presentation of spatial frequency. The dots above the PSTH represent the timing of the actual response spikes through each cycle of the presentation.

Similarly, those cells responding to increments of light from the green stimulus gun were termed Green-On cells (Figure 4.2), and those to decrements of light as Green-Off cells. The change of phase for the initial increment is evident in Figure 4.3. All of the above-mentioned cells are collectively grouped into the population of R-G cells.

Figure 4.1: Response of a Red-Off cell recorded in a PSTH. The stimulus presentation in the form of cone modulations is shown above the PSTH. The response can be seen to synchronise with decrements in L-cone modulation (in red). M-cone modulation (in green) produced no response. The response in the PSTH was an average of the aggregate responses within the ten bins of 0.25 ms each (in gray below).

A cell identified as Blue-On responded best to the dominant wavelength of the S-cone modulation from the blue stimulus gun (Figure 4.4). Likewise, a Blue-Off cell responded maximally to decrements of the S-cone modulation or to additive L-cone and M-cone modulation in the opposite phase to the S-cone
Figure 4.2: A cell responding to the On phase of the M-cone modulation (in green), labelled a Green-On cell. Responses were poor for increments during L-cone modulation (in red).

Figure 4.3: The response of a Green-Off cell. The reversal of phase at stimulus onset resulted in a decrement of the M-cone modulation (in green) and an “Off” response from the cell.
Figure 4.4: *Response of a Blue-On cell.* The 0.25 ms time bins (in gray below) showed robust responses to increments during S-cone modulation (in blue). There was no response from L-cone and M-cone modulation in phase (in yellow).

Figure 4.5: *The phase reversed response of a Blue-Off cell responding to decrements during S-cone modulation (in blue). Blue-Off cells may also respond to increments of the additive stimulation of L-cones and M-cones in the opposite phase (in yellow).*
modulating stimuli (Figure 4.5). The two blue cell types were categorized as B-Y cells. Methods to control against the presence of luminance artifacts were established (Figure 4.7).

The physiological properties of the recorded cells were analysed as described in Section 3.5. The optimum spatial frequency was quantified using a low frequency ratio \((LFR)\) and the optimum temporal frequency using a temporal frequency ratio \((TFR)\). The shape of the contrast function curves were described with respect to the maximum response \((R_m)\) and the contrast required to achieve half the maximum response \((b)\). The orientation and direction selectivities were measured using the orientation index \((OI)\) and the direction index \((DI)\), respectively. A calculation of the difference between the orientation preference and the radial angle that the cell made with the fovea gave an indication of the radial bias. The cells’ receptive field properties were measured by modelling the responses with the DOG from spatial frequency transfer functions using the optimum temporal frequency, contrast and orientation of the cell.

The orthodromic latencies were measured in cells where possible, to distinguish any difference in the latencies between the two cell groups. The physiological properties of the R-G and B-Y opponent cell groups were compared graphically and statistically. The findings may be compared to such previous work conducted in the New World marmoset monkey (Martin et al., 1997; White et al., 1998; Solomon et al., 1999; White et al., 2001; Szmajda et al., 2006).
4.3 Results

Of the 129 cells found within the dLGN of 4 macaque monkeys in 15 tracks, 63 were categorised as R-G cells and 25 as B-Y cells. To check for the presence of luminance artifacts, an achromatic cell (of the sample of four found in the parvocellular layers) with inputs from L-cones and M-cones in phase was compared to the B-Y responses. The remaining 36 cells which were not included in the analysis were either unresponsive to stimuli or lost before adequate data could be collected.

Most of the cells were located between $2^\circ$ to $12^\circ$ of the centre of the fovea. However, not all cells in each group contributed towards the analysis of the physiological properties if the responses were not sufficient. Hence, the number of cells in the sample for each physiological property were variable.

Many of the electrode tracks did not extend into the magnocellular layers. This was either because the line of electrode penetration was outside the magnocellular layers or recordings were intentionally suspended when the physiological indications were that the magnocellular layers had been entered.

4.3.1 Spatial frequency

The spatial frequency tuning curves were recorded as responses in impulses per second (imp/sec) against spatial frequency in cycles per degree (cyc/deg). Apart from the optimal spatial frequency, the spatial frequency curve was fitted with the DOG model from which four parameters were distinguished (Solomon et al., 2002). The centre sensitivity ($K_c$) component was the peak amplitude (in impulses per degree) of the sensitivity tuning curve. The centre radius ($r_c$) was the radius of the region from which the excitatory response was summated. The surround sensitivity ($K_s$) was the peak amplitude of the surround inhibitory area. The surround radius ($r_s$) was the radius of the inhibitory surround region (Solomon et al., 2002). See Section 3.5.1 for
details.

Figure 4.6 shows examples of typical spatial frequency transfer functions of four cell types upon presentation with drifting sine wave grating stimuli that were calibrated to modulate selective cone types. The spatial frequency transfer function of the first harmonic response were identified for different cell types upon presentation of stimuli that modulated the L-cones, M-cones and S-cones to varying degrees. The first column shows the responses of a Blue-On cell, the second column a Blue-Off cell, the third column a Red Off cell and the last column a Green On cell. The top row shows the responses of each cell type to an S-cone modulating stimuli, to which the Blue-On and Blue Off cells gave a vigorous response while the Red-Off and Green-On cells maintained only spontaneous activity.

The second row demonstrates the responses to an achromatic luminance grating modulating all three cone types in additive phase. The Blue-On cell produced a mild response, the Green-On and Blue-Off cells gave a better response while the Red-Off cell responded very well.

The last row shows responses to isoluminant M-cone and L-cone modulating (L-M chromatic) stimuli in opponent phase. There were minimal responses from the Blue-On and Blue-Off cells, while the Red-Off and Green-On cells responded vigorously.

Cells responding to both the achromatic and L-M chromatic stimuli were distinguished as being R-G cells if the responses to the L-M chromatic stimuli were as good as or better than the achromatic stimuli. The Red-On and Green-Off cells produced a moderate response from the luminance contrast in the achromatic stimuli and a vigorous response to the L-M chromatic stimuli. This was despite the cone contrast in the isoluminant L-M chromatic stimulus being a mere 16% compared to the 100% contrast modulation of the achromatic stimuli. However, this was expected since the parvocellular R-G cells receive opponent cone inputs from the L-cones and M-cones to the
antagonistic centre and surround regions of their receptive fields.

Figure 4.6: A spatial frequency transfer function showing the S-cone modulating stimuli evoking strong responses from the Blue-On and Blue-Off cells. These cells gave a minimal responses to the achromatic luminance modulation as well as to the L-M chromatic stimuli. The temporal frequency of the drift was 4 Hz while the orientation was optimised for maximum response.

The peak evoked discharge rates were calculated for the R-G and B-Y cells from the optimum stimuli presented. The R-G cells’ peak rate was obtained from the achromatic stimuli since more data were available from this stimuli. The B-Y cells’ peak rate was obtained from the S-cone isolating stimuli. The average for both groups was similar, with the R-G cells firing on average at \(21.42 \pm 9.47\) imp/sec (\(n = 54\)), while the B-Y cells averaged at \(23.13 \pm 12.06\) imp/sec (\(n = 23\)).
4.3.2 Luminance artifacts

The control for luminance artifacts in the S-cone isolating stimuli was established by comparison of responses from B-Y cells to achromatic cells of the parvocellular layers. Although B-Y cells do respond to achromatic stimuli, the luminance contrasts are expected to be very high for the observed responses to approach the responses produced from the S-cone isolating stimuli.

Figure 4.7 shows one such example of a Blue-On cell (column A) and Blue-Off cell (column C), along with an achromatic cell (column B) within the same track. The Blue-On cell was located at a visual eccentricity of 9.2° supero-temporally, the Blue-Off cell at 5° infero-temporally and the achromatic cell at 11° supero-temporally. Both contrast sensitivity functions of the S-cone input cells at their optimum spatial frequency showed an unsaturated contrast response. Only very high contrasts gave a response similar to that from an S-cone isolating stimuli. This minimised the possibility of a luminance artifact where low achromatic contrasts in the S-cone isolating stimuli could produce high response amplitudes. The Blue-Off cell which can respond especially vigorously to L-cone and M-cone stimulation in phase, was seen to respond well to the achromatic stimuli. This indicated that the achromatic stimuli, as well as the L-M chromatic stimuli, modulated only the L-cone and M-cone inputs.

The achromatic cell in Figure 4.7 (B) responded vigorously to the achromatic stimuli as expected, but was unresponsive to the S-cone isolating or L-M chromatic stimuli. This was due to the lack of chromatic opponent inputs to this achromatic cell. The achromatic cell was included for comparison to verify that presentation of stimuli using the same monitor and calibration resulted in a cell which did not respond to the S-cone isolating stimuli but did respond to the achromatic stimuli. This indicated the absence of luminance artifacts from the S-cone isolating stimuli. Furthermore, such
Figure 4.7: Contrast sensitivity functions of B-Y opponent cells compared with a cell classified as an achromatic cell, all recorded through the same eye of the monkey. Their retinal locations were within 4° of each other. The spatial frequency, drift frequency and orientation were optimised for each cell.
achromatic cells obtained in the same monkey driven through the same eye and having receptive fields close to the receptive fields of B-Y cells would be a control against the possibility of variation in macular pigmentation. Macular pigment would result in luminance artifacts where achromatic cells could respond to the S-cone isolating stimuli. Luminance artifacts may be present in cells at some recording sites and not in other cells at another different recording site in the same animal as a result of the variation in macular pigment across the retina.

The achromatic cell in this example was recorded through the same eye of the monkey as the other two B-Y cells. Its receptive field centre was within 3° and 4° from the receptive field centres of the Blue-On and Blue-Off cells, respectively. The minimal responses of this achromatic cell to the S-cone isolating and L-M chromatic stimuli, but a strong response to the achromatic stimuli, validated the assumption of the absence of luminance artifacts at this recording site.

4.3.3 Receptive field dimensions

The radii and sensitivity of the receptive field centre and surround regions were estimated using a DOG function (Rodieck, 1965). This was done by obtaining the response amplitudes for the spatial frequency transfer functions from sine wave gratings of optimal chromatic modulation, temporal frequency and orientation (Croner and Kaplan, 1995; White et al., 2001; Solomon, 2002).

The centre sensitivity of the receptive field was plotted against the centre radius of the cell for both the R-G and the B-Y cell populations. As can be seen from Figure 4.8, there is a linear relationship between the two variables for both populations. The lower centre sensitivity in the B-Y cell group (1.34 ± 6.24 imp/s/deg²; average [ave] ± standard deviation [SD], n = 34) as well as in selected R-G cells (R-G cell ave ± SD, 19.39 ± 30.56 imp/s/deg²; n
was the result of an increasing centre size, which caused dilution of sensitivity by summation over a large centre area. This suggested that the S-cone inputs into the B-Y cells were spaced further apart to result in a larger summation area than the majority of the R-G cells.

Figure 4.8: Double logarithmic plot of centre sensitivity ($K_c$) against centre radius ($r_c$) for the R-G and B-Y cell populations. Regression lines are shown for each group with 95% confidence limits. There was considerable overlap between the two cell populations.

The centre sensitivity was seen to be inversely proportional to the square of the radius with the regression slope close to $-2$ for each group. This indicated that the integrated sensitivity of the centre was the same across all centre sizes and also between the two cell types. This finding was similar for the individual cells and the two groups although the centre size varied.

The regression equations for the R-G cells ($r^2 = 0.94$, $n = 34$, $p < 0.001$) was

$$K_c = 0.08r_c^{-2.04} \quad (4.1)$$
and for the B-Y cells ($r^2 = 0.89, n = 16, p < 0.001$) was

$$K_c = 0.07 r_c^{-2.14}$$

(4.2)

where $K_c$ is centre sensitivity and $r_c$ is centre radius. For all cells together, the regression equation ($r^2 = 0.94, n = 50, p < 0.001$) was

$$K_c = 0.07 r_c^{-2.05}$$

(4.3)

The sample size for B-Y cells was not large enough to confirm that Blue-Off cells are less sensitive to contrasts of S-cone isolating stimuli than Blue-On cells, as has been found in a recent study (Tailby et al., 2008a).

The change of both centre and surround radii with increasing eccentricity is shown in Figure 4.9 and Figure 4.10 respectively. For both the R-G and B-Y populations, there was considerable overlap. The average centre radius of both cell types showed little change up to $10^\circ$ eccentricity. However, B-Y cells demonstrated a larger centre size (ave ± SD, 0.64 ± 0.42 degrees; n = 16) at any given eccentricity compared to the R-G cells (ave ± SD, 0.27 ± 0.34 degrees; n = 34; Wilcoxon rank sum test, $p < 0.01$). This finding has been noted earlier both for the macaque (Wiesel and Hubel, 1966; Schiller and Malpeli, 1978; Solomon et al., 2005) and for the marmoset (White et al., 2001; Tailby et al., 2008b). The average centre sizes of both cell types showed very little change within $10^\circ$ of eccentricity, where most of the sample came from. These findings for R-G opponent cells are consistent with the findings from an earlier study in the macaque (Derrington and Lennie, 1984).

The surround radius also did not vary greatly with eccentricity for both the R-G (3.37 ± 5.49 degrees; ave ± SD, n = 34) and B-Y (4.69 ± 11.41 degrees; ave ± SD, n = 16) cell populations. The surround radius of the two cell types overlapped in their distribution at all eccentricities.

The centre and surround radii are each graphed against eccentricity separately for the R-G and B-Y populations in Figure 4.11 and Figure 4.12,
Figure 4.9: Centre radius ($r_c$) as a function of increasing eccentricity for the R-G and B-Y cells groups. The B-Y cells tended to have a larger centre radius at any given eccentricity.

Figure 4.10: Surround radius ($r_s$) as a function of increasing eccentricity for R-G and B-Y cells groups. The two cell populations overlapped significantly.
respectively. Although the surround radius was obviously larger than the centre radius for the R-G population (by a factor of about 10 to 100) these variables seem to overlap somewhat in the B-Y cell group. This indicated a confirmation of the Type I spatially and chromatically antagonistic, selective centre-surround organisation proposed by Hubel and Wiesel (1968) for R-G cells within the macaque dLGN. It also indicated the presence of the Type II spatially mutual but chromatically antagonistic receptive field organisation in the B-Y cells.

![Graph showing centre and surround radii for R-G cells](image)

Figure 4.11: Centre and surround \( (r_c \& r_s) \) radii for the R-G cells as a function of increasing eccentricity. The surround radii may be larger by up to a factor of 10.

### 4.3.4 Non-linearity index

The non-linearity index was calculated for both the R-G (0.40 ± 0.10; ave ± SD, n = 34) and B-Y (0.38 ± 0.09; ave ± SD, n = 16) cell populations as an indication of the response summation of the cells. All cells were within the mid-range of the non-linearity index. There was considerable overlap between the R-G and B-Y cell populations as shown in Figure 4.13. Hence the linearity between the two cell populations was similar, returning a Wilcoxon
4.3.5 Low Frequency Ratio

A low frequency ratio (LFR) was calculated for all recorded cells as a measure of the cell’s preference for low spatial frequencies. Figure 4.14 shows this ratio for both the R-G and B-Y population of cells against the optimum spatial frequency preference of the cell.

