Subcortical Pathways for Colour Vision

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Abstract

Visual sub-modalities, such as colour, form and motion perception, are analysed in parallel by three visual “pathways” – the parvocellular (PC), magnocellular (MC) and koniocellular (KC) pathways. This thesis aims to further elucidate some properties of the subcortical pathways for colour vision. The experimental animal used throughout is a New World monkey, the common marmoset *Callithrix jacchus*.

Chapter 1 introduces the main anatomical and physiological properties of these parallel pathways.

Chapter 2 investigates mosaic properties of midget and parasol retinal ganglion cells. The functional specialization of these populations for colour and spatial vision was addressed, by comparing the mosaic of ganglion cells in dichromatic ("red-green colour blind") and trichromatic marmosets. Ganglion cells were labelled by photolytic amplification of retrograde tracer ("photofilling") following injections into the lateral geniculate nucleus, or by intracellular injection in an *in vitro* retinal preparation. The dendritic field size, shape and overlap of neighbouring cells were measured. Results show that in marmosets, both midget and parasol cells exhibit a radial bias, so that the long axis of the dendritic field points towards the fovea. The dendritic fields of midget ganglion cells from the same (ON or OFF) response-type array show very little overlap, consistent with the low coverage of the midget mosaic in humans. No large differences in radial bias, or overlap, were seen on comparing retinae from dichromatic and trichromatic animals. These data suggest that radial bias in ganglion cell populations is a consistent feature of the primate retina. Furthermore, they suggest that the mosaic properties of the midget cell population are associated with high spatial resolution rather than being specifically associated with trichromatic colour vision.

Chapter 3 investigates the anatomical segregation of the visual pathways at the level of the lateral geniculate nucleus. Ganglion cells were labelled from targeted injections of a retrograde tracer, and photofilled to reveal their dendritic morphology. After injections localised to the parvocellular layers of the geniculate (n=6), midget ganglion cells account for 95% of labelled retinal neurons; following an injection localised to the magnocellular layers, parasol ganglion cells were the most abundantly labelled neurons (79% of labelled cells). Injections that were >30% localised to the
konio cellular layers (n=4) labelled a higher proportion of widefield cells than any other injection (an average of 21%). Labelled widefield ganglion cell types included the small bistratified (n=31), sparse (n=13), and hedge (n=3) cells. Other cells with heterogeneous morphology were also labelled (n=9). This shows that a heterogeneous group of widefield ganglion cells project to the koniocellular layers of the lateral geniculate nucleus. Projection of small bistratified cells to the koniocellular layers is consistent with the idea that the koniocellular pathway is involved in the perception of blue-yellow colour.

Chapter 4 investigates directly the properties and location of blue-OFF responses in the subcortical visual pathway. In primates, signals for red-green color vision are carried together with signals for high-acuity spatial vision, by neurons in the parvocellular division of the subcortical pathway. It has been shown that ON-type signals for blue-yellow colour vision are carried by cells in a distinct, diffusely projecting (koniocellular) pathway, but the question whether blue-OFF signals are carried in the parvocellular or koniocellular pathway has not been resolved. Results detailed in Chapter 4 show that the blue-OFF responses, like blue-ON responses, are segregated to the koniocellular pathway. The blue-OFF cells show relatively large receptive fields, sluggish responses to maintained contrast, little sign of an inhibitory receptive field surround mechanism, and weak functional input from an intrinsic (melanopsin based) phototransductive mechanism. These properties are consistent with input from koniocellular or "W-like" ganglion cells in the retina, and suggest that blue-OFF cells, like blue-ON cells, form part of a primordial pathway for colour perception in primates.
**DECLARATION**

This is to certify that:

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

ii. Due acknowledgement has been made in the text to all other material used;

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

Brett Szmajda
Some of the results described in the present thesis have appeared in the following publications and abstracts:

Publications:


Abstracts:


The experiments for Chapter 2 were initiated during my Honours candidature and were completed during the first year of my PhD. During the course of my PhD, I also contributed to the following publications and abstracts, which involve experiments not described by this thesis:

Publications:

Abstracts:


ACKNOWLEDGEMENTS

No great work is accomplished alone. During the course of my PhD, I have become indebted to a number of people, all of whom have contributed to the work that you are reading.

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<td>angle relative to the fovea</td>
</tr>
<tr>
<td>ARO</td>
<td>angle relative to the optic disk</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>DAB</td>
<td>diaminobenzidine</td>
</tr>
<tr>
<td>DBD</td>
<td>dendritic branch density</td>
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<tr>
<td>DIC</td>
<td>differential interference contrast</td>
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<td>EEG</td>
<td>electroencephalogram</td>
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<td>GCL</td>
<td>ganglion cell layer</td>
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<td>HRP</td>
<td>horseradish peroxidase</td>
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<td>INL</td>
<td>inner nuclear layer</td>
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<td>IPL</td>
<td>inner plexiform layer</td>
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<tr>
<td>KC</td>
<td>koniocellular</td>
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<tr>
<td>L</td>
<td>long wavelength sensitive</td>
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<tr>
<td>LGN</td>
<td>dorsal lateral geniculate nucleus</td>
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<tr>
<td>M</td>
<td>medium wavelength sensitive</td>
</tr>
<tr>
<td>MC</td>
<td>magnocellular</td>
</tr>
<tr>
<td>ML</td>
<td>medium-long</td>
</tr>
<tr>
<td>NHMRC</td>
<td>National Health and Medical Research Council</td>
</tr>
<tr>
<td>PB</td>
<td>phosphate buffer</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate-buffered saline</td>
</tr>
<tr>
<td>PC</td>
<td>parvocellular</td>
</tr>
<tr>
<td>PSTH</td>
<td>peristimulus time histogram</td>
</tr>
<tr>
<td>S</td>
<td>short wavelength sensitive</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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Chapter 1

Introduction

In 1933, G.H. Bishop made recordings of compound action potentials from frog and rabbit optic nerve fibres. There were groupings of conduction velocity in these recordings, and Bishop suggested that the conduction velocity groupings might represent different types of cells. By analogy to the different types of somatosensory neurons, Bishop predicted that these different visual cell types might encode different aspects of the visual scene:

“In peripheral nerves a group of larger fibers mediates sensations of touch, including those permitting spatial discrimination, while a group of smaller fibers mediates pain and temperature ... The latter sensations are those predominantly characterized by an intensity factor. By analogy one might anticipate that the larger fibers of the optic nerve would also mediate that aspect of vision concerned with spatial discrimination or form, while the smaller fibers would be concerned with the quantitative factor of intensity.”

The hypothesis of parallel visual pathways has long since become embedded in the literature. The term ‘pathway’, in this sense, simply refers to a group of interconnected neurons. The two most common ways in which visual pathways have been defined are: anatomically, by examining the synaptic connections of subcortical visual neurons (examining how the visual system is ‘wired up’); or physiologically, by examining the differing response properties of cells (specifically, the properties of their receptive field – the region of visual space to which a cell responds).

The aim of this thesis is to elucidate further some properties of the subcortical pathways for colour vision. In this introduction, the anatomy of the early visual system will be discussed; this will be followed by a summary of the peculiarities of visual receptive fields. Next examined will be studies of parallel pathways in mammalian, non-primate visual systems; these will then be compared to the current knowledge of parallel pathways in primates.
1.1 Structure of the Subcortical Visual System

Figure 1.1 shows a schematic drawing of the primate retina, from Polyak (1941). Visual processing begins at the level of the photoreceptors, which convert the light signal into an electrical potential (for a review, see Baylor, 1996). There are four different types of photoreceptor in trichromatic primates, including humans: three types of ‘cone’ and one type of ‘rod’. Other primates (and nearly all other mammals) are dichromatic, meaning that they have two different types of cone; or monochromatic, meaning that they possess only one cone type.

Each photoreceptor type contains a different opsin – a protein that undergoes a conformational change in response to light – and each opsin type is sensitive to photons of a different range of wavelengths. Rods are further specialised for scotopic (night) vision and will not be considered here. In species with two or more cone types, the different spectral sensitivities of the different cone photoreceptors lay the foundation for colour vision: comparing the outputs of multiple cones means that wavelength disparities can potentially be disambiguated from changes in luminance.

Photoreceptors make synapses with bipolar cells and horizontal cells (Polyak, 1941; Boycott and Dowling, 1969). Selective wiring at the photoreceptor-bipolar synapse begins the segregation of visual signals into parallel pathways. Bipolar cells then make synapses with ganglion cells and amacrine cells (Polyak, 1941; Boycott and Dowling, 1969). The photoreceptor-bipolar and bipolar-ganglion cell synapses are modulated by (mostly inhibitory) inputs from horizontal and amacrine cells, respectively (for review, see Dacey, 1999).

Ganglion cells are the output neurons of the retina: their axons exit the eye via the optic nerve and project to a number of subcortical visual structures, including the superior colliculus, the pretectum, the suprachiasmatic nucleus, and the accessory optic nuclei (Rodieck and Watanabe, 1993; for a review, see Wässle, 2004). However, in primates, most ganglion cells (around 80%: Perry et al., 1984; Ahmad and Spear, 1993) project to a visual division of the thalamus: the dorsal lateral geniculate nucleus (LGN). From here, most axons project to the primary visual cortex (V1), and on to higher brain centres.
Figure 1.1. Outline of the cellular structures of the primate retina. (a) rods; (b) cones; (c) horizontal cells; (d-h) bipolar cells; (i-l) amacrine cells; (m-s) ganglion cells. Nomenclature for each retinal layer is indicated on the right. Modified from Polyak (1941).
This thesis examines cell properties at the geniculate and ganglion cell level. These structures are well-studied, and there is a partial segregation of the parallel pathways at these levels, as will be discussed in Section 1.4.