The majority of the B-Y cells were low pass filters as indicated by most of the LFRs being close to unity (0.99 ± 0.03; ave ± SD, n = 23). They preferred the lower spatial frequencies between 0.01 and 0.2 cyc/deg. The R-G cells, however, had LFRs over a wide range (0.70 ± 0.26; ave ± SD, n = 54) than the B-Y cells (Wilcoxon rank sum test, p < 0.01).

The optimum spatial frequency was spread out over spatial frequencies between 0.01 and 6 cyc/deg. Due to the considerable overlap of B-Y cells in their LFRs and optimum spatial frequencies in Figure 4.14, the number of cells with each ratio value is better shown as a histogram (Figure 4.15). A
Figure 4.13: Non-linearity index for the R-G and B-Y populations as a function of eccentricity. The data for the two cell groups overlapped considerably.

LFR of unity was reached by 75% of B-Y cells as opposed to only 26% of R-G cells. All of the B-Y cells had an LFR between 0.8 and 1, while only 46% of the R-G cells had a ratio of 0.8 or above (Wilcoxon rank sum test, \( p < 0.01 \)).

### 4.3.6 Temporal Frequency Ratio

The temporal frequency ratio (TFR) was calculated using a similar process as that used to calculate the LFR. Figure 4.16 shows the range of TFRs in the R-G and B-Y populations with no obvious preferences for the optimum temporal frequency. Cells with high TFR values were likely to be more sustained by responding to low temporal frequencies, while those responding to specific temporal frequencies of higher values would display transient qualities. These latter cells would also show a greater low temporal frequency attenuation due to a low TFR value. The R-G cells responded to temporal frequencies between 1 and 16 Hz (0.36 ± 0.19, ave ± SD, \( n = 47 \)). This could not be discriminated from the B-Y cells (0.34 ± 0.18, ave ± SD, \( n = 19 \)) responding to frequencies between 2 and 12 Hz (Wilcoxon rank sum test,
Figure 4.14: *LFR of cells shown against their optimum spatial frequency preference. Most of the B-Y cells were superimposed since they preferred low spatial frequencies as indicated by their low LFRs.*

Figure 4.15: *LFRs for the R-G and B-Y populations, where all the B-Y cells had an LFR between 0.8 and 1.*
4.3.7 Contrast gain

The slope of a contrast function curve as modelled by the Naka-Rushton fit can be quantified by the maximum response ($R_m$) and also the contrast value at half the maximum response ($b$). Those contrast response curves with high $R_m$ and high $b$ indicated a slower rate of saturation with increasing contrast, while a high $R_m$ and low $b$ suggested saturation at very low contrasts. Low $R_m$ values demonstrated a general depression in the response magnitude but the shape of the contrast saturation curves depended on the high or low $b$ values.

For cells with linear, unsaturated contrast functions, the $R_m$ value was taken to be the response in impulses per second at 100% contrast. The majority of cells in both the R-G and B-Y populations had unsaturated contrast functions as demonstrated by the $b$ values in the middle range (Figure 4.17).

The difference between the R-G (46.08 ± 15.90%; ave ± SD, $n = 42$) and
B-Y (47.08 ± 21.42%; ave ± SD, n = 15) cells was not significant (Wilcoxon rank sum test, p = 0.27). The $R_m$ for the R-G cells was on average 21.23 ± 10.93 imp/sec (n = 42), while that for the B-Y population was 19.49 ± 5.88 imp/sec (n = 15), which did not hold any statistical significance (Wilcoxon rank sum test, p = 0.29).

Figure 4.17: Indication of the contrast saturation function of recorded cells showing that most cells had an increase in response to higher contrasts without saturation of responses.

4.3.8 Orientation preference & Direction selectivity

The orientation preference of cells shown in Figure 4.18 is seen to be spread over a large range. The image shortening which may have resulted from the elevation of a tilted mirror was controlled by including cells in the analysis which were within a foveal eccentricity of 20°.

Figure 4.19 shows the orientation preference of cells quantified by the $OI$. Preferred orientations span the whole spectra for R-G cells, whereas there seems to be some predominance of cells tuned to orientations close to the horizontal in the B-Y group. Cells in the dLGN were originally described
to have circular receptive fields (Hubel and Wiesel, 1962). However, many cells showed a mild degree of orientation bias, consistent with reports of orientation biases in the cat (Levick and Thibos, 1980; Vidyasagar and Urbas, 1982) and the macaque (Passaglia et al., 2002). This behaviour is seen in both R-G (0.12 ± 0.11; ave ± SD, n = 47) and B-Y (0.13 ± 0.10; ave ± SD, n = 18) cell populations having mild orientation biases indicated by the low orientation indices (Wilcoxon rank sum test, p = 0.61).

The radial angle of cells from the fovea to their location in the visual field may bias their orientation preference to be the same as their radial angle, as has been reported in the cat (Levick and Thibos, 1980; Vidyasagar and Urbas, 1982; Soodak et al., 1987; Shou and Leventhal, 1989) and monkey (Passaglia et al., 2002). The difference between these two angles gave a measure of the radial bias. A small difference indicated a radial bias since the radial angle and the orientation preference were similar in value. Figure 4.20 shows a minimal radial bias in the dLGN cells that were recorded for this study.

Figure 4.18: Range of orientation preferences found in the macaque dLGN.

The direction preferences of cells recorded from both the R-G and B-Y populations ranged from 0° to 180° through the 22.5° steps of stimulus presentation. This is shown in Figure 4.21 as DI against the wide range of
Figure 4.19: *Low orientation selectivities for R-G and B-Y cells as indicated by the low orientation indices.*

Figure 4.20: *The absence of a strong radial bias in both the R-G and B-Y cell populations. There is a wide range of differences between the radial angle and the orientation preference.*
4.3.9 Orthodromic latencies

Orthodromic latency measurements to electrical stimulation of the optic chiasm for both R-G cells and B-Y cells were over a wide range of values. The latencies for the two cell groups overlapped considerably (Figure 4.22). Latencies for the contralateral and ipsilateral eyes are shown separately. As expected, ipsilateral latencies tended to be shorter. It was not always possible to evoke orthodromic action potentials in the dLGN cells with the pulsing current range and not all cells could be driven from both sides of the chiasm.

The average latency for the R-G cells was $4.10 \pm 0.75$ ms (ave ± sd, n = 15) compared to the slightly longer average latency for the B-Y cells of $4.80 \pm 1.08$ ms (ave ± sd, n = 10).

The average latency from the contralateral eye for the R-G cells ($4.40 \pm 0.53$ ms, ave ± sd, n = 6) was shorter than that for the B-Y cells ($5.22 \pm 0.88$ ms, ave ± sd, n = 5). However, this did not reach statistical significance (Wilcoxon rank sum test, $p = 0.23$).
The average ipsilaterally evoked spike response latency for the R-G cells was 3.90 ± 0.76 ms (ave ± sd, n = 9) and for the B-Y cells was 4.38 ± 0.97 ms (ave ± sd, n = 5). This difference was also not statistically significant (Wilcoxon rank sum test, p = 0.63).

Due to the overlap between the latencies of the R-G and B-Y cell groups, the differences in latency cannot be taken as a definitive criterion for classification of the two cell types. However, the extremes of the range could possibly be used as a guide, where latencies longer than 5.5 ms may be those of B-Y cells, while latencies shorter than 3 ms may be those of R-G cells.
4.4 Discussion

The physiological properties of B-Y cells as opposed to R-G cells in the dLGN of the Old World macaque monkey were determined in this study. The methods used involved the modulation of cone types with cone isolating stimuli. This is unique compared to the coloured lights used to find B-Y cells in an early study of the macaque dLGN (Schiller and Malpeli, 1978). The properties measured for the two cells groups included the centre and surround sizes, centre and surround sensitivities, non-linearity indices, spatial and temporal frequency preferences, contrast gain functions, orientation biases and direction selectivities. Minimal differences were observed in some of the properties between the R-G and B-Y cell groups.

The main difference was the lack of response to high spatial frequencies in the B-Y cells compared to the R-G cells. The B-Y cells overwhelmingly preferred low spatial frequencies in the population sample, such that all B-Y cells had a Low Frequency Ratio (LFR) between 0.8 and 1. This is a statistically significant difference (Wilcoxon rank sum test, $p < 0.01$) compared to the R-G cell population, where the ratios ranged from 0 to 1 (Figure 4.15). This indicated the presence of the Type II spatially mutual and chromatically antagonistic arrangement (Wiesel and Hubel, 1966) of the centre-surround regions in the receptive fields of B-Y cells within the dLGN of the macaque. The spatially antagonistic centre-surround organisation can, however, be seen in the Type I receptive field arrangement of the R-G cells. This results in the band pass high spatial frequency tuning functions and higher spatial frequency preferences in the R-G cells.

The receptive field centre sizes for the R-G and B-Y populations had some overlap. However, the B-Y cells generally had a larger centre radius than the R-G cells at any given eccentricity. This is also similar to findings in the marmoset for B-Y cells in koniocellular layers compared to the R-G of
the parvocellular layers (White et al., 2001). The small receptive field centre sizes of the R-G cells implicates a functional role in encoding high-acuity spatial information, as reported in the marmoset (Blessing et al., 2004). The larger size of the B-Y cells may indicate their role to not so much subserve spatial vision as to encode chromatic selectivity. The larger centre radius for the majority of B-Y cells may arise from the fact that the S-cones are spaced further apart on the retinal mosaic (Dacey and Lee, 1994).

The sensitivity profiles of the B-Y cells implied the presence of a large central excitatory region. The centre sensitivity decreased with increasing centre size for B-Y cells as it did for the R-G cells (Figure 4.8). However, the identical regressions for the graphs of both chromatic cell types (Figure 4.8) with a slope of -2 on double logarithmic co-ordinates indicated that the integrated sensitivity of the receptive field centre mechanism is independent of the centre size. This is similar to findings in the marmoset (White et al., 2001). Recent studies in the marmoset have also revealed that the integrated sensitivity of Blue-On cells with S-cone isolating stimuli is higher than that of parvocellular cells to modulation with achromatic gratings (Tailby et al., 2008b). Unless there is a genuine species difference, these reports may be confirmed in the macaque monkey with a larger sample size.

It has been reported that B-Y cells within the koniocellular layers of the marmoset have lower peak evoked discharge rates (White et al., 2001; Szmajda et al., 2006). The results from this study did not reveal a significant difference between the peak evoked discharge rate for the optimum stimuli presented between R-G and B-Y cells. The peak rates from the achromatic stimuli for the R-G opponent cells were comparable to the S-cone isolating stimuli for the B-Y cells in the macaque, contrary to findings in the marmoset (White et al., 2001; Szmajda et al., 2006).

The B-Y cells of the macaque had the same low response to high spatial frequencies as was reported in koniocellular dLGN neurons of the New World.
marmoset (White et al., 2001). The linearity of spatial summation was comparable between the B-Y and R-G cell populations of the macaque as was found in the above marmoset study (White et al., 2001). The preferences for low spatial frequencies and transfer of S-cone inputs through the dLGN for the B-Y cells of the macaque dLGN seem to be similar attributes to those of the B-Y cells found within the koniocellular layers of the marmoset (Martin et al., 1997; White et al., 1998; Solomon et al., 1999; White et al., 2001; Sz-majda et al., 2006). This may indicate that the B-Y cells of the macaque may be contained within the dLGN koniocellular regions as has been reported in the marmoset (Martin et al., 1997; Szmajda et al., 2006).

The orthodromic latencies in this study did not provide a significantly unique signature for the B-Y cells compared to the R-G cells. Even with a small sample size, there was considerable overlap between the two cell groups. However, the boundaries of the range may be used as a guide for classification of cells. Latencies greater than 5.5 ms may suggest a B-Y cell while those shorter than 3 ms may suggest a R-G cell. This is consistent with studies where latencies of R-G opponent parvocellular neurons were on average 2.4 ms in the macaque (Dreher et al., 1976).

The longer latencies in the B-Y cells may possibly arise from smaller cell bodies and thinner axons. Koniocellular layers have been suggested to contain smaller cell bodies and thinner axons than cells of the faster magnocellular pathway and the intermediate sized parvocellular R-G neurons (Dreher et al., 1976; Hendry and Reid, 2000). Hence, this may further indicate that the B-Y cells in the macaque dLGN may functionally be part of the koniocellular pathway.

Although there was significant overlap in the conduction velocities between the R-G and B-Y populations, it is consistent with overlapping findings of antidromically evoked latencies between R-G and B-Y ganglion cells from intra-ocular recordings (Solomon et al., 2005). However, studies in the
nocturnal prosimian primate *Galago* of the conduction velocities of afferents to the intercalated koniocellular layers of the dLGN revealed distinctively slow latencies (Irvin et al., 1986). This may be a result of “non-blue” axons of the koniocellular afferents since the *Galago* is known to lack a functional S-cone input pathway (Deegan and Jacobs, 1996).

The measurement of conduction velocities may be more useful for defining cell classes if the response latencies were measured over distances longer than merely from the optic chiasma to the dLGN. A location closer to the optic disc may be more suitable. The method of using more widely spaced landmarks between electrical stimulation and recording of response latency spikes has been more beneficial for the functional identification of “very slowly” conducting as opposed to “slowly” conducting retinogeniculate afferents in the cat Cleland et al. (1976).
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Chapter 5

Laminar distribution of S-cone input cells in the dLGN

5.1 Introduction

It is widely accepted that in both Old and New World monkeys the dLGN is segregated into several different layers (Malpeli and Baker, 1975; Leventhal et al., 1981) which encode different visual sub-modalities in parallel. The two main streams, named parvocellular and magnocellular, have been studied extensively in both the Old World and New World monkeys (Wiesel and Hubel, 1966; Dreher et al., 1976; Sherman et al., 1976; Norden and Kaas, 1978; Schiller and Malpeli, 1978; Hicks et al., 1983; Derrington et al., 1984; Martin et al., 1997; Yeh et al., 1995; White et al., 1998, 2001; Blessing et al., 2004).

Parvocellular neurons in the primate dLGN receive opponent inputs from the L-cones and M-cones of the retina through the midget ganglion cells (Perry et al., 1984), while the magnocellular neurons receive additive inputs from the L-cones and M-cones through parasol ganglion cells (reviewed in Dacey, 1999b; Masland and Martin, 2007; Werblin and Roska, 2007). The
claim that S-cone inputs are partially transmitted through the magnocellular stream in the macaque (Chatterjee and Callaway, 2003) has been disputed by the possible presence of luminance artifacts in the stimuli used for that study (Sun et al., 2006a).

The koniocellular pathway has eluded a strong functional correlation between anatomy and physiology since its first description (Kaas et al., 1978). This is particularly true in the Old World monkeys, due to the thinness and poor demarcation of these layers from the main parvocellular and magnocellular layers within the dLGN (Kaas et al., 1978; Hendry and Reid, 2000).

The koniocellar layers, particularly the centrally located broad layer K3, are wider and better defined in the diurnal New World marmoset monkey (Callithrix jacchus). Investigations which have anatomically reconstructed the sites of physiological recordings have implicated the koniocellular pathway in the transfer of blue-yellow chromatic signals (Martin et al., 1997; White et al., 2001; Szmajda et al., 2006). This may be the result of the S-cone signals received through the small bistratified ganglion cells of the retina which project to layer K3 in the marmoset (Szmajda et al., 2008).

In macaques, the S-cone On inputs are known to be transmitted to the dLGN through the small bistratified ganglion cells (Dacey and Lee, 1994). The S-cone Off signals were also proposed to be transmitted by a diffuse Off midget bipolar and ganglion cell pathway (Klug et al., 1992, 1993, 2003). However, this has been disputed with the finding that the retinal midget cell pathways do not contribute to the transmission of S-cone signals (Lee et al., 2005; Lee and Grunert, 2007).

The intrinsically photosensitive giant sparse ganglion cells (Klug et al., 2003) of the retina may also transmit S-cone Off signals through irradiance encoded by opponent rod inputs to luminance signals. The propagation of S-cone signals in the macaque has not been investigated to the level of the dLGN. Preliminary studies have indicated the small bistratified ganglion cells
carrying S-cone inputs may project to the middle koniocellular layers of the dLGN (Calkins and Hendry, 1996).

A pioneering study of the dLGN in the macaque (Schiller and Malpeli, 1978) reported that the middle layers of the dLGN contained a greater proportion of Off cells and a higher number of cells responding to increments and decrements of blue light (B-Y cells) than the dorsal layers. However, along with other functional studies of the macaque dLGN at the time (Wiesel and Hubel, 1966; Dreher et al., 1976; Hicks et al., 1983; Derrington et al., 1984) these B-Y cells were attributed to the middle parvocellular layers instead of the koniocellular layers which were yet to be distinguished. It would be interesting to locate physiologically identified B-Y cells responding to S-cone isolating stimuli within the dLGN to ascertain whether S-cone inputs are transmitted through the koniocellular layers in the macaque, similar to that reported in the marmoset (Martin et al., 1997; White et al., 2001; Szmajda et al., 2006).

The afferents from the koniocellular layers of the macaque dLGN to V1 have been well documented (Casagrande et al., 2007). The ventral koniocellular layers K1 and K2 were found to project to layers 1 and 3A of V1 in a simple synaptic fashion with few boutons. The dorsal koniocellular layers K3 to K6 terminated within the CO blobs of layer 3Bo of V1. These neurons had dense arbors with a complex projection pattern (Casagrande et al., 2007). This anatomical tracing was done through injections into specific koniocellular layers of the dLGN after physiological identification and were similar to koniocellular projection patterns to V1 in the owl and squirrel monkeys (Fitzpatrick et al., 1983; Diamond et al., 1985; Ding and Casagrande, 1997). However, the findings were not consistent with a study where V1 cortical injections traced projections back to the koniocellular layers in the macaque dLGN (Hendry and Yoshioka, 1994). Superficial cortical injections in V1 were traced to the dorsal koniocellular layers K4 to K6 in the study by Hendry &
Yoshioka (1994). Larger cortical injections in V1 revealed projections from all koniocellular layers in their findings.

The difference between the two studies was identified to be that the large cortical injections in V1 traced projections to layer K3 while the superficial injections traced projection to only the dorsal koniocellular layers (Hendry and Yoshioka, 1994). This led to the conclusion that the deeper cortical layers received projections from the ventral koniocellular layers. The projections from K1 to K3 may have been missed with the superficial injections in V1 due to the fineness of koniocellular collaterals in V1 (Casagrande et al., 2007). Hence, determining the cell types within the koniocellular layers of the macaque dLGN could relate the physiological properties of projections from ventral and dorsal koniocellular layers to those of their superficial or deep termination layers in V1.

Recordings from V1 cortical cells in supragranular, infragranular and the geniculo-recipient layers had revealed a population of cells which responded to both low contrast achromatic gratings and isoluminant chromatic stimuli at slow velocities (Vidyasagar et al., 2002). These findings indicated a convergence of the attributes of the magnocellular and parvocellular streams, respectively. These unique physiological responses in V1 may have also been from neurons of the koniocellular pathway alone, or in convergence with inputs of the magnocellular pathway. However, identification of neurons of the koniocellular pathway in V1 is not possible due to the complexity of the cortical networks and the intracortical connections. Hence, the best way to determine the physiological properties of koniocellular neurons is to identify the cell types within the koniocellular layers of the dLGN.

The projections of cells within the koniocellular layers of the dLGN have been reported to send direct inputs to area MT (Stepniewska et al., 1999; Sincich et al., 2004). This region of the dorso-parietal stream is not usually thought of as a primary visual area but involved more with higher order
visual processing (Felleman and Van Essen, 1991). Although various ontogenetic and architectural criteria indicate area MT to be more similar to a primary visual area than other ‘association’ areas of the cortex (Campbell, 1908; Bourne and Rosa, 2006) its usual description as a secondary visual area results from a later evolutionary specialization due to substantial inputs from V1. Retrograde tracers injected into area MT in the macaque revealed subcortical geniculate inputs bypassing V1 especially from mainly the koniocellular layers of the dLGN (Sincich et al., 2004). Hence, locating physiologically identified cells within the koniocellular layers would determine the nature of the inputs projecting to area MT and indicate this to be a primary visual area for certain stimuli. The direct extra-striate projections from these subcortical regions may play a role in blindsight (Sanders et al., 1974; Weiskrantz et al., 1974).

In this study, physiologically categorised B-Y cells were located anatomically within the macaque dLGN. This was done by histological reconstruction of electrode tracks after single-unit extracellular recordings from the dLGN. Explicit delineation of the koniocellular regions helped to establish whether the koniocellular pathway preferentially contained these B-Y cells with S-cone inputs. The locations of the B-Y cells were compared to the laminar placement of R-G opponent cells.

Of particular interest were the cell types within the koniocellular bridges extending into the parvocellular layers (Hendry and Yoshioka, 1994; Hendry and Reid, 2000). These bridges were included as part of the koniocellular regions for the analysis. Electrolytic lesions made during the recording process were used as a guide to locate cells within the dLGN sections. The histological reconstruction was also aided by information about cell depth, cell eccentricity and ocular dominance of the cells. Preliminary results from this study have been previously reported (Roy et al., 2007).
5.2 Methods

The procedures for the experiments conducted in this study are reported in Chapter 3. A brief description is given here. The macaque monkeys (*Macaca fascicularis*; n = 5) were prepared for electrophysiological recordings from the dLGN by appropriate anaesthesia and surgical intervention. The neurons within the dLGN were physiologically characterised according to the individual or combination of L-cone, M-cone and S-cone inputs, in or out of phase, which they received (see Section 4.2). The location of these recorded neurons within the dLGN layers were found through histological reconstruction of coronal sections stained for Nissl substance.

The animals were sacrificed with an overdose of sodium pentobarbitone (5 ml of Nembutal, 60 mg/ml, Merial Australia Pty Ltd) and intracardially perfused with 0.1M PBS and 4% PFA (BDH Lab Suppliers, England) in 0.1% PBS. The brain was submerged in 30% sucrose in 0.1M PB to prepare for histological sectioning. The brain was precisely blocked stereotaxically by aligning the curvature of the dorsal surface of the brain with the position and angle of the recording electrode as mapped onto a glass plate during the experiment. Coronal sections were made at a width of 50 µm from anterior to posterior using a Leica freezing microtome (Leica SM 2000R, Leica Microsystems, Nussloch, Germany). These sections were mounted onto slides before staining with Nissl substance and dehydration with various concentrations of ethanol. Alternate sections were stained with antibody against Calbindin (Figure 5.1) before being mounted on the slides and dehydrating with the various concentrations of alcohol. Slides were left to air-dry overnight before coverslips were mounted.

Reconstruction of the LGN sections was done using histological processing. Stacks of images were analysed from digital micrographs created using a Zeiss Axiocam digital camera. The dLGN sections from the experimental
animals corresponding to each track were aligned from posterior to anterior using the Adobe Illustrator image processing software package (Figure 5.2). The electrode tracks containing cells correlated with the physiological data (obtained using a purpose-written computer program in the Matlab language) were overlaid upon the corresponding sections according to the Horsley-Clark co-ordinates recorded for the track.

The shrinkage of the sections from histological processing ranged between 20% to 30% and was taken into account for each individual animal during track reconstructions. The position of lesions (6 - 10 μA for 6 - 10 s, electrode negative) made during electrophysiological recordings indicated the relative distance between cells in the same track after adjustment for the degree of shrinkage within the sections. Other factors such as the eye dominance, microdrive readings of cell depth during recording and cell eccentricity also
Figure 5.2: Consecutive dLGN sections were overlaid progressing posteriorly corresponding to a particular track for histological reconstruction. The most posterior section in this track was matched to Horsley-Clark stereotaxic coordinates of A7 anterior and L11 lateral. Arrow-heads indicate the start of recording tracks in each section and “L” is located next to a lesion.

Lesions typically created a circular atrophy region of coagulation and atrophy. The cell being recorded at the tip of the electrode may have been anywhere from the top to the bottom of the circular region caused by the lesion. Moreover, leaving a recording electrode in the brain for extended periods of time may have caused the electrode to sink or move relative to the brain. Hence, the exact depth of both the cells and the lesions may have had a small error.
There were 15 electrode tracks reconstructed in total. A normalised ratio was calculated to give an estimate of how far within the parvocellular layer a cell was located from the border of the koniocellular layer directly underneath. The ratio was calculated by the relative distance of the cell from the border of the koniocellular layer to the width of the overlying parvocellular layer. A ratio of unity indicated that the cell was on the dorsal edge of the main parvocellular layer, while a ratio of zero indicated a cell was located within the koniocellular layer itself.

Identification of the koniocellular bridges that have been reported to extend into and span across the main parvocellular layers between two adjacent koniocellular layers (Hendry and Yoshioka, 1994; Hendry and Reid, 2000) was also attempted. The dLGN sections were exposed to a low-pass spatial filter using a bitmap image manipulation software package (Adobe Photoshop) that effectively defocused the image. This procedure resulted in causing the intercalated layers and koniocellular bridges to appear as paler low-intensity regions due to the relatively small cell bodies and low cell densities within these regions (le Gros Clark, 1941; Ahmad and Spear, 1993; Hendry and Yoshioka, 1994). The koniocellular bridges were delineated using a standard Matlab image processing toolbox functions (imfilter, imopen, Mathworks, Natick, New Jersey, USA).

The recorded cells were localised within these koniocellular bridges using a double blind method, where the low pass image was overlaid with the electrode track containing the recorded cells. One of the investigators was asked to indicate whether the recorded cells were in the koniocellular layers and koniocellular bridges or outside these regions. This was done without the investigator being aware of the physiological categorisation of the cells as the R-G or B-Y types.
5.3 Results

The use of electrolytic lesions within the sections, along with the transition of eye dominance of the cell matched with the retinotopy and eye dominance of the dLGN layers, was essential to the histological reconstruction of the recorded cells. The cells were all between 2° and 12° of the fovea. Of the 92 cells recorded in the dLGN (from 15 tracks of four macaques) 63 were categorised as R-G cells because they responded to antagonistic L-cone and M-cone inputs and 25 were categorised as B-Y cells from responses to S-cone isolating stimuli.

Four achromatic cells, responding to L-cone and M-cone modulation in phase, were also found within the parvocellular layers. They were not included in the analysis but their responses were used in the control to guard against luminance artifacts (see Section 4.3.2). Recordings were not made from the magnocellular layers since the tracks were usually aligned too laterally to reach these layers. Since R-G and B-Y cells were targeted for this study, recordings were also intentionally stopped when physiological evidence indicated these layers had been reached. Four cells in K2 were excluded from the analysis so that the sample distribution remained dorsal to the magnocellular layers. Statistical analysis was conducted on 84 cells located within layers K3 to P6 of the dLGN.

5.3.1 Koniocellular bridges

The koniocellular regions that seemed likely to be koniocellular bridges within the parvocellular layers were demarcated objectively using an imaging procedure as shown in Figure 5.3. The koniocellular extensions and bridges were most obvious in the ventral parvocellular layers P3 & P4. The sequence of digital image processing (shown in Figure 5.3) to graphically define the koniocellular bridges (A) extending into the main layers within a Nissl stained
LGN section involved Gaussian filtering of the original Nissl-stained section to remove the higher frequencies (B). This was followed by eliminating the background (C) so that the koniocellular bridges (yellow arrows) became delineated. The treated image was superimposed with pseudocolouring onto the original section to clearly reveal the koniocellular bridges (D). The filtering reduced the contrast from the small bodied koniocellular neurons in the intercalated layers, as well as in the koniocellular bridges within the main parvocellular layers. The contrast from the larger cell bodies of the main magnocellular and parvocellular layers was left relatively intact. The koniocellular bridges were found predominantly throughout layers P3 and P4, where the intercalated koniocellular layers were poorly defined. The two ventral magnocellular layers and the dorsal-most parvocellular layers P5 and P6 were more compact and did not include as many koniocellular bridges. This low-pass filtering process may be applied to all dLGN sections for an objective means of identification of the koniocellular bridges.

Figure 5.4 demonstrates the localisation of a B-Y cell (black arrow) within such a koniocellular bridge revealed with the low pass spatial filtering (B) of the original section (A). This is supportive of the koniocellular regions containing B-Y cells which are perhaps not restricted to the classical koniocellular layers.

5.3.2 Laminar distribution of cells

Figure 5.5 shows examples of the reconstructed electrode tracks in the dLGN (one from each of the four macaques). The layers had been outlined using cues from the difference in staining density of the Nissl substance between cells in the main parvocellular and magnocellular layers compared to the lighter shading within the intercalated koniocellular layers. The koniocellular bridges are shown as areas of white within the main gray layers. The B-Y cells were located mostly within the koniocellular layers and bridges.
Figure 5.3: A section of the dLGN (A) imaged through a low-pass Gaussian filter (B). The background is eliminated (C) to reveal the koniocellular bridges (yellow arrows). This image is superimposed onto the original image to visualise the koniocellular bridges (D).
Figure 5.4: A reconstructed track within a section of the dLGN (A). When this section is seen through a low pass spatial filter (B), koniocellular bridges can be seen spanning across the middle parvocellular layers P3 and P4. The arrow-head indicates a location within a koniocellular bridge in which a B-Y cell was found.

Figure 5.6 shows the distribution of the R-G and B-Y across the different dLGN layers (pooled from all 15 tracks of all four monkeys). The cells were positioned according to their histologically defined laminar locations. Unfilled circles represented On cells; filled circles represented Off cells. The B-Y cells have been represented on a column separate to the R-G cells for clarity. The normalised ratio was used for the placement of the cells in Figure 5.6. Koniocellular layers and bridges were both included as part of the koniocellular region.

Analysis was conducted on a total of 61 R-G cells and 23 B-Y cells (excluding the four cells in layer K2). Out of these 84 cells, 42 of the R-G opponent cells and 19 of the B-Y cells were of the On centre type. Overall there was no indication of segregation of On and Off centre cells ($\chi^2 = 0.51$, $p > 0.48$) to the outer (K5, P5, K6, P6) or middle layers (K3, P3, K4, P4) respectively. When the R-G opponent cells alone were analysed, a mild tendency for such a segregation was noticed. It was found that 63% (12 out of
Figure 5.5: (A - D) Reconstructed tracks, one from each of the four monkeys, within Nissl-stained sections of the dLGN. The R-G cells were located in the parvocellular layers, while B-Y cells were located in both parvocellular layers and koniocellular regions. Only those koniocellular bridges within which cells were localised have been shown to extend into the parvocellular layers (P3 in A, B and D and P4 in C). Yellow bars represent lesions made within the track during electrophysiological recordings.