### 1.2 Receptive Fields of Visual Neurons

The receptive field of a visual neuron refers to the region in space that can elicit a change in the discharge rate of this cell (Kuffler, 1953). Two broad classes of visual cells are easily defined: those that respond to increments of light within their receptive field (ON cells), and those that respond to decrements of light within their receptive field (OFF cells). Furthermore, the receptive field of both ON and OFF cells has a characteristic structure, with an excitatory ‘centre’ region and a larger, concentric, inhibitory ‘surround’ region (Kuffler, 1953; Hubel and Wiesel, 1960). An example of this centre-surround receptive field structure is shown in Figure 1.2. The cell in Figure 1.2 is an ON cell, since the cell's response rate increases when light falls in the centre region of the receptive field, and responses are inhibited when light falls in the surround region. Kuffler's work in cat was expanded upon by De Valois (1965) and Wiesel and Hubel (1966), who studied the macaque, a trichromatic primate.

De Valois (1965) studied the chromatic properties of the receptive fields of LGN neurones. He found that LGN cells could be divided into two categories, based on their response to coloured stimuli: around a third were nonopponent, meaning they showed no chromatic response; these cells could be split into the two (ON and OFF) categories defined by Kuffler (1953). The remaining two-thirds of cells were spectrally opponent, meaning they exhibited an increased response to light of certain wavelengths, and a decreased response to light of other wavelengths. Based on this, spectrally opponent cells could be split into four different classes: red excitatory, green inhibitory (what De Valois called +R -G); green excitatory, red inhibitory (+G -R); blue excitatory, yellow inhibitory (+B -Y); and yellow excitatory, blue inhibitory (+Y -B).
Figure 1.2. Centre-surround receptive field structure of visual neurons. (A) The receptive field of visual neurons consists of an excitatory centre region, with a larger, concentric, inhibitory surround. The cell modelled here is an ON cell. An increment of light in the regions 1-5 will elicit the corresponding response shown in (B). An increment of light at positions 1 or 5 causes no change in the firing rate of the cell, as both positions are outside the receptive field. Positions 2 and 4 fall within the inhibitory surround, and thus an increment of light at either of these positions will decrease the firing rate of the cell. Position 3 falls within the centre region, and thus an increment of light at this position causes an increase in the firing rate of the cell. (C) The spatial response of visual neurons can be modelled by a difference-of-gaussians (DOG) model, with a typical output shown here. Modified from Wässle (1993).
Wiesel and Hubel (1966) expanded upon this work by defining several receptive field 'types', which differ in the spatial and/or chromatic organisation of their centre and surround subunits. Type I cells had a centre-surround organisation (like Kuffler’s cells), with the two regions receiving spatially and chromatically antagonistic input. The majority of type I cells had red-green opponent inputs. Type II cells also have chromatic opponency, but have a centre and surround that are spatially coextensive. Type II cells consisted mostly of blue-yellow opponent cells. Type III cells had receptive fields that showed spatial antagonism, but no chromatic antagonism. Finally, Type IV cells were similar to Type III cells, except that they demonstrated a chromatic input to the receptive field surround. Large spots of long-wavelength (red) light suppressed the spontaneous firing of Type IV cells, with cell activity ceasing completely for high-intensity red light (see also: Smith et al., 1992).

What is the anatomical basis of the physiological receptive field? It is probable that the two different components (centre and surround) are mediated by two separate retinal mechanisms. The receptive field surround appears to be mediated by inhibitory interneurons in the retina: the horizontal cells and amacrine cells. In experiments in the rabbit, Mangel and Miller (1987) showed that direct current injection into a horizontal cell changes the firing rate of ganglion cells within the horizontal cell’s receptive field (see also: Mangel, 1991; Martin, 1998). Amacrine cells also contribute to the receptive field surround, to a lesser degree (Flores-Herr et al., 2001).

Regarding the receptive field centre, Peichl and Wässle (1981) used intraocular recording techniques to record the receptive field centre size of ganglion cells in the cat retina. They then investigated the morphology of recorded ganglion cells, which they labelled with a neurofibrillar stain after eye removal. Recorded cells were found using small lesions made while recording, and also by comparing the blood vessel pattern in the stained tissue to the blood vessel pattern seen through an ophthalmoscope whilst recording. From this study, they found that the dendritic field size of ganglion cells is of similar magnitude to their receptive field size, implying that the direct excitatory pathway (cones to bipolar cells to ganglion cells) is the probable foundation of the centre response, and that ganglion cell dendritic field size is the anatomical correlate of the physiological receptive field centre size.
Since the direct excitatory pathway, from cones to ganglion cells, is responsible for the receptive field centre response, it follows that the anatomical connectivity of the inputs to a particular ganglion cell type – the cones and bipolar cells – will determine the physiological properties of that ganglion cell type. Ganglion cells with different connectivity should have different physiological properties. This is a foundation of parallel pathways: the specificity of a visual pathway to certain sub-modalities of vision (such as motion, colour, or form) is built up through specific connections to certain inputs, or alternately, by degree of convergence of inputs. The genesis of this idea came from physiological and anatomical work in the cat, which will be discussed next.

1.3 Parallel pathways in mammalian vision

Clear evidence of different functional types of retinal ganglion cell was demonstrated in cat in the 1960s. The first evidence came from Enroth-Cugell and Robson (1966), who demonstrated two classes of cells with a centre-surround receptive field distribution: there were cells that summated light linearly over their receptive fields (termed X cells), and cells that summated light in a non-linear fashion over their receptive fields (termed Y cells). To clarify this distinction: an increase in quantal flux at any position in the receptive field centre can be exactly balanced by a simultaneous decrease in flux at a different position. This will yield no net change in the action potential discharge rate of X cells (as the integral flux is equal to the mean light level), whereas the Y cell will always respond to the presentation of a stimulus, regardless of the phase.

Both X and Y cells were defined as brisk-responding by Cleland et al. (1971), who further showed that X cells show a tonic response to presented stimuli (their firing is sustained while a preferred stimulus is present), while Y cells show a phasic response to presented stimuli (for a maintained stimulus, they respond transiently, and then return to their baseline responsivity). Boycott and Wässle (1974) expanded this definition into the anatomical realm, by suggesting that the β- and α-ganglion cell types were the morphological correlates of the of the X and Y physiological typings, respectively; a hypothesis that was later proven to be correct (Cleland et al., 1975; Peichl and Wässle, 1981; Saito, 1983). Boycott and Wässle’s paper thus formed the basis of an idea.
mentioned in Section 1.2: that the anatomical connectivity of cells influences their physiological properties.

X cells are currently estimated to make up approximately 50% of cat ganglion cells, and Y cells approximately 5% (for review, see Troy and Shou, 2002). What about the remaining 45% of cat ganglion cells? These were found to be made up by a number of different cell types, which have non-standard receptive field properties (Stone and Fabian, 1966; Rodieck, 1967; Cleland and Levick, 1974b). Some features that these non-standard cells have in common are mostly sluggish temporal response properties, and many have a non centre-surround receptive field organization. A number of ganglion cell types fit these criteria: these were grouped under the umbrella term ‘W’ cells (Stone and Hoffmann, 1972). Since the X and Y designations refer to a single type of cell, while the W cell class is composed of multiple types of cells, the more general term W-group cells will be used to refer to these cells hereinafter. Following the terminology of Rodieck and Watanabe (1993), the term “group” refers to a collection of cell types with some common cell features.

W-group cells have since been found to encompass a large number of physiological types – at least seven, in the cat retina. Three of these cell types have a centre-surround receptive field structure: the colour coded cell (which responds antagonistically to blue vs. green light: Cleland and Levick, 1974b; Rowe and Stone, 1976), and the sluggish-sustained and sluggish-transient cell types (whose receptive field properties are implied by the names: Cleland and Levick, 1974a; Stone and Fukuda, 1974; Rowe and Stone, 1976). The remaining four types have a non-standard receptive field organisation: two types of direction selective cell (Stone and Fabian, 1966; Cleland and Levick, 1974b; Stone and Fukuda, 1974); a ‘suppressed by contrast’ cell type, which has a high basal level of firing that is attenuated upon stimulus presentation (Rodieck, 1967; Cleland and Levick, 1974b; Stone and Fukuda, 1974); and, finally, the ‘local edge detector’ cell type, which are primarily characterised by transient ON-OFF responses to stimuli presented anywhere in their receptive fields, low or zero maintained discharge in the absence of stimulation, and an insensitivity to large target stimuli (Stone and Hoffmann, 1972; Cleland and Levick, 1974b; Stone and
Fukuda, 1974). Table 1.1 summarises the receptive field properties of the cat, with comparison to rabbit and macaque.

**Table 1.1: Identified Receptive Field Types of the Cat, Rabbit, and Macaque**

<table>
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<th>Receptive field type</th>
<th>Cat*</th>
<th>Rabbit</th>
<th>Species</th>
<th>Macaque</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brisk sustained (X-like)</td>
<td>Refs 14; 5; 6</td>
<td>Refs 2; 4; 11</td>
<td>Refs 31; 15; 8; 13; 23; however, see section 1.5</td>
<td></td>
</tr>
<tr>
<td>Brisk transient (Y-like)</td>
<td>Refs 14; 5; 6</td>
<td>Refs 2; 4; 11</td>
<td>Refs 31; 15; 8; 13; 23; however, see section 1.5</td>
<td></td>
</tr>
<tr>
<td>Colour (B-Y)</td>
<td>Refs 7; 22</td>
<td>Ref 4</td>
<td>#</td>
<td>See brisk-sustained. Also, refs 10; 12; 17</td>
</tr>
<tr>
<td>Colour (R-G)</td>
<td>N/A</td>
<td>N/A</td>
<td>#</td>
<td></td>
</tr>
<tr>
<td>Direction selective</td>
<td>Refs 24; 7; 26</td>
<td>Refs 3; 18; 19; 20; reviewed in ref 28</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Local edge detector</td>
<td>Refs 25; 7; 26</td>
<td>Refs 18; 20; 1; 4</td>
<td>Refs 8; 9</td>
<td></td>
</tr>
<tr>
<td>Sluggish-sustained</td>
<td>Refs 6; 26; 22</td>
<td>Refs 4; 2</td>
<td>#</td>
<td></td>
</tr>
<tr>
<td>Sluggish-transient</td>
<td>Refs 6; 26; 22</td>
<td>Refs 4; 11; 2</td>
<td>#</td>
<td></td>
</tr>
<tr>
<td>Suppressed-by-contrast</td>
<td>Refs 21; 7; 26</td>
<td>Refs 18; 4; 1</td>
<td>Ref 24</td>
<td></td>
</tr>
</tbody>
</table>

*Cat receptive fields reviewed in ref 29.

References for Table 1.1:

1. (Amthor et al., 1989a)
2. (Amthor et al., 1989b)
3. (Barlow et al., 1964)
4. (Caldwell and Daw, 1978)
5. (Cleland et al., 1971)
6. (Cleland and Levick, 1974a)
7. (Cleland and Levick, 1974b)
8. (De Monasterio and Gouras, 1975)
9. (De Monasterio, 1978)
10. (De Valois, 1965)
11. (DeVries and Baylor, 1997)
12. (Derrington et al., 1984)
13. (Dreher et al., 1976)
14. (Enroth-Cugell and Robson, 1966)
15. (Gouras, 1968)
16. (Hendry and Reid, 2000)
17. (Lee et al., 1990)
18. (Levick, 1967)
19. (Oyster and Barlow, 1967)
20. (Oyster, 1968)
21. (Rodieck, 1967)
22. (Rowe and Stone, 1976)
23. (Shapley and Perry, 1986)
24. (Stone and Fabian, 1966)
25. (Stone and Hoffmann, 1972)
26. (Stone and Fukuda, 1974)
27. (Tailby et al., 2005)
28. (Taylor and Vaney, 2003)
29. (Troy and Shou, 2002)
30. (Valberg et al., 1986)
31. (Wiesel and Hubel, 1966)

A summary of the morphological homologues of each of the cat receptive field types can be found in Figure 1.3A. For comparison, the ganglion cells of the rabbit retina are shown in Figure 1.3B; known macaque ganglion cell types are shown in Figure 1.4. In cat, it is known that β- and α-ganglion cells are the morphological correlates of the X and Y receptive field types, as described earlier. δ- and ε-cells have been shown to have a sluggish-sustained receptive field type (δ-cells are OFF-responding, ε-cells are ON-responding: Leventhal et al., 1980; Stanford, 1987; Wässle...
et al., 1987; Dacey, 1989; Pu et al., 1994). There may also be other morphologies associated with the sluggish-sustained response type. \( \eta \)-cells are thought to represent the OFF-variety of sluggish-transient cells (Berson et al., 1999a); the morphological correlate of ON-sluggish-transient cells is unknown. Finally, the \( \zeta \)-, \( \theta \)-, and \( \lambda \)-cells all show physiological properties like the previously defined “local edge detector” cell type (Berson et al., 1998; Isayama et al., 1999; Isayama et al., 2000); and the \( \iota \)- and \( \kappa \)-cells are currently presumed to be the direction selective ganglion cells of the cat retina (Berson et al., 1997; Berson et al., 1999b).

In summary, ganglion cells from the cat retina can be sorted into types based on their functional properties; each physiological type described thus far arises from at least one morphologically distinct type of ganglion cell. The functional properties of these ganglion cells appear to be well conserved: the rabbit retina contains all of the major receptive field classes just described for the cat retina, as detailed in Table 1.1, and there are a number of morphologically and physiologically similar cell types in other mammalian species (like the mouse and the rat) that have not been covered here (see, for example: Hale et al., 1979; Peichl, 1991; Stone and Pinto, 1993). It is thus attractive to consider that there is a ‘blueprint’ of receptive field functionality, which is conserved between species. However, note that there are a number of receptive field types that are present in both the cat and the rabbit, but absent from the primate. The following section will examine the how the receptive field properties and morphology of primate retinal ganglion cells compares to that of the cat and rabbit.
Figure 1.3. Known retinal ganglion cell types in the cat and rabbit, and their presumed functional roles. A shows camera lucida drawings of ten known cat ganglion cell classes, and their groupings into distinct functional classes (indicated by the blue boxed text); these functional classes are described in Section 1.3. Cells are presented in wholemount view - as if looking down onto a flat-mounted retina. B shows a vertical view of the 11 different ganglion cell types found in the rabbit retina, with functional roles in green boxed text underneath the respective cell class (reviewed in Rockhill et al., 2002). Rabbit G3 cells are presumed to be the colour (BY) cells of the rabbit, based on their bistratified morphology, however this has not been confirmed with electrophysiological recordings. Cells in the cat and the rabbit demonstrate a general principle of ganglion cells: different morphological types of cell usually have different physiological properties. Modified from O’Brien et al., 2002 and Rockhill et al., 2002.
A. Cat

- Alpha
  - Brisk transient

- Beta
  - Brisk sustained

- Epsilon
  - Sluggish transient

- Delta
  - Sluggish sustained

- Zeta
  - Local edge detector

- Iota
  - Direction selective

B. Rabbit

- Local edge detector
- Colour (BY)?
- Brisk sustained
- Suppressed-by-contrast
- Direction selective
- Direction selective
- Brisk transient
Figure 1.4. Known retinal ganglion cell types in the macaque, and their presumed functional roles. Wholemount camera lucida drawings of the twelve known ganglion cell types are shown. Functional roles are indicated in red boxed text underneath the respective cell class; these functional types are described in Section 1.4. The hypothesised direction selective ganglion cell types are also shown - the recursive monostratified and bistratified cells. The physiological response profile of the recursive cell types has not been demonstrated, but they have similar morphology and coverage to the direction selective cells in other species. Modified from Dacey, 2004.
1.4 **Parallel Pathways in Primate**

This section will describe the three known retinogeniculate cell pathways that are described in all simian primates studied so far, including humans: two brisk (parvocellular, magnocellular) pathways, and one sluggish (koniocellular) pathway. Each sub-section will first outline the physiology of a ‘typical’ ganglion or LGN cell from this pathway. (Unless otherwise specified, when referring to the properties of neurons in a particular pathway, these physiological properties refer to cells at both the ganglion cell level, and the LGN level). Following the discussion of the physiology, the anatomical specialisations that are responsible for these functional response types are discussed. Comparisons between primate and other mammalian retinas will be discussed in Section 1.5.

In this section, pathways are named after the different laminae of the LGN. This is keeping with the convention of other authors, and is based on the principle that specific ganglion cell types project exclusively to certain divisions of the LGN. This is a convenient simplification: there is not a complete anatomical segregation of the parallel pathways at the level of the LGN. For example, the projection to the parvocellular layers is probably heterogeneous (Rodieck and Watanabe, 1993), though the dominant input is from the midget ganglion cell type, as described below (Section 1.4.1). Furthermore, koniocellular cells – labelled by a CaM II kinase immunostain – are found scattered throughout the parvocellular layers (Hendry and Yoshioka, 1994). Thus, what follows is a description of the physiological properties of the majority of cells in each pathway.

1.4.1 The Parvocellular Pathway

The parvocellular pathway accounts for 80% of retinal ganglion cell projections to the macaque LGN (Perry et al., 1984; Ahmad and Spear, 1993). Electrophysiological recordings from parvocellular neurons have shown that they have a number of characteristics that distinguish them from neurons of other pathways (Wiesel and Hubel, 1966; De Monasterio and Gouras, 1975; Dreher et al., 1976; Shapley and Perry, 1986; for reviews, see Lee, 1996 and Dacey, 2000). Parvocellular neurons exhibit tonic responses, and have a relatively small receptive field size, compared to magnocellular
and koniocellular cells. Some are red-green colour-opponent, meaning that they respond antagonistically to medium versus long-wavelength light. Parvocellular ganglion cells also have relatively slow axonal conduction velocities (approximately 2 m/s), compared to magnocellular cells (approximately 4 m/s: Gouras, 1969).

Parvocellular cells are thought to underlie our perception of red-green colour. In addition, the small receptive field size of parvocellular neurons (and their sensitivity to high spatial frequencies) means that this pathway is probably also responsible for our ability to discriminate fine detail. The role of the parvocellular pathway in both red-green colour discrimination and spatial vision is supported by lesion studies in macaque (Merigan and Eskin, 1986; Merigan, 1989; Schiller et al., 1990; Merigan et al., 1991). These studies have been done in two ways: either by oral administration of acrilamide monomer, which is selectively toxic to parvocellular cells (Merigan and Eskin, 1986; Merigan, 1989); or by injecting ibotenic acid into the LGN, which obliterates cells in the region of the injection site (Schiller et al., 1990; Merigan et al., 1991). Following parvocellular lesions, a 3-4 fold reduction in visual acuity is reported, as well as an approximately 1 log unit drop in chromatic sensitivity. However, note that it is unclear whether acrilamide monomer also affects koniocellular cells; additionally, ibotenic acid injections would also have lesioned the intercalated koniocellular layers of the LGN in ‘parvocellular-only’ lesions.