19) of the R-G Off cells and 43% (18 out of 42) of the R-G On cells were in the middle laminae, as has been previously reported (Schiller and Malpeli, 1978). However, this was not statistically significant ($\chi^2 = 2.16, p = 0.14$).

The R-G cells were almost equally distributed between the middle (30 out of 61) and outer layers (31 out of 61). However, most of the B-Y cells (20 out of 23) were found within the middle layers ($\chi^2 = 9.89, p = 0.02$). These
Figure 5.6: Pooled data for the distribution of cells (n = 88) throughout the layers of the LGN from all four monkeys. The R-G cell population has been displayed separate to the B-Y cells for clarity. Light gray columns located within and below the main gray layers represent koniocellular bridges and layers, respectively. The cells were placed along the schematised depth of the dLGN using a normalised ratio. All cells except 6 (3 R-G and 3 B-Y) were localised in the expected layer of eye dominance.
findings are consistent with a higher proportion of B-Y cells in the middle layers of the dLGN, as reported in an early study by Schiller & Malpeli (1978). Furthermore, 74% of the S-cone input cells (17 out of 23) were in the koniocellular regions and more than half of these cells (10 out of 17) were in the koniocellular bridges. In contrast, only 5% (3 out of 61) of the R-G opponent cells were located in all the koniocellular regions of the dLGN ($\chi^2 = 43.80, p < 0.001$). Considering the errors in estimation that could be potentially introduced due to the thinness of the intercalated layers, this is a remarkably high degree of segregation.
5.4 Discussion

This study involved the anatomical localisation of physiologically identified B-Y cells receiving S-cone inputs within the macaque dLGN. The results were compared to the location of R-G cells recorded at the same time. The majority of the B-Y cells were located in the middle layers (K3-P4) of the dLGN, especially within the koniocellular layers and bridges (74%). Only 5% of the R-G cells were located in the same regions.

The distribution pattern of B-Y cells within the koniocellular layers of the macaque dLGN was found to be similar to that described in the New World marmoset. It was reported that 77% (10 out of 13) of Blue-On cells (Martin et al., 1997) and 71% (5 out of 7) of Blue Off cells along with 69% (9 out of 13) of Blue On cells in another study (Szmajda et al., 2008) were located within the koniocellular layers of the New World marmoset.

The functional segregation between the parvocellular and koniocellular regions may have been previously underestimated due to the thinness and poor demarcation of the koniocellular layers within the macaque dLGN. Despite this noise of poor demarcation from the anatomical organisation, it was possible to ascertain from this study that there was a very significant tendency for B-Y cells to be located within the koniocellular regions of the macaque dLGN.

The determination of the segregation of B-Y cells was made possible by the inclusion of cells localized not only within the koniocellular layers, but also within the koniocellular bridges of the adjacent parvocellular layers. More than half the B-Y cells identified to be in the koniocellular regions were located within the koniocellular bridges (10 out of 17).

The inclusion of the koniocellular bridges may have enhanced the number of B-Y cells within the “koniocellular pathway”. However, if the distribution of the R-G and B-Y cells were evenly spread across the geniculate laminae,
it should not have affected the relative proportions of R-G and B-Y cells in the koniocellular regions.

This study in the macaque monkey strengthens the claim that S-cone signals are carried by a specific pathway to higher areas from the retina (Dacey and Lee, 1994; Hendry and Reid, 2000) as is the case in the New World marmoset (Martin et al., 1997; Szmacjda et al., 2008). The finding that the small bistratified cells carrying S-cone inputs from the marmoset retina project to koniocellular layer K3 (Szmacjda et al., 2008) may indicate the same pattern of input for the small bistratified cell in the macaque. Preliminary studies had suggested that the projections of small bistratified cells may terminate in the middle koniocellular layers of the macaque dLGN also (Calkins and Hendry, 1996). This would further support the existence of a similar functional role in specifically encoding blue-yellow chromatic signals between the Old World and New World monkeys.

The lack of R-G cells within the koniocellular regions of the dLGN agrees with claims that the cells of the parvocellular midget pathway may be biased against receiving S-cone inputs (Lee et al., 2005; Lee and Grunert, 2007). While the koniocellular pathway may specialise in the transmission of blue-yellow colour opponency, the R-G cells may be responsible for spatial detail as has been reported in the marmoset (Blessing et al., 2004). The minimal number of R-G cells within the koniocellular regions was supportive of these cells being involved mainly with the processing and transfer of high-acuity spatial frequency information where colour opponency is an associated supplement (Blessing et al., 2004).

The data in this study excluded the koniocellular layers ventral to the two magnocellular layers (K1 and K2) and any of the ventral koniocellular bridges which may have extended into neighbouring magnocellular regions. However, the only two B-Y cells that happened to be recorded ventral to K3 were both found in koniocellular layer K2. It remains to be determined whether the
S-cone inputs are segregated within the koniocellular regions of the ventral layers of the macaque dLGN as is the case in the dorsal layers. Projections from the ventral koniocellular layers to V1 layers 1 to 3A (Casagrande et al., 2007) including the CO blobs of layer 2/3 in V1 may implicate a physiology for chromatic encoding within the ventral koniocellular layers.

The B-Y cells found in the middle koniocellular layers K3 and K4 in the present study are likely to project to the CO blobs of layer 3Bo in V1 (Casagrande et al., 2007) where they would be involved with chromatic processing in the cortex (Hendry and Yoshioka, 1994; Hendry and Casagrande, 1996; Hendry et al., 1997; Yoshioka and Hendry, 1999). The B-Y signals from these layer 2/3 projections may be further intermixed with magnocellular and parvocellular afferents within layer 4B in V1 (DeYoe and Van Essen, 1985; Shipp and Zeki, 1985). These signals may be transmitted to both the dorsal stream through the V2 thick stripes to area MT and also to the ventral stream through the pale and thin stripes to V4, respectively (Shipp and Zeki, 1985; Zeki and Shipp, 1989; Nassi and Callaway, 2007).

The finding of koniocellular bridges extending into the main parvocellular layers raises the question as to whether cells within these regions are morphologically the same as those in the intercalated koniocellular layers proper. The results from this study suggested that the bridges were extensions of the intercalated layers. Based on the physiological nature of the cone inputs they received, these bridges may be encompassed as part of the koniocellular submodality in that they largely included B-Y cells which selectively carried S-cone inputs in the dLGN. The method of spatial low-pass filtering of sections stained with Nissl substance enhanced the visibility of both the koniocellular layers and the assumed koniocellular bridges extending into the parvocellular layers. Studies using neurochemical tracers for identification of dLGN cells within the koniocellular bridges, as has been done for the koniocellular layers (Hendry and Yoshioka, 1994; Hendry and Calkins, 1999) would
help to correlate cells revealed by spatial filtering within these koniocellular regions as being morphologically part of the koniocellular pathway.

Physiological evidence has been previously presented for S-cone inputs possibly converging with signals from the magnocellular pathway onto single cells at the level of V1 (Vidyasagar et al., 2002). However, the findings from the present study suggests that the S-cone inputs at the subcortical level are still segregated to the koniocellular pathway in the macaque, separate from the magnocellular and parvocellular channels within the dLGN. This segregation may also provide a site for selective modulation of the blue-yellow pathway by both cortical and subcortical extra-retinal inputs (Casagrande et al., 2005; Schütz et al., 2008).

Another important functional implication of the segregation of B-Y cells within the koniocellular regions is the transmission of S-cone signals to the dorso-parietal stream through direct koniocellular projections to area MT that has been anatomically revealed (Sincich et al., 2004). Findings from a human psychophysical study have suggested very fast responses to S-cone isolating stimuli in area MT (Morand et al., 2000). Recent work has also demonstrated an involvement of the B-Y opponent system in directing spatial attention (possibly mediated by the dorsal stream) to a greater extent than the R-G opponent pathway (Li et al., 2007). Such a segregation of the R-G and B-Y pathways may also have implications for studies that could potentially address various psychophysical phenomena and clinical conditions, such as blindsight (Sanders et al., 1974; Weiskrantz et al., 1974).

The location of the koniocellular bridges may also have implications for understanding the phylogenetic development of the geniculate laminae in the macaque. In the New World monkeys the dLGN is divided into three main sections of a dorsal parvocellular, a middle koniocellular and a ventral magnocellular section (Spatz, 1978). The parvocellular and magnocellular sections are subdivided into a single contralateral and ipsilateral layer. In
Old World monkeys, however, the parvocellular section has four major layers (Kaas et al., 1978; Malpeli and Baker, 1975).

The primordial primate pattern for geniculate lamination prior to the separation of the evolutionary branches of the Old World and New World monkeys may be that observed in the structure of the dLGN in the New World monkey. It has been proposed that the folding over of the same continuous contralateral and ipsilateral parvocellular layers leads to the impression of the two extra parvocellular layers seen in cross-sections of the dLGN in Old World monkeys (Kaas et al., 1978). However, there is a possibility that a later development in the Old World monkeys may have resulted in the duplication of the parvocellular laminae, which could be linked to the partial segregation of the Off and On cells into the ventral and dorsal pairs of parvocellular laminae, respectively (Schiller and Malpeli, 1978). If this was the case, it is possible that the duplication of the parvocellular laminae might have happened in a ventral direction, extending into the broad primordial koniocellular section. The fact that the koniocellular bridges were found predominantly within the ventral parvocellular laminae in this study, would support this theory. This may also explain the functional finding that most cells located within the koniocellular regions were Blue-On rather than Blue-Off cells. In the sample reported for this thesis, the Blue-On cells usually produced an Off response to achromatic stimuli. Thus, a propensity for a natural segregation to contain Off responses within the ventral laminae may have driven the phylogenetic migration of the parvocellular Off laminae ventrally. The resulting koniocellular bridges extending into the main parvocellular layers might be the remnants of such an evolutionary migration.
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Chapter 6

Responses in area MT with cortical inactivation

6.1 Introduction

The middle temporal area (MT) in the primate is located within the posterior bank of the superior temporal sulcus (Gattass and Gross, 1981; Van Essen et al., 1981). It has been shown to be extensively involved with higher order motion processing (Dubner and Zeki, 1971; Gattass and Gross, 1981; Van Essen et al., 1981; Desimone and Ungerleider, 1986). Many of the neurons found in this region are tuned for the speed, direction and orientation of the stimuli (Maunsell and Van Essen, 1983a; Albright, 1984; Albright et al., 1984; Mikami et al., 1986a; Rodman and Albright, 1987, 1989; Dobkins and Albright, 1994).

The inputs to area MT are predominantly magnocellular in nature (Maunsell and Van Essen, 1983c; Shipp and Zeki, 1989; Maunsell et al., 1990; DeYoe et al., 1994). The signals from the ventral two magnocellular layers of the dLGN travel through layer 4Cα to layer 4B of V1 (Shipp and Zeki, 1989). Cells in layer 4B of V1, which can send inputs directly to area MT (Nassi
and Callaway, 2007), also receive projections from parvo-recipient layer 4C/β (Yabuta et al., 2001). The layer 4B cells projecting directly to area MT are located amongst cells which send inputs to the CO thick stripes of V2 and area V3 (Maunsell and Van Essen, 1983c; Burkhalter et al., 1986; Livingstone and Hubel, 1987; Shipp and Zeki, 1989; Zeki and Shipp, 1989; Levitt et al., 1994a,b; Sincich and Horton, 2003; Nassi and Callaway, 2006, 2007). The signals from V3 to area MT are magnocellular in nature, involving higher order form analysis as well as processing of orientation and direction (Levitt et al., 1994a,b). The timing of signals to V3 and area MT shortly after inputs to V1 from the dLGN (Schmolesky et al., 1998) strongly indicates a domination of the magnocellular stream in these areas. However, there has been recent evidence for parvocellular signals reaching area MT from layer 4C/β of V1 most likely through a polysynaptic input via 4B pyramidal cells into the CO thick stripes of V2 (Nassi and Callaway, 2006; Nassi et al., 2006; Nassi and Callaway, 2007). A parvocellular input has also been shown to reach area MT disynaptically from the dLGN through the layer 6 Meynert cells in V1, bypassing layer 4C/β in V1 (Nassi et al., 2006).

Recent work has demonstrated some selectivity for chromatic motion in area MT (Dobkins and Albright, 1994; Croner and Albright, 1999; Sedemmann et al., 1999; Barberini et al., 2005). The use of moving stimuli (Saito et al., 1989; Dobkins and Albright, 1994; Gegenfurtner et al., 1994; Sedemmann et al., 1999; Thiele et al., 1999; Barberini et al., 2005; Riecansky et al., 2005) and psychophysics (Croner and Albright, 1999; Riecansky et al., 2005) have indicated a role for chromatic borders in discrimination of motion direction (Saito et al., 1989; Dobkins and Albright, 1994; Riecansky et al., 2005). S-cone signals were also reported to produce strong direction selective responses along with an additive contribution to luminance signals for encoding direction selectivity (Barberini et al., 2005).

Human psychophysical studies using S-cone isolating stimuli have re-
revealed two exclusive groups with fast and slow velocity inputs to area MT (Morand et al., 2000). The fast pathway had response latencies between 40 and 75 ms, while the slow pathway had latencies between 175 and 240 ms. The psychophysical evidence from this human study has not yet been supported by anatomical or neurophysiological investigations. However, a study in the macaque monkey using retrograde tracer injected into area MT followed the anatomical pathway of input sources into this area (Sincich et al., 2004). The findings revealed a significant proportion of cells in the intercalated koniocellular layers of the dLGN which provided a direct input to area MT (Sincich et al., 2004). However, this direct input to area MT from the LGN is much smaller in comparison to the geniculate input to the cortex and hence its role in perception is yet to be defined. The physiological nature of these projection cells in terms of their cone inputs has not been investigated. A minority of S-cone signals found to display opponency to luminance signals in area MT have been suggested to be the result of a blue-yellow opponent pathway (Barberini et al., 2005).

It may be possible that the fast S-cone input pathway determined in the human psychophysical study (Morand et al., 2000) may in fact be carried by the direct koniocellular input from the macaque dLGN to area MT as revealed with retrograde anatomical tracing (Sincich et al., 2004). To investigate this possibility, V1 may be reversibly inactivated to inhibit all feedforward signals except for the direct inputs from subcortical areas to area MT. The nature of cone inputs from responses in area MT while V1 is inactivated could reveal whether the direct subcortical inputs into this area include signals from S-cones. However, the exact source of the direct subcortical inputs cannot be determined with certainty, since there are sources other than the dLGN, such as the inferior pulvinar (Standage and Benevento, 1983), that can potentially carry signals to area MT.

Direct subcortical pathways to area MT which altogether bypass V1 may
be responsible for the residual vision observed occasionally in visual fields of people with cortical damage in V1. This produces the ability to detect, localise and discriminate the direction, orientation, motion, size and shape of the visual stimuli without acknowledging awareness of the visual stimuli as seen in blindsight (Weiskrantz et al., 1974).

The latencies of area MT inputs can also be investigated to provide information about the source of these inputs. The conduction velocity of the neuronal axons affect the response latencies so that axons of large diameters projecting from V1 to area MT result in fast latencies (Rockland, 1995). Previous studies had suggested that there was an anatomical basis for functional differences in signal transmission where two distinct fast and slow conduction pathways were responsible for encoding the fast and slow components of stimulus speed, respectively (ffytche et al., 1995; Zeki, 1998). Using magnetoencephalography (MEG) and EEG in normal human subjects, it was revealed that a fast conduction pathway encoded stimuli moving faster than 6 degrees per second. A slow conduction velocity pathway encoded stimuli moving slower than 6 degrees per second (ffytche et al., 1995). This study by ffytche et al. (1995) proposed that unlike the slow conduction velocity pathway which possibly traveled through V1, the fast conduction velocity pathway bypassed V1 completely on its way to providing a direct input to area MT (referred to as V5 in the study). This may have led to the preservation of the ability to detect fast motion in blindsight (Weiskrantz et al., 1974; ffytche et al., 1995; Zeki, 1998; Sorenson and Rodman, 1999).

The finding of fast and slow conduction velocity pathways for fast and slow motion respectively was disputed by a recent study where latencies were determined after irreversibly inactivating V1 in the macaque monkey with electrolytic lesions (Azzopardi et al., 2003). While there was evidence of fast and slow conduction velocity pathways to area MT in the contralateral unlesioned hemisphere, there were longer response latencies and also
the preservation of sensitivity to both fast and slow movements with long response latencies in the lesioned hemisphere (Azzopardi et al., 2003). The resulting preservation of the slow motion (along with fast motion) was taken as evidence that the slow conduction velocity pathway does not exclusively pass through V1 on its way to area MT. This contradicts the suggestion (ffytche et al., 1995) that slow motion signals are transferred only through V1 by a slow conduction velocity pathway. Furthermore, the presence of longer latencies to cells selective to fast or slow motion after V1 lesions suggests that the slow conduction velocity pathway encodes both fast and slow motion signals.

The present study was conducted to determine the cone inputs to cells of area MT before and during inactivation of V1 by reversible cooling using a Peltier device or by iontophoresis of the inhibitory transmitter γ-aminobutyric acid (GABA). The latencies of each type of cone input with and without inactivation of V1 were measured using different cone modulating stimuli presented as flashing square wave gratings. The response latencies of area MT cells during reversible V1 inactivation were also recorded to compare with reports of longer response latencies in area MT during V1 inactivation in the lesioned macaque brain (Azzopardi et al., 2003).
6.2 Methods

The procedures for this study are discussed in detail in Chapter 3. A brief overview is given here. The effect of cooling V1 on the responses of area MT cells was investigated after topographically matching the visual receptive fields of cells being inactivated in V1 to overlap the area of the visual field from which area MT recordings were made. V1 was reversibly cooled using a Peltier device (8 mm x 9 mm) positioned on the cortical surface aligned posterior to the lunate sulcus. This resulted in inactivating a region approximately 2° to 6° temporal to the vertical meridian and 2° to 6° below the horizontal meridian in the contralateral visual field. The positioning of the Peltier device upon the cortex also cooled the fundus of the calcarine sulcus of V1 located about 5 mm beneath the cortical surface. This area represents a region between 15° and 25° eccentricity in the infero-temporal contralateral visual field (Van Essen et al., 1984). The temperature gradient within the calcarine sulcus directly beneath the Peltier device was determined with a digital thermister to ascertain that the temperature was sufficient for inactivation of responses. A temperature of 12°C or below was taken to be sufficient to inactivate the cortical area (Volgushev et al., 2000).

Inactivation of V1 using GABA iontophoresis was also employed in one of the eight macaques used for this study. This allowed precise focal inactivation of topographically matching receptive field regions of the cells in V1 and area MT. The GABA was delivered with a 200 nA current through four micropipettes attached to the electrode penetrating V1. The method was verified by noting a significant suppression of neural activity in V1 cells from the recording electrode (attached to the GABA pipettes within V1) during GABA iontophoresis. The affected receptive field region of the V1 site being inactivated was calculated as a function of the magnification factor ($M$), the receptive Field Size ($FS$) of cells in the area and the Field Size Scatter
Determining the affected region in V1 aided in restricting the extent of the visual stimulus to fall within the visual field of the area MT cell in a location likely to be inactivated by the GABA application.

For the recordings in area MT, six different stimulus conditions (see Figure 3.10) were generated on the Barco monitor using “Open GL” commands controlled by a custom software (EXPO, Peter Lennie). The first stimulus condition was a blank gray luminance-averaged background to detect baseline spontaneous activity. The second stimulus condition modulated L-cones, M-cones and S-cones in phase (L+M+S). The third, fourth and fifth stimulus conditions individually modulated the L-cones, M-cones or S-cones, respectively. The last stimulus condition was the modulation of L-cones and M-cones in phase (L+M). The cone contrast for each stimuli is shown in Table 6.1 with the dominant wavelength of the red (L), green (M) and blue (S) guns shown in brackets.

**Table 6.1: Cone contrasts for the various stimuli presented during area MT recordings**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>L (558 nm)</th>
<th>M (530 nm)</th>
<th>S (423 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L+M+S</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>L</td>
<td>0.08</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M</td>
<td>0</td>
<td>0.08</td>
<td>0</td>
</tr>
<tr>
<td>S</td>
<td>0</td>
<td>0</td>
<td>0.80</td>
</tr>
<tr>
<td>L+M</td>
<td>0.5</td>
<td>0.5</td>
<td>0</td>
</tr>
</tbody>
</table>

The stimuli were flashed as square wave gratings at a rate of 2 Hz and the peri-stimulus time histograms (PSTHs) were first constructed with a bin-width of 1 ms. The analysis involved identifying the 20 ms bin that showed the highest peak averaged response over 17 to 20 passes of the stimulus presentation.

The peak response was compared statistically to ensure the response was
significantly above the average of the spontaneous activity for that stimulus presentation using a student’s t-test (significance value \( p < 0.05 \)). Responses which were considered to be significantly above the spontaneous activity at the normal body temperature (control responses) were compared statistically to those when the cortex was cooled at 6°C to 12°C (inactivated responses). In the case of GABA inactivation, this was the comparison between the responses before GABA iontophoresis (control responses) to the responses during GABA iontophoresis (inactivated responses). A second student’s t-test was used for this comparison to determine whether the control responses were significantly different to the responses during V1 inactivation (significance value \( p < 0.05 \)). If this was the case, this cell was categorised as having a significant change of response during V1 inactivation. Cells with comparatively different responses during the control and inactivated conditions were sometimes categorised to be cells without a significant change in response during inactivation. Since this was due to the large standard deviations within the control response or response during V1 inactivation, the statistical analysis ensured these cells were not categorised as showing a significant change in response during inactivation.

The responses during V1 inactivation were normalized to the control responses for each stimulus condition to give a response ratio, so the relative change in response for each cell could be quantified. The recovery response after rewarming of the cortex was also recorded where possible.

The response latency for each stimulus type for the control and inactivated conditions was found using a criteria (Maunsell and Gibson, 1992) of identifying three consecutive one millisecond bins which gave a response greater than 3, 3 and 2 standard deviations above the average of the spontaneous activity. The criterion for response latency was sometimes not met due to the lack of 3 consecutive response bins although an actual response averaged over 20 ms seemed to be present.
A control for the measure of whether the Peltier cooling device was directly inactivating area MT instead of the intended V1 region alone was established. This was done by recording the presence of both altered and unaltered latencies of area MT cell responses in those cells not topographically located in the visual field region corresponding to the area of V1 cooling. Figure 6.1 shows an example where the cell is located at a non-foveal visual field eccentricity of 9.8° peripherally to the fovea and is not affected by cooling. Had the cooling effect spread directly to area MT the non-foveal visual fields would be more likely to be affected since the representation of peripheral area MT is closer to the cooled region of V1 than the representation of foveal area MT. Another cell recorded from the same track at the same eccentricity showed a change in response latencies during cooling.

![Figure 6.1: The response latencies for a non-foveal area MT cell located in a topographically non-corresponding part of the V1 region being cooled. The latencies available before, during and after V1 inactivation with Peltier cooling are shown. There is no effect of cooling on the response latencies. A non-foveal area MT cell being directly cooled would show a change in latency.](image)

The effective cooling of the V2 stripes within the lunate sulcus by place-
ment of the Peltier device directly posterior to the lunate sulcus on the V1 cortex could lead to further disruption of signals to area MT. The V2 stripes are known to provide direct inputs to area MT (Shipp and Zeki, 1985; Ungerleider and Desimone, 1986b; Bullier and Girard, 1988; Nassi and Callaway, 2007), transmitting signals from layer 4B of V1.

The tissue from area MT was processed and stained for Nissl body and myelin after coronal sectioning in 50 \(\mu\)m steps using a freezing microtome (Leica SM 2000R, Leica Microsystems, Nussloch, Germany). Electrolytic lesions were made during area MT recordings (6 - 10 \(\mu\)A for 6 - 10 s, electrode negative) and the electrode tracks were reconstructed with the help of these (Figure 6.2).

![Figure 6.2](image)

**Figure 6.2:** *A coronal section of the macaque superior temporal sulcus stained for Nissl substance. Recordings were made from two tracks (arrow-heads) in this region of area MT. Lesions marked “L” along the depth of the tracks were used as a guide to identify recording locations.*

The sections stained for myelin with a silver stain were also used to identify densely myelinated regions which are typical of area MT within the superior temporal sulcus (Van Essen et al., 1981, 1984).
6.3 Results

6.3.1 General findings

The cone inputs to recorded area MT cells were revealed using stimuli of varying cone modulations as described in Section 6.2. Cells showing responses to S-cone isolating stimuli were also tested with L+M modulation in-phase at different contrasts to check that the responses to S-cone stimulation were not due to luminance artifacts (see Section 4.3.1).

Figure 6.3 shows an example of a cell’s responses before cooling (A), and during cooling (B). It is evident that cooling had little effect on this cell. For comparison, Figure 6.4 shows a cell greatly affected by V1 cooling (B), where the responses to all the stimuli were reduced. The error bars on the PSTHs represent the standard mean error.

6.3.2 Eccentricities

Since the Peltier device could only be placed on the dorsal surface of V1, the area that could be most easily inactivated was usually within 2° to 6° eccentricity from the centre of the fovea. A number of penetrations were made until the appropriate part of area MT could be located. However, since the cooling may have affected eccentricities between 15° to 25° within the calcarine sulcus inferior to the V1 cortical surface, recordings were also made from area MT cells which had receptive fields extending infero-laterally up to 18° eccentricity.

The eccentricities of the centres of the receptive fields of the recorded area MT cells are shown in Figure 6.5. Due to the difficulties of matching the topographically corresponding inactivated regions of V1 to the visuotopic location of the area MT cells being recorded, only data from four of the eight macaque monkeys were analysed in this study. The visual field of V1
Figure 6.3: Example of a cell in area MT which did not show a significant change in response during inactivation of V1. The responses before inactivation (A) and during inactivation (B) were fairly similar. The square wave stimulus grating was flashed at 2 Hz. Cone types were modulated in combination or individually, as labelled above each PSTH. The optimum orientation and aperture size were used. The stimulus was phase reversed and the responses folded into one cycle where the bin-width was 20 ms.
Figure 6.4: Example of a cell’s responses in area MT before V1 inactivation (A) and with highly reduced response during inactivation (B). The square wave grating was flashed at 2 Hz and the responses were folded into one cycle. All three cone types were modulated in various combinations or individually, as labelled above each PSTH. The bin-width was 20 ms.
corresponding to the placement of the Peltier cooling device on the cortical
surface was plotted to be approximately from azimuth 2° to 6°, and elevation
-2° to -6°. During inactivation with GABA iontophoresis in V1, the area of
inactivation was much smaller but the receptive fields of the V1 cells being
inactivated were matched to be within the receptive fields of the area MT
cells being recorded (Figure 6.5). The stimuli were restricted to specifically
overlap the V1 receptive fields being inactivated.

Figure 6.5: Eccentricities of cells recorded from area MT in the four monkeys.
The placement of the Peltier device on the surface of V1 corresponded to a
visual field region approximately 2° to 6° azimuth and -2° to -6° elevation
from the fovea. The GABA inactivation focally overlapped the receptive fields
of the cells recorded in animal VC22.

6.3.3 Methods of Inactivation

The spread of cooling from the Peltier device may have inactivated parts
of the thick, pale and thin stripes in the horizontal meridian of V2. This
area of V2 next to the V1 and V2 border is enclosed along the lunate sulcus
(Bullier and Kennedy, 1983) and may have been affected to a depth of about 6 to 8 mm from the surface. Thermisters driven to this depth registered temperatures down to 12°. A comparison of response ratios between the methods of Peltier cooling and GABA iontophoresis found that there was no statistically significant difference (student’s t-test, p = 0.25). The L+M+S stimulus condition was used for this analysis since it usually yielded the maximum response.
6.3.4 Responses to different cone modulating stimuli

The responses to the varied stimulus conditions are not directly comparable due to the different cone contrasts in each condition (Table 6.1), which was inevitable due to technical limitations. Therefore, the responses for each condition have been shown separately. Table 6.2 shows the average response for the control and inactivated conditions. It includes the whole sample and responses to all the different cone modulating stimuli. The most vigorous responses were from modulation of all three cone types in phase (L+M+S), as well as L+M modulation in phase. The difference between responses before and during V1 inactivation was significant for the L+M+S and L-cone modulating stimuli (paired t-test).

Table 6.2: Comparison of responses in the control and inactivated conditions for the different stimuli. Stimuli with higher cone contrasts usually showed better responses than stimuli with lower cone contrasts.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Control</th>
<th>Inactivated</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>L+M+S</td>
<td>34.44 ± 19.42</td>
<td>30.27 ± 20.69</td>
<td>0.02</td>
</tr>
<tr>
<td>(n = 28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>25.40 ± 14.23</td>
<td>21.80 ± 14.11</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>(n = 16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>28.93 ± 16.08</td>
<td>24.36 ± 19.87</td>
<td>0.14</td>
</tr>
<tr>
<td>(n = 14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>20.98 ± 12.09</td>
<td>18.61 ± 10.69</td>
<td>0.20</td>
</tr>
<tr>
<td>(n = 17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L+M</td>
<td>31.85 ± 19.54</td>
<td>28.65 ± 22.25</td>
<td>0.13</td>
</tr>
<tr>
<td>(n = 27)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 6.6 shows the control responses and responses during V1 inactivation of area MT cells for the presentation of the L+M+S stimuli (with all
three cones having a contrast of 100%). This stimulus yielded the maximum response in 23 of the 28 area MT cells recorded.

Nineteen area MT cells showed no change in response with inactivation when presented with the L+M+S stimuli, either with Peltier cooling or GABA iontophoresis. Nine cells showed a significant reduction in their responses during V1 inactivation. The average control response for the cells with no significant change in response during V1 inactivation was 37.22 ± 20.37 imp/sec (average [ave] ± standard deviation [sd]; n = 19). The average response during V1 inactivation for these cells was 37.27 ± 20.24 imp/sec (ave ± sd; n = 19). The difference in these values was not statistically significant (paired t-test, p = 0.97).

For the group of cells that did show a change in response during V1 inactivation using any method, the average control response was 28.57 ± 16.81 imp/sec (ave ± sd; n = 9) and the response during V1 inactivation was 15.50 ± 12.72 imp/sec (ave ± sd; n = 9). This difference was statistically significant (paired t-test, p < 0.01).

The data for cells responding to the L condition (cone contrast 0.08) is shown in Figure 6.7. Two of the 16 cells underwent a significant reduction in response during V1 inactivation.