The physiological properties of ganglion cells are influenced by anatomical specialisations of the ganglion cells, and the bipolar cells they receive input from. To demonstrate how this applies to the parvocellular pathway, a diagram of the retinal connectivity of the parvocellular pathway is provided in Figure 1.5A and B. There are three types of cone photoreceptor: long- (L), medium- (M), and short-wavelength sensitive (S). The parvocellular pathway begins where L and M cones contact midget bipolar cells. Midget bipolar cells then make synapses with midget ganglion cells. Close to the fovea (the point of maximum visual acuity), midget bipolar cells contact only one cone per bipolar cell, and midget ganglion cells contact only one bipolar cell per ganglion cell (Figure 1.5A; Polyak, 1941; Boycott and Dowling, 1969; Kolb, 1970; Kolb and Dekorver, 1991; Calkins et al., 1994).
Figure 1.5. Retinal connectivity of the three well-understood visual circuits. (A) The central (‘private-line’) connectivity of the parvocellular pathway. Each L or M cone is contacted by an ON and OFF midget bipolar cell, which then contact an ON and OFF midget ganglion cell, respectively. Since there is no convergence in this pathway, chromatic information is preserved. (B) The peripheral connectivity of the parvocellular pathway. Note that if random wiring is assumed, the increase in convergence causes a loss of chromatic information. (C) The magnocellular pathway. Diffuse bipolar cells contact multiple cones. Parasol ganglion cells then sum from multiple diffuse bipolar cells. (D) The blue-ON/yellow-OFF pathway (thought to be a subset of the koniocellular pathway). The small bistratified cell receives differing input to its inner (ON) and outer (OFF) tiers: the blue cone bipolar, and diffuse bipolar cells, respectively. The differing cone inputs of the blue cone bipolar cell and the diffuse bipolar cell gives the small bistratified ganglion cell its unique blue-ON/yellow-OFF physiology. Modified from Martin (1998).
This ‘private line’ connection means that foveal ganglion cells will have a chromatic response similar to the (L or M) cone that they receive input from. Thus, it is easy to see a simple reason for the red-green chromatic selectivity of foveal parvocellular neurons: these private line connections mean that the receptive field centre(s) of midget bipolar and midget ganglion cell will have the same chromatic properties as the cone that they are connected to, making them cone-selective by default. The physiological ramifications of this are established quantitatively in the random wiring model (Lennie et al., 1991; Dacey and Packer, 2003), which suggests that red-green opponency is inevitable in a cell that has a centre that is composed of only one L or M cone, even when the surround is drawn indiscriminately from a mix of L and M cones.

More peripherally (further from the fovea), the dendritic field size of midget ganglion and midget bipolar cells increases (see Figure 1.5B). Thus, there is potentially greater convergence at the midget bipolar and midget ganglion cell level – each midget bipolar cell will contact more cones, and each midget ganglion cell will contact more midget bipolar cells (Boycott and Dowling, 1969). This leads to two possibilities. One possibility is that the midget bipolar cells, and midget ganglion cells, might indiscriminately contact cells within their dendritic field, leading to mixing of cone-specific colour information (this would be the prediction of the random wiring model, as it assumes no specificity of contacts between L and M cones). If there is a mixture of cone inputs to both the centre and the surround, red-green opponency will be destroyed (Lennie et al., 1991). The alternate proposition is that there is a segregation of L and M signals to specific sub-classes of midget bipolar and midget ganglion cells (the ‘selective wiring’ hypothesis). In this case, the ‘purity’ of the centre is conserved – the centre receives input from only L or M cones – meaning that the cell will be red-green opponent, as described above. Both the random and selective wiring hypotheses have some support: there are a number of physiological, anatomical and psychophysical studies which imply red-green spectral mixing in the peripheral retina, supporting random wiring (Derrington et al., 1984; Mullen and Kingdom, 1996; Diller et al., 2004; Mullen et al., 2005; Jusuf et al., 2006a); and there are, likewise, studies implying a specificity in red-green connections in peripheral retina (De Monasterio and Gouras,
1975; Abramov et al., 1991; Yeh et al., 1995b; Lee et al., 1998; White et al., 1998; Martin et al., 2001; Reid and Shapley, 2002; Blessing et al., 2004; Solomon et al., 2005; Vakrou et al., 2005).

Having considered the anatomical connectivity of this cell class, one can anticipate the receptive field structure of parvocellular ganglion cells. In a selective wiring system, the receptive field structure of parvocellular ganglion cells would resemble the “Type 1” receptive field structure of Wiesel and Hubel (1966), containing a centre and surround that is both spatially and chromatically opponent. The centre and surround would each receive input from only one cone type, and this would not change from central to peripheral retina. However, in a random wiring system, the receptive field structure of parvocellular ganglion cells would be more varied: cells might have mixed centres or surrounds, or both (except in the fovea, where the centre mechanisms are derived from the inputs from only one cone). Chapter 2 of this thesis addresses this question of random versus selective wiring, by examining the morphology of midget ganglion cells. Specifically, the question addressed is whether the coverage and dendritic field orientation of the midget ganglion cell mosaic is different in dichromatic (red-green colour blind) and trichromatic (colour normal) marmoset monkeys. The hypothesis is that any morphological specialization related specifically to red-green colour vision should be present only in trichromatic animals.

1.4.2 The Magnocellular Pathway

The magnocellular pathway comprises approximately 10% of the retinal input to the geniculate (Perry et al., 1984; Ahmad and Spear, 1993). Magnocellular neurons usually respond in a phasic manner; the axonal conduction speed of magnocellular ganglion cells is relatively rapid, compared to parvocellular neurons (as discussed above, in Section 1.4.1); and magnocellular receptive fields are relatively large – at least double the receptive field size of parvocellular receptive fields at equivalent eccentricity (Wiesel and Hubel, 1966; De Monasterio and Gouras, 1975; Dreher et al., 1976; Shapley and Perry, 1986; for reviews, see Lee, 1996 and Dacey, 2000). The receptive field structure of most magnocellular neurons resembles the “Type 3” or “Type 4” neurons of Wiesel and Hubel’s (1966) original classification: they have a typical centre-surround receptive field structure, which is not chromatically opponent at high temporal
frequencies. However, electrophysiological recordings at low temporal frequencies have shown that while the receptive field centre of magnocellular cells probably sums inputs from both L and M cones, the surround most likely has a chromatic input (Smith et al., 1992).

Magnocellular cells are thought to have a role in motion, flicker, and luminance detection. It has also been argued that the magnocellular pathway might also be responsible for high-acuity vision at low contrasts (Lee, 1993), as magnocellular cells have a similar spatial resolution to parvocellular neurons, despite larger receptive field sizes (Blakemore and Vital-Durand, 1986; Crook et al., 1988). Ibotenic acid lesions of the magnocellular layers of the LGN cause a deficit in high-temporal frequency flicker and motion perception (Schiller et al., 1990; Merigan et al., 1991). Note that, like the parvocellular lesions with ibotenic acid, there would also have been lesioning to the intercalated koniocellular layers of the LGN in what these authors called ‘magnocellular-only’ lesions.

Like the parvocellular pathway, the magnocellular pathway is anatomically segregated from the first retinal synapse, as shown in Figure 1.5C. The first synapse in the magnocellular pathway is from cones to diffuse bipolar cells. Of the six main types of diffuse bipolar cell (DB1-DB6), it is thought up to four of these types (DB2-DB5) are members of the magnocellular pathway. DB2 and DB3 are OFF-type bipolar cells; DB4 and DB5 are ON-type bipolar cells (Boycott and Wässle, 1991). All four of these diffuse bipolar cell types receive input from all cones (Hopkins and Boycott, 1995), though this input would naturally be biased towards M and L cones (since S-cones make up only 10% of the cone population in primates: Martin and Grünert, 1999). The axons of DB2 and DB3 cells contact OFF parasol ganglion cells, and the axons of DB4 and DB5 cells are thought to contact ON parasol cells (for a review, see Wässle, 1999).

Even close to the fovea, parasol ganglion cells are thought to contact multiple diffuse bipolar cells, due to their large dendritic field size. Diffuse bipolar cells contact 5-10 cones within their dendritic field (Boycott and Wässle, 1991). This convergence of inputs at both the cone-bipolar and bipolar-ganglion cell synapses means that magnocellular cells are less likely to carry a cone-selective signal (since it is likely that
they will receive input from different classes of cones). This could help to explain why magnocellular cells prefer luminance over colour stimuli, as mentioned above.

1.4.3 The Koniocellular Pathways

The koniocellular pathways account for 10% of ganglion cell projections to the primate LGN (Solomon, 2002). Originally, cells in the koniocellular layers of the LGN were presumed to be interneurons (Le Gros Clark, 1941). Only in the last fifteen years has it become apparent that cells in the koniocellular layers of the LGN may be responsible for some aspects of our visual perception, including the processing of the blue-yellow axis of colour vision (for reviews, see Casagrande, 1994; Hendry and Reid, 2000).

The anatomical and physiological properties of cells belonging to the koniocellular pathways are studied in Chapter 3 and Chapter 4; the introduction and discussion of these chapters provides a detailed examination of the morphology and physiology of koniocellular neurons, and thus these will only be briefly considered here.

The koniocellular pathways are thought to be composed of a heterogeneous collection of cells, in contrast to the parvocellular or magnocellular pathways, which are dominated by a single class of cell. Evidence of this is found in the heterogeneity of spatial, chromatic and temporal properties, in cells recorded from the koniocellular layers of the prosimian *Galago* (Norton and Casagrande, 1982; Irvin et al., 1986) and a New World primate, the marmoset (White et al., 2001). Koniocellular cells are distinguishable by their sluggish temporal properties, much like the *W-group* of cat ganglion cells described in Section 1.3. Koniocellular cells also tend to have larger receptive fields than either parvocellular or magnocellular cells (in *Galago*, mean centre size of koniocellular cells is approximately double that of magnocellular cells); and low peak and maintained discharge rates (in marmoset, most koniocellular cells had <60 impulses/sec peak and <5 impulses/sec maintained). In *Galago*, many koniocellular cells also respond to either aural or tactile stimuli (Irvin et al., 1986; Casagrande, 1994); it is not known whether the same holds true in marmoset.