The average control response for the cells with no change in response using any method of V1 inactivation was 25.48 ± 15.20 imp/sec (ave ± sd; n = 14). The average response during V1 inactivation for these cells was 22.66 ± 14.69 imp/sec (ave ± sd; n = 14). The difference in these values was statistically significant (paired t-test, p = 0.03).

For the cells that did show a change in response during V1 inactivation using any method, the average control response was 24.84 imp/sec (n = 2) and the average response during V1 inactivation was 15.78 imp/sec (n = 2).

Of the data collected for the M condition (cone contrast 0.08) one cell showed a marked increase in response during V1 inactivation (Figure 6.8).
Figure 6.6: Control response versus response during V1 inactivation of area MT cells when presented with the L+M+S stimulus. Those cells affected by V1 inactivation lie below the identity line. This was the optimum stimulus for the majority of cells recorded.

Figure 6.7: Responses before and during V1 inactivation for cells responding to L-cone modulation. Two cells showed a significant change in response during V1 inactivation.

This may be due to a release in inhibition in V1 with inactivation to facilitate this input to area MT. Three cells showed a reduction in response during V1 inactivation.
Ten cells showed no significant change in response during V1 inactivation by any method. The average control response for these cells was $26.25 \pm 17.50$ imp/sec (ave ± sd; n = 10). The average response during V1 inactivation for these cells was $21.19 \pm 13.87$ imp/sec (ave ± sd; n = 10). The difference in these values was statistically significant (paired t-test, p = 0.01).

For the cells that showed a significant change in response during V1 inactivation using any method, the average control response was $35.63 \pm 10.87$ imp/sec (ave ± sd; n = 4) and during V1 inactivation was $32.30 \pm 31.87$ imp/sec (ave ± sd; n = 4). The difference in these values was not statistically significant (paired t-test, p = 0.77). The lack of statistical significance between control responses and responses during V1 inactivation for the cells showing a significant change in response during V1 inactivation may be a result of a large SD in the responses during V1 inactivation within the small sample size.

Figure 6.8: Responses before and during V1 inactivation of cells responding to M-cone modulation. One cell had a significant increase in response with V1 inactivation.

For the S condition (cone contrast 0.80), there was one cell which showed an increase in response during V1 inactivation (Figure 6.9). Three cells
showed a significant reduction in response with V1 inactivation.

There were 13 cells which did not show any change in response during V1 inactivation. The average control response for the cells with no change in response during V1 inactivation was $20.47 \pm 12.13$ imp/sec (ave ± sd; n = 13). The average response during V1 inactivation for these cells were $19.44 \pm 10.37$ imp/sec (ave ± sd; n = 13). This difference was not statistically significant (paired t-test, p = 0.41).

For the cells that showed a significant change in response during V1 inactivation using any method, the average control response was $22.65 \pm 13.64$ imp/sec (ave ± sd; n = 4). The average response during V1 inactivation for these cells was $15.91 \pm 12.92$ imp/sec (ave ± sd; n = 4). This difference was not statistically significant (paired t-test, p = 0.39).

Figure 6.10 shows the data for cells responding to the L+M stimulus (cone contrast 0.5 for each cone). Out of the nine cells showing a change in response during V1 inactivation, one showed a facilitation of the response. The means of V1 inactivation for this cell was GABA iontophoresis. The

![Graph](image.png)

Figure 6.9: Cell responses before and during inactivation of V1 to S-cone modulation. While most cells which were affected by V1 inactivation showed a reduction in response, one cell had an increased response.
two cells showing similar facilitation in the previous M-cone modulating and S-cone modulating conditions, respectively, were a result of V1 inactivation with Peltier cooling.

Figure 6.10: Cell responses before and during V1 inactivation for the L+M condition showed a tendency for reduced responses during V1 inactivation. The cell showing an increased response during V1 inactivation overlapped the receptive field of a V1 cell under the effect of GABA iontophoresis.

Eighteen cells showed no significant change in response during V1 inactivation. The average control response for these cells was $32.02 \pm 22.10$ imp/sec (ave $\pm$ sd; n = 18). The average response during V1 inactivation for these cells was $32.52 \pm 23.80$ imp/sec (ave $\pm$ sd; n = 18). This difference was not statistically significant (paired t-test, p = 0.67).

For the cells that did show a change in response during V1 inactivation using either method, the average control response was $31.52 \pm 14.23$ imp/sec (ave $\pm$ sd; n = 9). The average response during V1 inactivation for these cells was $20.93 \pm 17.44$ imp/sec (ave $\pm$ sd; n = 9). The difference in these values was not statistically significant (paired t-test, p = 0.06).

Inactivation of V1 with Peltier cooling and precise focal GABA iontophoresis revealed a preservation of all three cone inputs to varying extents.
within the receptive fields of area MT cells which were directly overlapping with the inactivated V1 region. This is a profound finding as it indicates that these cone signals do in fact bypass V1 and send inputs directly to area MT from subcortical areas.
6.3.5 Response ratios with changing eccentricity

The inactivation of V1 with Peltier cooling revealed area MT cells affected at eccentricities slightly further than the foveal receptive fields directly targeted with the Peltier placement over V1. While the V1 regions expected to be directly cooled by the Peltier were within 2° to 6° temporal and inferior to the fovea of the contralateral eye, visual eccentricities up to 8° were affected. This may have resulted from the cooling effect spreading to the V1 cortex surrounding the Peltier device. The cooling effect may have also spread to inactivate regions of the calcarine sulcus representing regions of the visual field from approximately 15° up to 25° infero-temporal to the fovea (Van Es- sen et al., 1984). Cells at these eccentricities were recorded but did not show a significant reduction in response during cooling.

A response ratio of the response during V1 inactivation to the control response was calculated. A response ratio of unity would indicate a preservation of the control response. A value less than unity would indicate a reduction in response during V1 inactivation by Peltier cooling or GABA iontophoresis in V1. Response ratios greater than unity indicated an increase in response during V1 inactivation. Better responses may result from a decrease in inhibitory inputs during V1 inactivation, leading to the facilitation of alternative inputs to area MT. There was no significant difference in the response ratio between the two methods of V1 inactivation (student’s t-test, p > 0.15 for each condition, individual values not shown).

Shown in Table 6.3 are the average response ratios from the whole population of cells responding to each stimulus presentation. The cells responding to the S-cone modulating stimuli had an average drop in response to 0.92 of the control response.

The response ratios of cells for the L+M+S condition are shown in Figure 6.11. The cells with no significant change in response during V1 inactivation had an average response ratio of 1 ± 0.16 (ave ± sd; n = 19).
The nine cells which showed a significant reduction in response during V1 inactivation had receptive field eccentricities ranging from 3.5° to 7.6°. Their average response ratio was 0.48 ± 0.21 (n = 9). The difference in response ratio between the cells with and without a significant change in response was statistically significant (student’s t-test, p < 0.01).

Similar graphs for the response ratios with changing eccentricity are shown for the L, M and S conditions (Figures 6.12, 6.13, 6.14).

For the L condition, the average response ratio of cells with no significant change in response during V1 inactivation was 0.87 ± 0.15 (ave ± sd; n = 14). Those cells which did show a significant reduction in response with V1 inactivation had an average response ratio of 0.61 ± 0.27 (ave ± sd; n = 2). The difference in response ratio between the cells with and without a significant change in response during V1 inactivation was not statistically significant (student’s t-test, p = 0.19).

The cells responding to the M condition without a change in response during V1 inactivation had an average response ratio of 0.81 ± 0.09 (ave ± sd; n = 10). Those showing a significant change in response during V1 inactivation had an average response ratio of 0.79 ± 0.52 (ave ± sd; n = 4). The difference in response ratio between the cells with and without a
Figure 6.11: *Response ratios with changing eccentricity for the L+M+S condition. Cells both with and without a change in response during V1 inactivation overlapped in eccentricity.*

A significant change in response during V1 inactivation was not statistically significant (student’s t-test, p = 0.48).

With the S condition, the average response ratio for cells showing no change in response during V1 inactivation was $0.99 \pm 0.23$ (ave $\pm$ sd; n = 13). Those cells which showed a change in response during V1 inactivation had an average response ratio of $0.71 \pm 0.54$ (ave $\pm$ sd; n = 4). The difference in response ratios between the cells with and without a significant change in response during V1 inactivation was not statistically significant (student’s t-test, p = 0.19).

Although the cells showing no change in response during V1 inactivation were located across a range of eccentricities for each of the L, M and S stimulus conditions, the lack of cells with a change in response during V1 inactivation prevented a direct comparison to be made.

Figure 6.15 shows the cells with and without a change in response during V1 inactivation to be largely overlapping in eccentricity for the L+M condi-
Figure 6.12: Response ratios with changing eccentricity for L-cone modulation. The two cells with a significant change in response during V1 inactivation were located within 6° of the fovea.

Figure 6.13: Response ratios of cells responding to M-cone modulation. The cells showing a significant change in response during V1 inactivation were between 3° and 8° of foveal eccentricity.
Figure 6.14: Response ratios of cell at various eccentricities to S-cone modulation. Cells with a significant change in response during V1 inactivation were located centrally.

The average response ratio for the cells without a change in response during inactivation was $1 \pm 0.18$ (ave $\pm$ sd; $n = 18$) and for those with a significant change in response was $0.62 \pm 0.45$ (ave $\pm$ sd; $n = 9$). The difference in response ratio between the cells which showed a significant change in response and the cells which did not during V1 inactivation was statistically significant (student’s t-test, $p = 0.02$).

Area MT cells affected during V1 inactivation for all conditions were between $3^\circ$ and $8^\circ$ of visual eccentricity, corresponding well with the visuotopic location of the areas of V1 targeted for inactivation. These cells overlapped in eccentricity with the cells showing no significant change of response during V1 inactivation. These latter cells were located also located at non-foveal ($>8^\circ$) eccentricities.
Figure 6.15: *Response ratios as a function of eccentricity for the L+M condition. Cells with and without a change of response during V1 inactivation were at overlapping eccentricities.*

### 6.3.6 Latencies of area MT cells with V1 cooling

Response latencies to the differing stimulus conditions were calculated using a previously used criteria (Maunsell and Gibson, 1992). The latencies for the control responses are reported in the following results unless otherwise stated.

The average control latencies for the whole population of cells responding to each stimulus are shown in Table 6.4. The shortest onset response latencies were for the L+M+S or the L+M stimuli. These values were consistent with the onset latencies for area MT cells from a recent study (Bair et al., 2002) which were found to average 40 ms compared to the offset latency average of 29 ms. The response latencies to individual cone modulations (L or M or S) varied since the data available for these groups was limited. The latencies for stronger responses were expected to be faster since the criterion for reliably determining the latency was met sooner.
Table 6.4: Control response latencies (in ms) for all cells responding to each stimulus condition.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>No. of Cells</th>
<th>Response latency Average ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>L+M+S</td>
<td>19</td>
<td>38.47 ± 30.87</td>
</tr>
<tr>
<td>L</td>
<td>4</td>
<td>79.75 ± 56.91</td>
</tr>
<tr>
<td>M</td>
<td>10</td>
<td>54.50 ± 45.20</td>
</tr>
<tr>
<td>S</td>
<td>5</td>
<td>49.60 ± 19.67</td>
</tr>
<tr>
<td>L+M</td>
<td>21</td>
<td>37.86 ± 26.76</td>
</tr>
</tbody>
</table>

The control latencies were correlated with eccentricity to determine whether there was a relationship between the distance from the fovea and the latency of responses to stimulus presentations.

Figure 6.16 shows the fastest latency of the two response phases plotted against eccentricity. The average latency for cells with no significant change in response during V1 inactivation was 30.77 ± 18.62 ms (ave ± sd; n = 13).

The cells which showed a significant change in response during V1 inactivation had an average latency of 55.17 ± 45.93 ms (ave ± sd; n = 6). The difference in response latency between these two groups did not, however, reach statistical significance (student’s t-test, p = 0.13). The cells with latencies longer than 80 ms were between 4° and 8° of the fovea. Two of these three cells were affected by V1 inactivation.

The criteria (as in Maunsell & Gibson, 1992) for reliably determining response latency resulted in fewer cells responding to the L, M and S conditions being able to reach the criteria than those cells responding to the L+M+S condition. The latencies from the available data are shown in Figure 6.17, Figure 6.18 and Figure 6.19. Data only for cells which were unchanged in response during V1 inactivation were available for the L condition, which had an average latency of 79.75 ± 56.91 ms (ave ± sd; n = 4).
Figure 6.16: Response latencies for the L+M+S condition. Fast latencies were recorded at all eccentricities.

The M condition showed cells which did not show a significant change in response during V1 inactivation to have an average latency of 53.29 ± 47.46 ms (ave ± sd; n = 7). Those that did show a significant change in response during V1 inactivation had an average latency of 57.33 ± 49.17 ms (ave ± sd; n = 3). The difference in response latencies between these two groups was not statistically significant (student’s t-test, p = 0.46).

The average latency during the S condition for cells not showing a significant change in response during V1 inactivation was 49.25 ± 22.69 ms (ave ± sd; n = 4) and the cell which did show a change during V1 inactivation had a latency of 51 ms. The latencies from L+M+S modulation in phase for those cells showing S-cone inputs have also been displayed (Figure 6.20) since there was more latency data available for the L+M+S stimulus. There is considerable overlap between cells that were significantly affected by V1 inactivation (50.8 ± 50.2 ms, ave ± sd; n = 7) and those that were not (34.0 ± 22.1 ms, ave ± sd; n = 4). The difference between these two groups is not statistically significant (student’s t-test, p = 0.23). Fast latencies are evident for both these cell groups at central and non-foveal (> 8°) eccentricities.
Figure 6.17: Response latencies to L-cone modulation for cells which were not affected during inactivation of V1.

Figure 6.18: Response latencies to M-cone modulation. The response latencies of the cells were fairly similar with changing eccentricity.
Figure 6.19: Response latencies to S-cone modulation. The response latencies of cells did not differ greatly with increasing eccentricity.

Figure 6.20: Response latencies of cells with S-cone inputs for the L+M+S stimulus. The distribution of latencies for cells with a significant change in response during V1 inactivation was similar to those cells which were not affected. Fast latencies were present at all eccentricities.
Figure 6.21: *Latencies as a function of eccentricity for responses to the L+M stimulus. The latencies of the cells with and without a significant change in response during V1 inactivation were overlapping.*

For the L+M condition, the latency of the 15 cells unaffected by V1 inactivation and the six cells which were significantly affected were located in the same range of eccentricity. The average latency for the former group was 33.93 ± 19.98 ms (ave ± sd; n = 15) and the latter group was 56.33 ± 40.17 ms (ave ± sd; n = 6). The difference in response latencies between these two groups was not statistically significant (student’s t-test, p = 0.15).

There was a rough segregation of latencies into two groups which were faster or slower than 70 ms for cells located at eccentricities less than 10° for all five stimulus conditions. Latencies at non-foveal eccentricities were usually faster than 70 ms. Cells with responses that were and were not significantly affected during V1 inactivation were present in both the fast and slow latency groups. Note that the 70 ms cut-off was an arbitrary criterion rather than one based on any statistically valid division. Bimodal statistical significance was not conducted due to only a sample of 3 cells with latencies slower than 70 ms to the different stimuli.
The control latencies from the cells responding to the five stimulus condition are shown in Figure 6.22. The star under a cell indicates a significant change in response during V1 inactivation for the optimum stimulus condition or the majority of conditions it responded to. The rough segregation into the fast and slow latency groups can be seen. Latencies ranged from 17 ms to 157 ms. Onset latencies in area MT have been reported (Bair et al., 2002) to be $40 \pm 4.8$ ms (ave $\pm$ sd) and offset latencies slightly faster at $29 \pm 5.2$ ms (ave $\pm$ sd).

![Figure 6.22: The control response latency of cells for different stimulus conditions. The star under the cell indicates a significant change of response during V1 inactivation. Latencies ranged from 17 ms to 157 ms. There was a rough segregation into two groups of cells with response latencies either faster or slower than 70 ms.](image)

The cells with long latencies were all affected by V1 inactivation and could indicate an indirect projection through V1 to area MT. It is important to note that the cells in Figure 6.22 with a star showed a significant
change in response during V1 inactivation either for the maximum responses recorded or with the majority of conditions. However, the response for some of the L-cone or M-cone conditions did not show a statistically significant change during V1 inactivation. Moreover, six cells which were affected by V1 inactivation had latencies faster than 70 ms. Hence, long and short latencies may include responses to stimuli which are both affected or not affected significantly during V1 inactivation.

There were two instances where the response latencies from the S-cone modulation lagged those from the L+M+S and L+M stimuli by 20 ms. One of these cells demonstrated a significant change in response during V1 inactivation. The response latencies available for the individual cone modulation (L or M or S) stimuli were limited and usually slower compared to the response latencies of the L+M+S or L+M modulations in phase.

Latencies are shown as a function of the response ratios in Figure 6.23. The response ratios and latencies to the stimulus condition (usually L+M+S) which gave the maximum response were used. Responses during V1 inactivation that were too low for the criteria for response latency to be met (Figure 3.11 are not represented on the graph.

The response ratios were close to or above 1 for the cells with no significant change in response during V1 inactivation. The latencies were seen to be usually faster than 70 ms for this group (unfilled shapes in Figure 6.23) with the one exception. Since only the maximum responses were used, latencies to other stimulus presentations that may have been slower than 70 ms are not shown. The cells with a significant change in response during V1 inactivation show a rough segregation of latencies into fast and slow groups.

The percentage change in latency was calculated for all those cells where latency data were available for both the control and inactivated conditions. Figure 6.24 shows the percentage change in latency as a function of the control latency for cells which were and were not significantly affected by V1.
inactivation during each stimulus presentation. The rough segregation into two groups of control response latencies above or below 70 ms can be seen. Neither group categorically demonstrated a consistent change in response latency during V1 inactivation.

There were four instances where a response from the area MT cell showed between 40% and 80% reduction in latency. However, these were all from cells which did not show a significant change in response during V1 inactivation (Figure 6.24). Those area MT cells significantly affected by V1 inactivation (filled shapes) showed a similar percentage of change in latency as those that were not. Control latency and stimulus condition were not reliable indicators of whether response latencies would be affected by V1 inactivation.

The percentage change in latency as a function of the percentage change in response to the stimulus giving the maximum response (usually L+M+S) is shown in Figure 6.25.

The percentage change in latencies was both positive (slower latencies) and negative (faster latencies) in cells both with and without a significant

Figure 6.23: The response latencies as a function of response ratios for the maximum responses available, which was usually for the L+M stimulus.
Figure 6.24: Percentage change in response latency between latencies before and during V1 inactivation for each stimulus condition. A significant change in response during V1 inactivation (filled shapes) was not correlated with a particular percentage change in latency.
Figure 6.25: The percentage change in latency as a function of percentage change in response for the maximum response. Cells both with and without a significant change in response during V1 inactivation showed a change in latency. The change could be both towards faster or slower latencies.

change in response during inactivation. Hence, inactivated latencies were measured to be both faster and slower in this study as opposed to the shift towards only longer latencies in area MT cells seen with V1 lesions in the macaque (Azzopardi et al., 2003).

The cut-off value of 70 ms between the fast and slow groups suggested by this study is similar to the limit of the fast latencies in an earlier human psychophysical study (Morand et al., 2000). The results from this study indicated that area MT cells with a response latency slower than 70 ms are more likely to be affected by V1 inactivation.

Other inputs with long latencies to these cells may not be affected by V1 inactivation. Cells with latencies faster than 70 ms can include cells both with and without a significant change in response during V1 inactivation. Latencies of subcortical inputs to area MT which are already bypassing V1 would not have changed during V1 inactivation.
Of the 28 cells recorded in area MT, 17 were shown to have S-cone inputs. Inactivation of V1 affected the S-cone inputs to only four of these cells. The effect of V1 inactivation on all cone inputs to these four affected cells are shown in Figure 6.26. The majority of responses are reduced. The four responses from the different stimuli lying above the identity line are from the same cell. Hence, the overall response of the cell to different stimuli during inactivation is similar to the response to S-cone modulation.

Figure 6.26: Responses to different stimuli for the four cells with S-cone inputs which showed a significant change in response during V1 inactivation. The responses were usually reduced for the different stimuli with V1 inactivation, indicating that all cone inputs were affected in a similar manner to the S-cone inputs. The responses above the identity line are from a cell also showing a better response to S-cone modulation during V1 inactivation.

The latency of these responses is shown in Figure 6.27. The criteria (Maunsell and Gibson, 1992) for a reliable latency value was not met for most of the individual cone modulations.

The rough segregation into latencies faster or slower than 70 ms is also
Figure 6.27: The response latencies to the different stimuli of the four cells with S-cone inputs which were affected during V1 inactivation.

observed here. The L+M+S and L+M inputs from the cell which improved during V1 inactivation (response ratios above 1) were in the fast latency group. A larger sample size may reveal a trend in the distribution of latencies as a function of the response ratio in affected cells with S-cone inputs.
6.4 Discussion

The major input to area MT is known to be from the magnocellular division of the dLGN, which provides additive L-cone and M-cone inputs into this area for luminance perception (Allman and Kaas, 1971; Zeki, 1974; Maunsell and Van Essen, 1983a,b; Albright, 1984). Reduction of area MT activity has been reported after inactivation of the magnocellular layers of the dLGN alone and more so in conjunction with parvocellular layers (Maunsell et al., 1990). Inactivation of the parvocellular layers alone has minimal effect on area MT responses (Maunsell et al., 1990).

Previous studies of chromatic signals for motion processing have involved the use of moving stimuli (Saito et al., 1989; Dobkins and Albright, 1994; Gegenfurtner et al., 1994; Seidemann et al., 1999; Thiele et al., 1999; Barberini et al., 2005; Riecansky et al., 2005) and psychophysics (Croner and Albright, 1999; Riecansky et al., 2005). Area MT neurons were previously thought to be largely unresponsive (Gegenfurtner et al., 1994; Riecansky et al., 2005) and gain minimal benefit from chromatic signals (Thiele et al., 1999). However, chromatic borders were found to aid in discrimination of motion direction (Saito et al., 1989; Dobkins and Albright, 1994; Riecansky et al., 2005). S-cone signals in particular have been shown to produce strong direction-selective responses although the contrast sensitivity to these signals were much lower than to luminance contrast (Seidemann et al., 1999). S-cone signals have been suggested to contribute additively to luminance signals for encoding direction selectivity (Barberini et al., 2005). Some opponency to luminance signals indicated that a minority of the S-cone inputs may be carried to area MT by a blue-yellow opponent pathway (Barberini et al., 2005). However, these previous results are not directly comparable to the present study in which flashing square wave cone modulating gratings were used.

The findings in this study revealed a significant reduction in responses of
some area MT cells during V1 inactivation and confirmed the success of the methods used for V1 inactivation by Peltier cooling or GABA iontophoresis. However, the majority of area MT neurons were unaffected by either one or the other method of inactivation under the same circumstances as those neurons which were affected. The inactivation was aimed at the direct and indirect projections from V1 to area MT (Shipp and Zeki, 1989; Zeki and Shipp, 1989). Inactivation of V1 has been shown to reduce the responses of all cell types in the thick, thin and pale stripes of V2 (Schiller and Malpeli, 1977; Girard and Bullier, 1989). Hence, inputs to area MT from both V1 and V2 would have been affected in the present study, due to inactivation of V1 through GABA iontophoresis or by direct cooling using a Peltier device.

About two thirds of the cells recorded in area MT received S-cone inputs and the quarter of these cells which were affected significantly by V1 inactivation also had their other cone inputs affected in the same way (Figure 6.26). This may suggest a common source of all cone inputs for these cells, possibly after integration of signals in layer 4B of V1 (Yabuta et al., 2001) and the thick and pale stripes of V2 (Sincich and Horton, 2002, 2003).

The S-cone inputs which survived V1 inactivation were consistent with the anatomical demarcation of a direct koniocellular input to area MT from the dLGN in macaque monkeys using a retrograde tracer (Sincich et al., 2004). This was also consistent with the findings reported in Chapter 5 that the S-cone signals were mainly transmitted by the koniocellular subdivision of the dLGN. The survival of both fast and slow S-cone inputs to area MT after V1 inactivation strengthens support for the presence of a fast S-cone pathway to area MT seen in human psychophysical studies (Morand et al., 2000) which may be due to a direct S-cone input bypassing V1 to human area MT. However, the transmission of the preserved L-cone and M-cone signals alone in cases without S-cone inputs through the koniocellular pathway is unlikely from the findings in Chapter 5.
The preservation of responses from L-cone and M-cone modulation during V1 inactivation may be of some significance. Selective inactivation of the magnocellular layers of the dLGN have been shown to completely abolish the responses in a few area MT neurons (Maunsell et al., 1990). However, there have been previous reports of preservation of direction selectivity and binocularity in area MT with V1 inactivation (Bullier and Girard, 1988; Girard and Bullier, 1989; Rodman and Albright, 1989; Rodman et al., 1989). The minimal effects of inactivation on responses in area MT to additive L-cone and M-cone modulation found in the present study suggest a possible magnocellular pathway from the dLGN bypassing V1 en-route to area MT. This may be a result of displaced magnocellular cells in the koniocellular layers, especially by displaced ipsilateral retinal afferents of magnocellular layer M2 within koniocellular layer K1 (Fitzpatrick et al., 1983; Yoshioka and Hendry, 1999).

Achromatic parvocellular and koniocellular neurons may also be responsible for these signals. In the anatomical study that revealed a direct projection from the koniocellular layers to the dLGN (Sincich et al., 2004), about two thirds of the cells labelled by the retrograde tracer from area MT in both the koniocellular and the parvocellular layers could also be double labelled for the protein α-Cam II kinase. This protein is known to be the morphological marker for koniocellular neurons, whether or not they are located in the koniocellular layers (Hendry and Yoshioka, 1994). Hence, the remaining third of the cells projecting to area MT which were not double labelled for the α-Cam II kinase protein may be of the magnocellular and parvocellular functional classes carrying L-cone and M-cone inputs in phase.

There is also a minor direct projection from mainly the dLGN magnocellular and parvocellular layers to V2 (Yukie and Iwai, 1981). The projection of these cells from V2 (in regions not affected by the Peltier device in this study) to area MT may have abundant collaterals to innervate a great ma-
jority of the area MT cells shown to receive L-cone and M-cone inputs. The responses in V2 have been shown to diminish with V1 inactivation (Schiller and Malpeli, 1977; Girard and Bullier, 1989). However, this may be due to an effect on the two-thirds of the inputs V1 provides to V2 (Schiller and Malpeli, 1977; Girard and Bullier, 1989). The direct subcortical inputs may largely survive this inactivation.

The number of inputs transmitted by these routes from the dLGN directly to area MT or via V2, though limited, may be sufficient for blindsight (Sanders et al., 1974; Weiskrantz et al., 1974). Blindsight has been reported in humans (Sanders et al., 1974; Weiskrantz et al., 1974; Cowey and Stogerig, 1991; Kentridge et al., 1999; Sahraie et al., 2003, 2006) and monkeys (Rosa et al., 2000; Moore et al., 2001) after V1 damage. Certain cells in area MT have been reported to have shorter response latencies than cells in V1 (Raiguel et al., 1989, 1999), strengthening the suggestion that inputs to area MT may be directly from subcortical areas bypassing V1. In fact, many cells in the present study had particularly short latencies. The fastest onset response latency (from responses to usually the L+M+S stimulus) was recorded to be shorter than 35 ms for an overwhelming 81% of cells (17 out of 21).

The superior colliculus is known to receive direct retinal inputs (Hubel et al., 1975; Pollack and Hickey, 1979). These in turn may bypass the dLGN and project to area MT through the pulvinar (Standage and Benevento, 1983). The superior colliculus has been additionally shown to send inputs through the inferior and lateral pulvinar (Benevento and Standage, 1983; Weller et al., 2002). Inactivation of the superior colliculus does not diminish responses in area MT such as the binocularity, orientation and direction selectivities of the cells (Rodman et al., 1990; Girard et al., 1992). However, inactivation of V1 in combination with the inactivation of the superior colliculus does abolish all responses in area MT (Rodman et al., 1990). In
light of these findings, the superior colliculus may not be the obvious source of direct subcortical inputs to area MT, but in some way may augment the inputs to area MT. The present findings that many inputs, whether carried by L-cone, M-cone or S-cone input cells, survive V1 inactivation may suggest that the dLGN may also provide a robust input that is enhanced by the collicular input through the pulvinar. The pulvinar may additionally send inputs to lower layer 3 in V2 (Livingstone and Hubel, 1982). These may contribute to the preserved signals in area MT in a similar manner to the direct projections from the dLGN to V2.

Besides the tectopulvinar connections, area MT sends and receives inputs to and from the claustrum of the basal ganglia (part of telencephalon) and the pons (part of metencephalon) (Weller et al., 2002; Kaas and Lyon, 2007). If the superior colliculus and koniocellular regions of the dLGN do not transmit L-cone and M-cone signals directly to area MT, the claustrum and pons may be alternative sources. The inputs into these regions would then be expected to be similar to the characteristics of the dorso-parietal stream in encoding the motion and location of objects. Inactivation of V1 affects regions of the infero-temporal stream such as V2, V3 and V4 (Schiller and Malpeli, 1977; Bullier and Girard, 1988; Girard and Bullier, 1989; Girard et al., 1991) responsible for encoding form and colour. This may explain the finding that low spatial frequencies below four cycles per degree (Sahraie et al., 2003) and the motion attributes of objects (Weiskrantz et al., 1995) can still be discriminated in blindsight.

In this study, the area of direct Peltier cooling overlapped with a region corresponding to approximately 2° to 6° horizontally and vertically in the contralateral visual field inferior to the fovea. The cells within the calcarine sulcus corresponded to non-foveal eccentricities of 15° to 20° in the contralateral inferior visual field (Van Essen et al., 1984). The temperature gradients measured in this region during the experiments were sufficient for
inactivation of the cells within the fundus of the calcarine sulcus. However, the two area MT cells at non-foveal eccentricities were not affected during inactivation.

There were seven instances amongst the varying stimuli presentations and conditions in this study where the response was absent in the control condition but appeared during inactivation of V1. The response ratios and latencies of these dormant cell responses were not significantly different from those cells which had initial control responses (with or without a significant change in response during V1 inactivation). There is a possibility that these dormant cells receive inhibition in the control condition from V1. The dormant cells may have the same excitatory input as those cells which were unaffected by V1 inactivation.