Due to the heterogeneity of physiological responses, it is likely that the koniocellular pathways are responsible for processing multiple visual sub-modalities (hence why they are referred to here as the koniocellular *pathways*, plural). The responses of koniocellular cells to auditory or tactile stimuli hint that a koniocellular...
pathway might be involved in polysensory integration. Based on a direct projection of koniocellular cells to visual area V5 (Morand et al., 2000; Sincich et al., 2004; Barberini et al., 2005), a koniocellular pathway might also have a role in rapid motion processing, and blindsight (a condition where blind people can visually sense objects without consciously ‘seeing’ them). However, the best-supported koniocellular pathway is a pathway involved in the processing of the blue-yellow axis of colour vision.

Blue-ON/yellow-OFF responses have been demonstrated in the small- and large-bistratified ganglion cells (see Figure 1.4: Dacey and Lee, 1994; Dacey et al., 2003). The anatomical connections of the large bistratified cell are unknown. The anatomy of the small bistratified cell is better-studied, and suggests that the blue-ON/yellow-OFF physiology is caused by the different cone-specific connections of each dendritic tier (Figure 1.5D). It is known that each of the two dendritic tiers receives input from a separate bipolar cell type: the inner tier (closer to the ganglion cell layer) receives input from the blue-cone bipolar cell, and diffuse bipolar cell(s) (most probably DB2/DB3) provide input to the outer tier (closer to the inner nuclear layer: Ghosh et al., 1997; Calkins et al., 1998; Ghosh and Grünert, 1999). The blue-cone bipolar cell selectively contacts S-cones (Mariani, 1984; Kouyama and Marshak, 1992). The DB3 cell is assumed to receive a mixed L/M cone input.

Thus, the small bistratified ganglion cell receives a blue-ON signal to its inner tier, and receives a yellow-OFF signal to its outer tier. It is likely that a similar situation is true for the large bistratified cell also. These two cell types are presumed to project to the koniocellular layers of the LGN, because cells with a blue-ON/yellow-OFF signature tend to be recorded from the koniocellular layers of the marmoset (Martin et al., 1997). However, the projection of small bistratified cells has not yet been analysed. Part of the work described in Chapter 3 aimed to anatomically test the prediction that small and large bistratified ganglion cells project to the koniocellular layers of the LGN.

The koniocellular pathways are also segregated in their projection to the primary visual cortex (V1). It is well established that koniocellular axons project to the more superficial layers of cortex (in Brodmann’s (1905) terminology, layers 1-4A), while the primary projection of parvocellular and magnocellular axons are layers 4Cβ and 4Cα, respectively (Livingstone and Hubel, 1982; Fitzpatrick et al., 1983; Lachica and
Casagrande, 1992; Hendry and Reid, 2000). Recent evidence indicates that the koniocellular projection to cortex might be heterogeneous, as at least three separate axon populations are found in the superficial layers of V1 following koniocellular injections (Casagrande et al., 2007).

Recordings made in V1 from LGN afferents (Chatterjee and Callaway, 2003) show discrete populations of colour-sensitive neurons that are localised to specific cortical layers: red-green cells are found in layer 4Cβ, achromatic cells in 4Cα, blue-ON cells in lower layer 2/3 and upper 4A, and blue-OFF cells in layer 4A only. Cells that are blue-OFF responding are seldom reported in the LGN (Hubel and Wiesel, 1960; Derrington et al., 1984; Valberg et al., 1986); and some controversy exists over the source of blue-OFF signals in the retina (Klug et al., 2003; Dacey, 2004; Dacey et al., 2005; Lee et al., 2005). In an attempt to resolve some of these disparate findings, the study in Chapter 4 examined the spatial properties and anatomical position of blue-OFF cells in the marmoset LGN.

1.5 TRANS-SPECIES COMPARISONS

Throughout this chapter, brisk sustained and brisk transient cells from all species have been treated as if they were perfectly homologous. This is clearly a simplification. Some of the first classification studies in macaque and owl monkey LGN suggested that parvocellular neurons are equivalent to the X cells of the cat, and magnocellular neurons are equivalent to the Y cells (Dreher et al., 1976; Sherman et al., 1976). These two studies defined X and Y cells on the basis of their sustained vs. transient response properties, and orthodromic/antidromic latencies. However, when Kaplan and Shapley (1982) applied the original test for X and Y cells in macaque – the test of linear summation vs. non-linear summation – they found that by this definition, all parvocellular cells and 75% of magnocellular cells could be classified as X cells, with the remaining 25% of magnocellular cells classified as Y cells. Further differences can be drawn between X cells of the cat and parvocellular neurons: for example, some parvocellular neurons show a red-green colour-opponent response, while X cells show no chromatic selectivity. This has led to the suggestion that parvocellular cells are a primate-specific evolution for red-green colour vision (Shapley and Perry, 1986).
Chapter 1 – Introduction

This uncertain homology highlights the difficulties with comparing cells types defined using a purely physiological classification scheme. Thus, a modern distinction of different cell types combines both anatomical and functional classifications, like these guidelines from Troy and Shou (2002):

- “All ganglion cells of a particular type must have physiological properties that render them distinct from all other ganglion cells.
- All cells of one type have a similar morphology that is distinctive, including the pattern of stratification of their dendritic arbors within the retina’s inner plexiform layer.
- The projection of axons of one cell type follow a standard pattern.
- The array of cells of one type form a pseudo-regular and well-organised mosaic when viewed in the plane of the retina.”

In addition, note that a ‘natural type’ of ganglion cell (as defined by Rodieck and Brening, 1983) can consist of ganglion cells that show a systematic change in their morphological properties – for example, the steady increase in ganglion cell size as distance from the fovea increases.

The task of classification of ganglion cell types is made considerably simpler in species where a census has been made of all retinal ganglion cells, like that made recently in the rabbit (Rockhill et al., 2002). Rockhill and colleagues used a number of techniques – intracellular injection, photofilling, and particle-mediated gene transfer – to label 734 ganglion cells in rabbit retina. They identified 11 ‘natural types’ of ganglion cell, which they believe account for the majority of the ganglion cell types in the rabbit retina (see Figure 1.3). The task remaining in rabbit is now to consolidate the seven known (and any unknown) physiological receptive field types with the identified anatomical types of ganglion cells.

By comparison, the understanding of the ganglion cell types in the primate retina is limited. Three major classes of receptive field have been extensively studied – brisk sustained, brisk transient and blue-ON – and linked to four major classes of retinal ganglion cells – the midget, parasol and small/large bistatified ganglion cells, respectively. As summarised above in Table 1.1, there are at least three different receptive field types – direction selective, sluggish sustained and sluggish transient – that exist in both cat and rabbit retinas (Barlow et al., 1964; Stone and Fabian, 1966; Levick, 1967; Oyster and Barlow, 1967; Oyster, 1968; Cleland and Levick, 1974a, b;
Stone and Fukuda, 1974; Rowe and Stone, 1976; Caldwell and Daw, 1978; Amthor et al., 1989b; DeVries and Baylor, 1997), that have not been identified in macaque retina (though there are indications that there may be direction selective ganglion cells in macaque: see Telkes et al., 2000). Two further cat/rabbit receptive field types have been scarcely identified in macaque: the local edge detector, and the suppressed-by-contrast cell types (Levick, 1967; Rodieck, 1967; Oyster, 1968; Stone and Hoffmann, 1972; Cleland and Levick, 1974b; Stone and Fukuda, 1974; De Monasterio and Gouras, 1975; Caldwell and Daw, 1978; De Monasterio, 1978; Amthor et al., 1989a; Tailby et al., 2005). Thus, two possibilities can be speculated: first, cats and rabbits might have both evolved these five cell types (direction selective, local edge detector, sluggish-sustained, sluggish-transient, and suppressed-by-contrast) independently; in this case, these five cell types should not be found in primate. Second, these five cell types could be part of a mammalian ‘blueprint’ of receptive field function, as speculated earlier; in this case, we may find similar classes in the macaque.

Of course, even starting from a common blueprint, some cell types may have been later modified or even removed by evolution; this may happen if the role of a particular cell type was supplanted by a newer cell type. For example, it has been proposed that the “local edge detector” cell class may mediate spatial vision in rabbits (at low temporal frequencies), instead of the “X-like” cell type (van Wyk et al., 2006). It is possible that in primates, the evolution of the midget-parvocellular system replaced the function of the local edge detectors; these local edge detector cells may then have been lost to further evolution.

It is understandable that efforts have focused on elucidating the properties of the midget (parvocellular) and parasol (magnocellular) ganglion cells in primate – combined, these two cell types account for 90% of LGN projections (Perry et al., 1984; Ahmad and Spear, 1993). However, consider that a typical macaque retina has approximately 1.5 million ganglion cells (Perry and Cowey, 1985); 10% of this total (150,000 cells) amounts to approximately the same number of cells as in an entire cat retina (Hughes and Wässle, 1976). The remaining 10% of macaque ganglion cells – those cells found in the koniocellular pathway – could thus represent as great a diversity of ganglion cells as the diversity of ganglion cells in the entire cat retina. Naturally, an
array of 150,000 ganglion cells would have a lower spatial resolving power than parvocellular cells (due to a sparser sampling of the cone mosaic). The visual acuity of the cat, for example, is around one-tenth of the visual acuity of humans (at 16 candelas/metre\(^2\): Pasternak and Merigan, 1981). But the projected roles of the koniocellular pathway (as detailed in Section 1.4.3) do not depend on spatial acuity.

It is only recently that tools have become available allowing a census of all the ganglion cells in an entire retina (such as photofilling, and “gene-gun” techniques). These tools are just beginning to be applied to primate retina (e.g. Dacey, 2004), but a lot of research remains to be done before there is a complete picture of the ganglion cell types of the primate.

### 1.6 AIMS OF THIS THESIS

This thesis aims to investigate colour-specific connectivity in the midget ganglion cell class; the projection of widefield ganglion cells to the LGN; and the physiology of blue-OFF responding geniculate neurones. These three projects, which will form the basis of subsequent chapters of this thesis, explore the anatomical and physiological differences between the three known visual ‘pathways’ – the parvocellular, koniocellular, and magnocellular pathways. The overall aim is to expand our knowledge of the different classes of ganglion cell that project to the LGN, and refine our knowledge of the specialisations of the different pathways. These goals will be achieved by using a combination of anatomical and physiological techniques.

The experimental animal used throughout is the marmoset. The marmoset is a type of New-World primate, which exhibits a sex-linked polymorphism of colour vision: all males are dichromats, but about two-thirds of females are trichromats (Jacobs, 1983). Also, in marmosets, the koniocellular layers of the LGN are well-segregated from the magnocellular and parvocellular layers (Kaas et al., 1978; Goodchild and Martin, 1998), allowing easier study of this pathway. Ganglion cell types defined thus far in the marmoset resemble those of the macaque (see Figure 1.6: Ghosh et al., 1996).

The questions addressed in the remaining chapters are:

- **Chapter 2**: Are the coverage and orientation of midget and parasol cell dendritic fields altered in dichromatic (‘red-green colour blind’) animals,
compared to trichromatic (‘colour normal’) animals? Essentially, does a drive to establish colour-specific connections change the morphology of the dendritic fields of midget and parasol ganglion cells? To find out, the mosaic properties of ganglion cells were examined.

- **Chapter 3**: What ganglion cell types project to the koniocellular layers of the LGN? This problem was addressed by injecting a retrograde tracer into various LGN subdivisions, and classifying the labelled retinal ganglion cells.

- **Chapter 4**: Are blue-OFF cells a subset of the midget-parvocellular pathway? Or are they a subset of the koniocellular pathway, like blue-ON cells? Recent studies have provided evidence for both (Klug et al., 2003; Dacey et al., 2005). Chapter 4 examines the physiological properties of blue-OFF LGN neurones, with the hypothesis that if blue-OFF neurons are a subset of the parvocellular pathway, then blue-OFF cells should have similar spatial response properties to parvocellular cells.
Figure 1.6. Known ganglion cell types in the marmoset. Camera lucida drawings of marmoset ganglion cells are shown, presented in wholemount view. The outer tier of the small bistratified cell is drawn in red. Boxed numbers underneath each cell indicate the distance from the fovea (eccentricity) for that cell. The main three types of ganglion cell found in macaque - the midget, parasol, and small bistratified cells - are all present in marmoset, and bear a similar morphology to their macaque counterparts. Also identified are hedge cells and parvocellular giant cells, which resemble the macaque T and parvocellular giant ganglion cell types (Rodieck and Watanabe, 1993). Modified from Ghosh et al., 1996.
Chapter 2

Mosaic properties of midget and parasol ganglion cells in the marmoset retina

2.1 Introduction

The neuronal signals that underlie spatial and colour vision in primates are transmitted to the brain in parallel, by distinct populations of ganglion cells. Functional specialization in these populations depends on the sampling of the receptor array by individual neurons, and on the spatial relationship (mosaic properties) of each population of neurons. Experiments described in this chapter address the morphology and mosaic properties of midget and parasol ganglion cells in the primate retina.

Midget and parasol cells are the two main classes of retinal ganglion cells in primate (Polyak, 1941; Leventhal et al., 1981; Perry et al., 1984; Rodieck et al., 1985; Watanabe and Rodieck, 1989; Ghosh et al., 1996). They can be distinguished by numerous morphological criteria – for example, midget cells have smaller dendritic fields, and smaller somata, than parasol cells at any given eccentricity. Midget cells project to the parvocellular layers of the lateral geniculate nucleus. This population is considered to contribute signals for high-acuity spatial vision at high image contrast, as well as providing input for the red-green axis of colour vision in trichromatic (“normal colour vision”) primates. Parasol cells project to the magnocellular layers of the lateral geniculate nucleus. They form the dominant input to cortical pathways for motion perception, and also contribute to spatial vision at low image contrast.

Two well-studied mosaic properties of ganglion cell arrays are orientation bias and dendritic field coverage. The dendritic fields of parasol cells in human (Rodieck et al., 1985), of both parasol and midget cells in macaque (Schall et al., 1986a; Watanabe and Rodieck, 1989), and of most cat ganglion cells (Leventhal and Schall, 1983) exhibit radial orientation bias – that is, the long axis of the dendritic field tends to point towards the fovea or area centralis. Such radial bias may serve as a 'blueprint' for establishing the orientation selectivity map in primary visual cortex (Leventhal, 1983; Schall et al. 1986b; Shou and Leventhal, 1989; however, see Vidyasagar & Urban, 1982).
anatomical data are also broadly consistent with physiological studies showing that most ganglion cells and geniculate relay cells respond best to radially oriented lines (Levick and Thibos, 1982; Smith et al., 1990; Shou et al., 1995; but see also Passaglia et al., 2002).

The term “coverage” describes the overlap of dendritic fields among the members of a given cell class (McIlwain, 1986). The coverage factor for an array is calculated by multiplying the areal density by the dendritic field area of cells in the same region (Wässle and Riemann, 1978). A coverage factor of 1 represents a cell mosaic that tiles the retina evenly, with no gaps between the dendritic fields of cells. Higher coverage implies greater overlap between neighbouring cells, while coverage lower than 1 would describe a cell mosaic that contains “gaps”.

In cat retina, the coverage factor for alpha ganglion cells is 1.4-1.7, and the coverage factor for beta class cells is 2.8-3.1 (Wässle et al., 1981c; Wässle et al., 1981b). These values refer to either the ON- or the OFF- subpopulation of each class, since ON- and OFF- subpopulations tile the retina independently (Wässle et al., 1981a; Dacey, 1993b; Lohmann and Wong, 2001). Consistent with this result, the parasol and small bistratified cell classes in primate retina also tile the retina as overlapping arrays, with coverage values from 1.8-3.4 (Perry and Cowey, 1985; Silveira and Perry, 1991; Dacey and Brace, 1992; Dacey, 1993b; Grünert et al., 1993; Yamada et al., 1996). By contrast, Dacey (1993b) showed that the dendritic trees of neighbouring midget cells in human retina do not overlap but interdigitate, to form a uniform mosaic with a coverage no greater than 1. A second difference between midget and parasol pathways is that the radial bias in midget-parvocellular pathway cells may be weaker than that of parasol cells (Rodieck et al., 1985; Smith et al., 1990). It has been speculated that these distinct features of the midget pathway are a sign of colour-selective connections of midget ganglion cells with the input matrix of midget bipolar cells (Wässle and Boycott, 1991; Dacey, 1993b; Martin et al., 2001). This idea has indirect support from demonstrations that midget cells in peripheral primate retina show red-green colour-selectivity (Dacey, 1994; Martin et al., 2001; but see also Diller et al., 2004).

Now, if limited overlap in the midget array is a result of developmental processes for producing colour-selectivity, then midget ganglion cells in dichromatic (“red-green
colour blind”) animals would lack any driving force for the development of such circuitry. Accordingly, the midget mosaic in dichromatic animals might be expected to revert to the common mammalian pattern of overlapping, radially oriented dendritic fields. Alternatively, if the low overlap is simply associated with the high sampling density of the midget array, as required for transmitting signals for high-acuity spatial vision, then the same kind of mosaic properties should be present in both dichromatic and trichromatic individuals.

In common with many species of New World primates, marmosets exhibit a sex-linked polymorphism of colour vision whereby all males are dichromats, but about two-thirds of the females are trichromats (Jacobs, 1983). In the current study, the retinas of dichromatic and trichromatic marmosets were compared. The working hypothesis is that any morphological specialization related specifically to red-green colour vision should be present only in trichromatic animals.

A previous study of dichromatic Cebus monkeys (Yamada et al., 1996) showed that the dendritic morphology and density of midget and parasol cells in this species are comparable to those in macaque, but these authors did not specifically compare dichromatic with trichromatic animals. In the present study, both parasol and midget ganglion cells in marmosets are shown to exhibit a foveal bias with respect to their dendritic field orientation, but no difference in dendritic field morphology or orientation was seen in dichromatic compared to trichromatic animals. Further, it is shown that the dendritic fields of neighbouring midget cells in dichromatic and trichromatic marmosets show a low degree of overlap, consistent with a coverage factor close to unity. The conclusion is that the retinal connectivity which supports trichromatic colour vision does not alter the dendritic field morphology of ganglion cells, and suggest that low coverage in the midget mosaic might be a consequence of specialization for transmitting high spatial resolution signals. Such specialization may nevertheless have supported the expression of trichromatic colour vision, by restricting the number of cones providing input to each member of the midget mosaic. This anatomical arrangement would thereby minimize the spectral mixing of cone inputs to midget ganglion cells.
2.2 METHODS

Data were obtained from six adult marmosets (*Callithrix jacchus*), body weight 290-420 g. Three of the animals were female. The colour vision phenotype of the female animals was predicted by polymerase chain reaction amplification of cone opsin-encoding genes, and confirmed electrophysiologically by functional identification of cone inputs to single neurons in the lateral geniculate nucleus (Solomon, 2002; Blessing et al., 2004). Two of the females were trichromats. Additional data were obtained from re-analysis of intracellularly injected retinal ganglion cells from two male and four female marmosets, from the study by Ghosh et al. (1996). The colour vision phenotype of these animals is unknown. Animals were obtained from the Australian National Health and Medical Research Council (NHMRC) combined breeding facility. All procedures used conform to the provisions of the Australian NHMRC code of practice for the use and care of animals.