This study also quantified the latency of responses to stimulation of the three cones individually and in various combinations of cone contrast. A previous study in area MT (Bair et al., 2002) reported onset latencies of $40 \pm 4.8$ ms (ave $\pm$ sd) and slightly faster offset latencies of $29 \pm 5.2$ ms (ave $\pm$ sd). The latencies in the current study in comparison ranged from 17 to 157 ms, with some segregation of cells with response latencies above or below 70 ms (Figure 6.22). Although cells with latencies above 70 ms showed a significant reduction in response after V1 inactivation (suggesting a long route through V1), responses with long latencies from certain stimuli in these cells were unaffected with V1 inactivation. However, six cells with responses affected during inactivation had latencies faster than 70 ms. Hence, cells with slower latencies were most likely to be affected by V1 inactivation but those with faster latencies may or may not be. It is likely that most of the cells with latencies longer than 70 ms receive inputs through V1 since they were affected by V1 inactivation. Cells with latencies faster than 70 ms may or may not project through V1 to area MT.

It has been suggested that area MT receives inputs from separate fast
and slow conduction pathways (ffytche et al., 1995; Zeki, 1998). The fast conduction pathway was proposed to be directly from the dLGN carrying information about fast motion, while the slow conduction pathway through V1 conveys slow movement information. It was, however, suggested in another study that the fast and slow pathways were not specific to fast and slow motion, respectively (Azzopardi et al., 2003). The findings from the study by Azzopardi et al., (2003) report a shift towards longer response latencies with V1 inactivation, although slow motion and long latencies to slow or fast motion were not selectively affected.

The percentage change in latencies before and during inactivation in the present study did not differ greatly between cells that were affected by reversible inactivation of V1 (using either method of inactivation) and cells that were not (Figure 6.24). Many cells that were unaffected by V1 inactivation and were in the faster latency group had up to a 30% change in latency with no overall trend. Those cells affected by V1 inactivation in the faster latency group did not have a large percentage change in latency. Five of the eight cells unaffected by inactivation were 20% to 80% slower, but the remaining three cells had minimal percentage change in latency. Four of the six cells in the slower group that were affected by inactivation maintained unchanged latencies. Hence, the longer response latencies reported by the earlier study (Azzopardi et al., 2003) was not consistent with the present study. The effect on slow or fast motion during V1 inactivation was not investigated in these procedures.

The latencies from the receptive field surround have been reported to be longer by about 16 ms than those in the central excitatory region (Perge et al., 2005). The response latencies determined in the present study were, in contrast, usually faster than 75 ms at non-foveal eccentricities. Slower latencies were usually found closer to the fovea. Differences in latencies could arise from other factors such as stimulus size, contrast, duration and
onset (Bair et al., 2002). Great care was taken to standardise recordings in this study to minimise the above-mentioned variations.

It seems that the direct koniocellular projection from the dLGN to area MT may provide the surviving S-cone inputs to area MT during V1 inactivation. The exact route of the preserved responses from modulation of L-cones and M-cones remains to be determined. Since both methods of inactivation in this study did not demonstrate large effects on these L-cone and M-cone inputs, subcortical regions such as the dLGN (perhaps projecting through V2) and the inferior and lateral pulvinar are likely sources of these signals. Moreover, the contribution of inter-hemispheric projections from the intact cerebral hemisphere should not be underestimated. There was a greater reduction of response reported in area MT with sectioning of the corpus callosum in combination with reversible inactivation of V1 than with V1 inactivation alone (Girard et al., 1992). The investigation of inter-hemispheric projections may require studies with the extension of inactivation of V1 in both hemispheres to record the cone inputs and response latencies in area MT cells.
Chapter 7

Summary & Conclusions

The parvocellular and magnocellular streams of the visual pathway have been studied extensively in both Old World and New World primates (Wiesel and Hubel, 1966; Dreher et al., 1976; Sherman et al., 1976; Norden and Kaas, 1978; Schiller and Malpeli, 1978; Hicks et al., 1983; Derrington et al., 1984; Yeh et al., 1995; Martin et al., 1997; White et al., 1998; Blessing et al., 2004). However, the koniocellular stream has eluded thorough investigation in the Old World monkeys due to the unclear demarcation and relative thinness of the intercalated koniocellular layers within the dLGN. Studies in the New World marmoset have been more successful due to the broadness of the koniocellular layers between the main parvocellular and magnocellular layers in the dLGN of this animal (Martin et al., 1997; White et al., 1998; Solomon et al., 1999; White et al., 2001; Szmajda et al., 2006).

The studies in this thesis were aimed at determining the physiological properties of B-Y cells compared to R-G cells in the dLGN of the Old World macaque monkey. The conduction velocities of these cell types were also recorded. The distribution of B-Y cells within the macaque dLGN were investigated to determine whether these cells were segregated within the koniocellular regions, including the koniocellular layers and the koniocellular bridges that span the parvocellular layers.
The present study revealed that B-Y cells had a preference for lower spatial frequencies compared to R-G cells. They also had larger receptive fields at any given eccentricity compared to the R-G cells (Figure 4.15). The temporal preferences, orientation selectivities and contrast gains were similar between the two cell populations. The integrated sensitivity of the centre mechanisms for both cell types was found to be independent of the centre radius (see Figure 4.8).

The latencies to orthodromic stimulation of the B-Y cells overlapped considerably with those of the R-G cells (see Figure 4.22). A definite criterion to identify the cell groups using their latencies could not be determined. However, cells with longer latencies were more likely to be B-Y cells. This should be verified with a larger sample size.

Of remarkable interest was the finding that the B-Y cells seemed to be segregated to the koniocellular regions of the dLGN. The koniocellular bridges extending into the parvocellular layers were included as part of the koniocellular regions after they were demarcated through low-pass filtering using an image processing tool (see Figure 5.3). About three quarters of all B-Y cells were located in the koniocellular regions and of these more than half were within the koniocellular bridges. The only B-Y cells found ventral to K3 were also within the koniocellular layer K2. Hence, more detailed studies extending into the ventral layers (K1, M1, K2, M2) of the dLGN could ascertain whether a similar segregation of B-Y cells exists in the ventral layers also.

In contrast, the majority of R-G cells were located evenly throughout the parvocellular layers. The slight tendency for R-G On centre cells to be in the ventral two parvocellular layers compared to R-G Off centre cells was consistent with an earlier study (Schiller and Malpeli, 1978). However, very few R-G cells were located in the koniocellular regions. Hence, inclusion of these koniocellular bridges as part of the koniocellular regions revealed a
remarkable segregation of B-Y cells in the macaque to mainly koniocellular regions. Although there may be a convergence of the S-cone inputs with magnocellular signals at the level of the V1 cortical cells (Vidyasagar et al., 2002), the S-cone inputs remain largely segregated within the dLGN. Studies using a neurochemical marker together with the technique of low pass filtering can help to establish the validity of the low pass filtering method used to identify all koniocellular regions within the macaque dLGN. The functional role of the koniocellular regions to mainly contain B-Y signals can also be verified.

The koniocellular bridges were more visible in the middle parvocellular layers P3 and P4. This may be a consequence of an evolutionary development in the Old World macaque. The two extra parvocellular layers in the macaque may have extended ventrally into the main koniocellular layer from the primordial structure of only two parvocellular layers, one contralateral and one ipsilateral, seen in the New World marmoset. This would result in the high prevalence of koniocellular bridges from the original main koniocellular layer. The tendency for Off cells to be located in the middle layers (Schiller and Malpeli, 1978) is consistent with the large number of Blue On cells found in the middle laminae which mostly responded to decrements of achromatic luminance in the present study. The scarcity of B-Y cells in the dorsal parvocellular layers may be a result of the primordial arrangement of only a thin koniocellular layer which may have carried the S-cone signals in this region. The broad middle koniocellular region in the primordial arrangement, into which the parvocellular layers may have extended ventrally, could have contained more B-Y cells. This is also consistent with the greater number of B-Y cells within the middle regions of the dLGN found in the present study.

An early study revealing B-Y cells in the middle regions of the dLGN of the macaque (Schiller and Malpeli, 1978) may have recorded cells from
these koniocellular bridges as well as the thinly demarcated koniocellular layers. However, since only blue filters were used for the presentation of colour stimuli in the early study, luminance artifacts may have resulted in responses from achromatic cells within the parvocellular layers. A control for these artifacts was established in the present study (see Figure 4.7) with the knowledge that S-cone modulating stimuli are unlikely to cause more than 10% modulation of L-cones and M-cones (Sun et al., 2006b).

The B-Y cells within the koniocellular layers of the macaque dLGN are likely to receive inputs from the small bistratified ganglion cells in the retina, through which S-cone signals are exclusively carried (Dacey and Lee, 1994). In the marmoset it has been shown that the small bistratified cells project to koniocellular layer K3 of the dLGN (Szmajda et al., 2008). This layer contains the majority of cells receiving S-cone signals from which physiological properties of the koniocellular layers have been recorded (Martin et al., 1997; White et al., 1998, 2001; Szmajda et al., 2006). Hence, there may be similar terminations of the small bistratified ganglion cell carrying S-cone inputs into specific koniocellular regions of the dLGN. This supports the existence of a separate pathway for conveying S-cone signals in both Old and New World monkeys and may perhaps be a common functional feature for all primates.

The S-cone inputs of the B-Y cells that are mostly segregated to the koniocellular regions may be included in the direct anatomical projection bypassing V1 to area MT from the koniocellular layers of the dLGN (Sincich et al., 2004). Human psychophysical studies have shown that spatial attention may be augmented by S-cone input signals without enhancement from luminance increments (Li et al., 2007), further indicating a segregation of S-cone input signals through the koniocellular pathway to the dorsal visual stream. Fast S-cone signals to area MT have also been reported in a human psychophysical study using S-cone isolating stimuli (Morand et al., 2000). Hence, further studies in this thesis involved recording responses in area MT.
of the macaque while inactivating V1 to reveal whether S-cone inputs are carried directly to area MT. If this was the case, investigations could be carried out as to whether this is through a fast conduction pathway.

The findings from area MT recordings revealed a reduction in responses of some area MT cells during inactivation of V1, confirming the success of the methods used. However, all three cone inputs were unaffected by V1 inactivation in the majority of the cells. Area MT is known to receive inputs through V1 or via V2 and V3 from the magnocellular division of the dLGN (Allman and Kaas, 1971; Zeki, 1974; Maunsell and Van Essen, 1983a,b; Albright, 1984). Responses were noticeably reduced with inactivation of the magnocellular layers of the dLGN (Maunsell et al., 1990). The inactivation of V1 has been shown to reduce the responses of all cell types in the thick, thin and pale stripes of V2 (Schiller and Malpeli, 1977; Girard and Bullier, 1989). Hence, cooling with the Peltier device would have inactivated inputs to area MT from both the V1 cortical surface and the stripes of V2 adjoining the lunate sulcus (Shipp and Zeki, 1985; Ungerleider and Desimone, 1986b; Bullier and Girard, 1988; Shipp and Zeki, 1989; Levitt et al., 1994a) directly anterior to the placement of the Peltier device.

The survival of the S-cone inputs during V1 inactivation was consistent with the direct anatomical projection from the koniocellular layers of the dLGN to area MT reported in the macaque (Sincich et al., 2004). The presence of S-cone inputs seen in human psychophysical studies (Morand et al., 2000) was also supported by the preservation of both fast and slow latencies to flashing S-cone modulating stimuli. Another notable finding from this study was the maintenance of responses to L-cone and M-cone modulation during V1 inactivation. The source of these signals may provide the majority of inputs for blindsight (Sanders et al., 1974; Weiskrantz et al., 1974; Cowey and Stoerig, 1991; Kentridge et al., 1999; Sahraie et al., 2003, 2006).
Much of the perception in blindsight is related to low spatial frequencies and object motion (Weiskrantz et al., 1995; Sahraie et al., 2003). Some area MT cells have shorter response latencies than cells in V1 (Raiguel et al., 1989, 1999). It is possible that cells with short latencies which encode motion information may bypass V1 (Ifytche et al., 1995; Zeki, 1998). Some of these cells may be preserved in blindsight for the perception of motion. The majority of response latencies of cells to the stimuli yielding the maximum responses in the present study were faster than 35 ms. This may indicate some of these cells with short latencies in area MT that were unaffected by V1 inactivation received inputs which bypass V1. However, it has been suggested that cells with fast and slow conduction velocities may not necessarily be specific to fast and slow motion, respectively (Azzopardi et al., 2003). Latencies to slow and fast motion were not investigated in the present study. Investigation of this property in area MT cells during reversible inactivation of V1 may reveal a link between latency and motion.

The response latencies found in this study were comparable to a previous study of area MT (Bair et al., 2002). Although the present study had a range of latencies, there was a tendency for segregation of cells with response latencies above or below 70 ms (see Figure 6.22). The fast latency group contained cells with and without significant changes in responses during inactivation. However, all three cells with latencies longer than 70 ms were affected during V1 inactivation. This may suggest that cells with very slow latencies received projections through V1, whereas cells with fast latencies may or may not have bypassed V1. The latencies of cells both with and without a significant change in response during V1 inactivation were not seen to get longer during V1 inactivation.

The subcortical sources of the L-cone and M-cone inputs during V1 inactivation, present in cases without S-cone inputs, are yet to be identified. From findings in the first part of this thesis, it may seem unlikely to be through
koniocellular projection from the dLGN which carry predominantly S-cone signals. However, the inputs may still arrive from achromatic koniocellular neurons through this pathway. Magnocellular cells within the koniocellular layers may have a contribution, most notably from those cells in koniocellular layer K1 which are displaced ipsilateral cells from magnocellular layer M2 (Fitzpatrick et al., 1983; Yoshioka and Hendry, 1999). Cells projecting directly to area MT from the dLGN but not double labelled with α-CaM II kinase in the study by Sincich et al., (2004) may be conveying magnocellular and achromatic parvocellular inputs. There is additionally a minimal direct projection to V2 from the dLGN (Yukie and Iwai, 1981) and the pulvinar (Livingstone and Hubel, 1982) that could transmit a small proportion of inputs to area MT followed by extensive collaterals within area MT.

There have been reports of unaffected direction selectivity and binocularity in area MT with V1 inactivation alone and in combination with superior colliculus removal (Bullier and Girard, 1988; Girard and Bullier, 1989; Rodman and Albright, 1989; Rodman et al., 1989). The superior colliculus may not itself be the source of the preserved inputs but may augment other inputs to area MT via its projection to the inferior and lateral pulvinar (Benevento and Standage, 1983; Standage and Benevento, 1983; Stepniewska et al., 1999; Weller et al., 2002). Apart from the tectopulvinar projections, a likely source of inputs to area MT may be through the inter-hemispheric transfer of intact signals through the corpus callosum. Sectioning of this structure reportedly lead to further reduction of responses in area MT with V1 inactivation (Rodman et al., 1989; Girard et al., 1992).

Further physiological studies in area MT, possibly with the combined inactivation of dLGN and V1 together, may reveal other direct geniculo-cortical inputs to area MT. This may require the involvement of both hemispheres during inactivation of input sources to area MT to investigate the extent to which the corpus callosum aids in preserving the area MT responses. These
findings may be correlated with anatomical studies tracing projections from area MT to various subcortical regions apart from the dLGN to ascertain the sources of blindsight.
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