Animals were anaesthetized with isoflurane (Forthane, Abbott, Sydney, Australia; ICI, 1-2%) and intramuscular ketamine (30 mg/kg) and xylazine hydrochloride (Rompun, Bayer Animal Health Australia, Pymble, NSW; 2-4 µg/kg). Body temperature was maintained near 38 °C by means of a thermistor-controlled heating blanket. The trachea and a femoral vein or a tail vein were cannulated. Anaesthesia was maintained using sufentanil citrate (Sufenta-Forte, Janssen-Cilag, Beerse, Belgium; 4-8 µg/kg), infused at a rate of 0.5-1.5 ml/h in dextrose ringer solution (Baxter, Sydney, Australia), and a 70%: 30% mixture of NO₂: carbogen (5% CO₂ in O₂). The electroencephalogram (EEG) and electrocardiogram were monitored to ensure adequate depth of anaesthesia. Criteria for adequate anaesthesia were dominance of low-frequency (< 8 Hz) components of the EEG and stability of EEG signal and heart rate under intermittently applied noxious stimuli such as tail– or paw-pinch.

The animal was mounted in a stereotaxic frame. A craniotomy was made over the dorsal lateral geniculate nucleus (LGN). The eyes were protected with oxygen-permeable contact lenses. The positions of the fovea and the optic disk were mapped with a fundus camera. A microelectrode (parylene-coated tungsten or glass-coated steel, impedance 5MΩ; F. Haere Co., Bowdoinham, ME) was lowered to the position of the LGN, and multiunit visually evoked activity was recorded.
The recording location was classified as either the parvocellular, magnocellular or koniocellular layers, based on the contrast, movement and chromatic selectivity of the single and/or multi-unit visually evoked activity. In addition, the dominant eye and position of the multiunit receptive field within the visual field was noted. The recording electrode was withdrawn and replaced with a micropipette (tip ~80 µm; drawn from a glass capillary tube with 0.86 mm inner diameter, 1.5 mm outer diameter) filled with a solution of 2 or 10% biotinillated dextran, tetramethylrhodamine conjugate (“Microruby”; Molecular Probes, Eugene, OR). The micropipette was lowered to the same depth below cortex as the preferred region found with the electrode, and multi-unit activity was recorded to confirm the visual field location. Injections were made by iontophoresis using a positive current (5-15 µA) for 30 to 45 minutes.

Following these injections, 65 to 90 hours were allowed for retrograde transport of the tracer. Single-unit recordings made from the LGN during this time confirmed the colour vision phenotype of the animal (Blessing et al., 2004). The animal was then overdosed with sodium pentobarbitone (80-150 mg/kg, i.v.). The eyes were removed and processed as described below. The animal was perfused with 0.25 l of saline (0.9% NaCl) followed by 0.3 l of freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer (PB), then 0.25 l of 10% sucrose in PB. The brain was removed, and postfixed in 4% paraformaldehyde in PB for 12 hours, before being transferred to 30% sucrose for 24-72 hours. The brain was sectioned in the coronal plane using a freezing microtome. Alternate sections were stained for Nissl substance or mounted in Mowiol (Hoechst, Sydney, Australia: Harlow and Lane, 1988).

The LGN injection sites were reconstructed as follows. Photomicrographs of the fluorescent and neighbouring Nissl sections were acquired using a Zeiss Axiocam HRc colour digital camera mounted on a Zeiss Axioplan 2 microscope, using a 2.5x objective. Outlines of the LGN laminae and the injection site were traced from these photomicrographs, using the Adobe Illustrator graphics program (San Jose, CA, USA), and a drawing tablet (Wacom, Vancouver, WA, USA). These outlines were then overlaid to give a three-dimensional outline of the injection site.
2.2.1 In vitro photofilling and histology

The retina was removed from the eye and placed in carboxygenated Ames’ tissue culture medium (Sigma, St Louis, MO, USA). Each retina was cut into 2-4 segments. Each segment was placed, photoreceptor side down, onto a piece of Millipore filter, then transferred to a superfusion chamber (Warner Instruments, Hamden, CT, USA). The chamber was maintained at 37.5°C and the tissue was superfused with carboxygenated Ames’ medium, at a rate of 1-2 ml/min. The chamber was mounted on the stage of a Zeiss Axioskop 2 fixed stage microscope (Zeiss, Oberkochen, Germany). The retina was viewed through a 40x water immersion objective (Zeiss). Cells were photofilled by illumination (normally for 1-2 minutes) with a dual-band Lucifer Yellow/Rhodamine filter (Chroma filter set 51016), as described by Dacey et al., (2003). Following photofilling, the retinal piece was removed from the superfusion chamber and fixed for two hours in 4% paraformaldehyde in 0.1 M PB at 4 °C, then mounted and coverslipped in Mowiol. Alternatively, fixed pieces were processed for visualization of diaminobenzidine hydrochloride (DAB; Sigma), using the avidin-biotin method, as follows. Retinal pieces were incubated in 0.2% Triton X-100 in 0.1 M PB for 3 hours. The tissue was rinsed in 0.1 M PB, then incubated in the avidin-biotin complex (ABC Elite; Vector Laboratories, Burlingame, CA, USA) overnight, and then rinsed again. Labelled cells were visualized by incubation in 0.05% DAB and 0.01% hydrogen peroxide in 0.1 M PB and subsequently intensified with nitro blue tetrazolium (Vaney, 1992). Following this reaction, pieces were rinsed in phosphate-buffered saline, then mounted in Mowiol.

Some data were re-analysed from the work of Ghosh et al (1996). The protocol that Ghosh et al (1996) used for intracellular injection of cells is briefly summarised here. First, the retina was dissected, placed onto a Millipore filter, and placed into a superfusion chamber as per the preparations for photofilling (above). Ganglion cell somata were identified using Acridine Orange. Individual cells were selected under visual control and penetrated with glass micropipettes, which were filled with a solution of Lucifer Yellow and Neurobiotin. Following injection, the retinal pieces were fixed in paraformaldehyde, processed to visualise neurobiotin, then mounted in Mowiol or Ultramount.
2.2.2 Analysis

A total of 577 cells (404 midget cells, 173 parasol cells) was analysed. Of these, 317 midget cells were labelled using the photofilling method, and 87 midget and all of the parasol cells were re-analysed from material used in Ghosh et al (1996). Only cells that were completely labelled were analysed. No correction was made for the minimal tissue shrinkage due to paraformaldehyde fixation and Mowiol mounting. Dendritic field size and orientation was analysed either using a computer-assisted camera lucida system, or from a Z-stack of digital images at 0.3-0.5 µm spacing, acquired using a Zeiss Axiocam HRc colour digital camera (Zeiss). Custom software written in MatLAB (Image processing toolbox, Mathworks, Natick, MA, USA), was used to analyse each stack of images.

The dendritic field dimensions of each cell were measured by tracing a polygon around the outermost tips of the dendritic field. The major and minor axes of an ellipse with the same area as the polygon were calculated. The average of the major and minor axes is the dendritic field diameter. The orientation of the major axis of this ellipse was measured and compared to the polar angle of the cell – defined as the angle of a line joining the cell to the fovea, relative to a line between the fovea and optic disk. Analogous procedures were used to calculate dendritic field orientation relative to the optic disk.

Because this study was not concerned with the effects of anatomical distortions around the fovea, cells within 2 mm of the fovea were excluded from the orientation analysis. For a subset of cells (140 parasol, 54 midget), the orientation of coherent labelled bundles of ganglion cell axons in the neighbourhood of the labelled cell was also measured.

Dendritic overlap was measured between photofilled midget cells with adjacent or overlapping dendritic fields. To minimise experimenter bias, each cell in a pair was first outlined individually, without reference to the dendritic field outline of the neighbouring cell. Where dendritic fields overlapped, the dendritic field of each ganglion cell could be readily identified by differences in staining quality between the two cells, or by tracing individual dendrites though the Z-stack of images. However, when defining the
dendritic field, some small imprecision where neighbouring dendritic fields meet cannot be ruled out.

After outlining both cells in a pair individually, the degree of overlap between the two polygons was calculated by pixel-wise logic and integration. The degree of overlap was expressed as the percentage of the dendritic field area that is covered by its neighbour.

### 2.3 Results

Figure 2.1 shows examples of midget and parasol cells. The midget cell in Figure 2.1A and the parasol cell in Figure 2.1B were intracellularly injected (Ghosh et al., 1996). As described previously (Polyak, 1941; Leventhal et al., 1981; Perry et al., 1984; Rodieck et al., 1985; Watanabe and Rodieck, 1989; Ghosh et al., 1996), at the same eccentricity, midget cells have smaller dendritic fields and somal sizes than parasol cells. Additionally, the soma of midget ganglion cells tends to be located towards the edge of their dendritic field (Ghosh et al., 1996). The cells in Figure 2.1C, D, E were photofilled following retrograde labelling with microruby. Figure 2.1C shows an isolated midget cell, Figure 2.1D shows an isolated parasol cell, and Figure 2.1E shows a group of labelled foveal midget cells including four cells with neighbouring dendritic fields. Well-labelled photofilled cells were morphologically indistinguishable from intracellularly injected cells. Dendritic field size measurements showed no systematic difference between photofilled cells and injected cells at equivalent eccentricities, so the data for the following analyses were pooled.

In Figure 2.2A, the dendritic field diameter of midget and parasol cells is plotted against eccentricity. The aspect ratio of these cells (ratio of the major : minor axis) is shown in Figure 2.2B. Higher aspect ratios represent more elliptical dendritic fields. For the region above 2 mm eccentricity, midget cells have a significantly higher aspect ratio than that of parasol cells (aspect ratio, mean ± S.D.: midget cells, 1.47 ± 0.32, n = 265; parasol cells, 1.34 ± 0.21, n = 169; p < 0.001, Wilcoxon rank-sum). This difference is consistent with measurements made in macaque retina (Martin et al., 2001). For confirmed trichromatic animals, the mean aspect ratio of midget cells (1.37 ± 0.28, n = 35) is slightly lower than for confirmed dichromats (1.48 ± 0.32, n = 120, p = 0.05, Wilcoxon rank-sum).
Figure 2.1. Micrographs of marmoset midget and parasol retinal ganglion cells labelled by intracellular injection or photofilling techniques. (A), injected midget ganglion cell, 3.8 mm eccentricity. (B), injected parasol ganglion cell, 3.6 mm eccentricity. (C), photofilled midget ganglion cell, 5.1 mm eccentricity. (D), photofilled parasol ganglion cell, 1.4 mm eccentricity. (E), Foveal midget cells, labelled by photofilling. Eccentricity: 0.5 mm. Scale bars: (A), (B) = 25 μm; (C), (D) = 20 μm; (E) = 10 μm.
Figure 2.2. Quantification of parasol and midget ganglion cell morphology in marmosets. (A), dendritic field diameter as a function of eccentricity. Midget cells (n=404) and parasol cells (n=173) form non-overlapping distributions. Parasol cell diameters are close to three times those of midget cells at the same eccentricity. (B), aspect ratios of the best-fitting ellipses for each cell. The graphics next to the y-axis show ellipses with the corresponding aspect ratio. At eccentricities above 2 mm, midget cells have a higher aspect ratio than parasol cells. For both dendritic field diameter and aspect ratio, midget cells from trichromatic marmosets (Tri) are similar to midget cells from dichromatic marmosets (Di).
2.3.1 Analysis of dendritic field orientation

The angular deviation of the dendritic field major axis from a line joining the centroid of the dendritic field to the fovea was measured. This will be referred to hereinafter as the angle relative to the fovea (ARF). Analogous calculation was used to derive the angle relative to the optic disk (ARO). Both the ARF and the ARO range from -90° to +90°, with negative angles referring to clockwise deviations from zero, and positive angles referring to counter-clockwise deviations.

Histograms of dendritic field orientations of parasol and midget cells are shown in Figure 2.3A, B. The two histograms on each plot show the ARF and the ARO for each cell class. No clear differences were seen in the dendritic field orientation of male or female animals. All of the distributions are broadly centred on a mean of zero (Table 2.1), suggesting that there is a weak but consistent bias for all dendritic fields to point to the central retina.

It is difficult to distinguish whether the distributions are centred on the fovea or optic disk, because the ARF and ARO are strongly correlated (correlation coefficient 0.91, $r^2 = 0.82$). These possibilities were distinguished as follows.

Firstly, circular statistics were applied to the data, using two variants of the Rayleigh test of circular uniformity. The first test determines whether a distribution differs significantly from a uniform distribution (in Figure 2.3, a uniform distribution would be a flat histogram). The second test determines whether the mean of a data set differs from zero. The test statistics (Table 2.1) showed consistently lower p values for ARF than for ARO, consistent with the idea that the foveal orientation bias is stronger than the optic disk orientation bias.
Figure 2.3. Orientation bias of ganglion cell dendritic field and ganglion cell axons in marmosets. (A, B), distributions of angle relative to the fovea (ARF, shaded histograms) and angle relative to the optic disk (ARO, open histograms) for dendritic fields of parasol cells (A) and midget cells (B). The ARF and ARO for labelled axon bundles is shown in (C). The dendritic fields of both midget and parasol cells show a weak bias towards the fovea. Axons show a strong bias towards the optic disk. Parameters for each of these distributions can be found in Table 1.
Chapter 2 – Mosaic properties of ganglion cells

Table 2.1: Dendritic field and axonal orientation

<table>
<thead>
<tr>
<th></th>
<th>Mean Angle (deg) ± SD</th>
<th>Rayleigh test 1 (z)</th>
<th>Pz</th>
<th>Rayleigh test 2 (u)</th>
<th>Pu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasol dendritic field ARF (n=169)</td>
<td>15.4 ± 36.7</td>
<td>5.38</td>
<td>p&lt;0.005</td>
<td>2.82</td>
<td>p&lt;0.0025</td>
</tr>
<tr>
<td>Parasol dendritic field ARO (n=169)</td>
<td>14.4 ± 38.1</td>
<td>2.32</td>
<td>p&lt;0.1</td>
<td>1.89</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Midget dendritic field ARF (n=295)</td>
<td>-12.2 ± 37.1</td>
<td>6.84</td>
<td>p&lt;0.005</td>
<td>3.37</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Midget dendritic field ARO (n=157)</td>
<td>-14.2 ± 37.4</td>
<td>3.46</td>
<td>p&lt;0.05</td>
<td>2.31</td>
<td>p&lt;0.025</td>
</tr>
<tr>
<td>Axonal ARF (n=194)</td>
<td>-11.6 ± 23.2</td>
<td>87.64</td>
<td>p&lt;0.001</td>
<td>12.17</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Axonal ARO (n=154)</td>
<td>-2.9 ± 18.5</td>
<td>121.50</td>
<td>p&lt;0.001</td>
<td>15.51</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

ARF = Angle relative to the fovea; ARO = Angle relative to the optic disk

Secondly, at the position of 194 labelled cells, the heading of local axon bundles was measured. Comparison of these data (Figure 2.3C) with the dendritic field data shows that the axon bundles are clearly biased towards the optic disk (mean orientation ± S.D. = -2.9 ± 18.5, z = 121.5, u = 15.5, Pz < 0.001, Pu < 0.001), whereas the dendritic fields are more weakly biased toward the fovea. Consistently, the Rayleigh u value for ARO is greater than for ARF for axon bundles, and both statistics show much higher values for axon bundles than for dendritic fields. In summary, the data suggest that dendritic fields of both midget and parasol cells show a weak radial bias toward the fovea, with no clear difference between dichromatic and trichromatic retinas.

2.3.2 Analysis of coverage

The overlap of the dendritic fields of pairs of neighbouring midget cells was measured. Only photofilled material was used for this analysis. Neighbours were defined as any two ganglion cells with adjacent or overlapping dendritic fields. In total, 28 ON/ON, 19 OFF/OFF and 43 ON/OFF pairs from three dichromatic animals (two male and one female) were analysed, and 14 ON/ON and 7 OFF/OFF pairs from two trichromatic animals were analysed. Because the ON and OFF mosaics are considered to be spatially independent (Wässle et al., 1981a; Dacey, 1993b; Lohmann and Wong, 2001) measuring ‘neighbours’ from different mosaics should serve as a control, because the
dendrites of an ON/OFF pair normally occupy separate compartments in the inner plexiform layer.

An example of an ON/OFF pair is shown in Figure 2.4A, B. The dendritic fields of the two cells stratify at distinct levels in the inner plexiform layer, and show substantial spatial overlap. By contrast, Figure 2.4C shows three neighbouring cells from the same (ON) type mosaic, which show a much more limited degree of overlap. The quantitative analysis described below showed 69% mean dendritic field overlap for the ON/OFF pair, and 3.6% mean overlap for the three ON cells. Data from the other cell pairs examined were consistent with this pattern, as follows.

Figure 2.5A shows a *camera lucida* drawing of the trio of ON cells from Figure 2.4C. The dendritic fields of these cells were outlined by a polygon enclosing the outermost tips of the dendritic tree (Figure 2.5B), and the proportion of the dendritic field that is covered by each neighbour was computed. The position of the centroid (centre of mass) of each dendritic field was also calculated (cross marks in Figure 2.4C). Note that dendritic fields were not outlined with typical convex hulls, instead showing concavities in some regions. This was because convex hulls would unnecessarily overestimate the coverage of the midget ganglion cells. There is thus some subjectivity involved when defining the dendritic field; the experimenters attempted to mitigate this as much as possible by tracing dendritic fields of neighbouring cells individually, without reference to the dendritic field outline of the neighbouring cell.

Figure 2.5C shows 16 same-mosaic pairs, with the overlap between pairs and the positions of the cell centroids indicated. The cell pair with the smallest centroid separation is at the top-left, with increasing centroid separation reading to the right and downwards. For all cases, the overlap is less than 10%, with no clear relationship between overlap and the distance between dendritic fields.
Figure 2.4. Dendritic field overlap of neighbouring midget cells. (A) shows an ON/OFF pair from a dichromatic marmoset. The focus is on the dendrites of the ON cell. (B) shows the same field, with the focus on the dendrites of the OFF cell. There is a large degree of overlap between the dendritic fields of these two cells. (C), a group of three ON cells with neighbouring dendritic fields, from a dichromatic marmoset. Neighbouring ON cells show little overlap of dendritic fields. Scale bars = 10 μm. Scale bar in B applies to A,B.
Figure 2.5. Quantification of dendritic field overlap in midget cells. (A) shows a camera lucida drawing of the three neighbouring ON midget cells from Figure 4. The outline of the dendritic field of each cell is shown in (B). (C), outline drawings of the 16 cell pairs with the smallest separation between their dendritic fields. OFF/OFF pairs = dark grey, ON/ON pairs = light grey. Boxes marked with asterisks show midget pairs from confirmed trichromatic animals. The centroid of the dendritic field of each cell is marked with an X. Scale bars: (B) = 20 \( \mu \text{m} \); (C) = 50 \( \mu \text{m} \). Scale bar in B applies to A, B.
Figure 2.6 summarizes the data from 42 ON/ON, 26 OFF/OFF and 43 ON/OFF pairs. Figure 2.6A shows overlap as a function of the inter-centroid distance. It should be noted that each pair contributes two values to this plot. Cells from the same mosaic show very little overlap (mean: 2.4%, SD 2.3%; mean dendritic field size: 35.6 µm, SD 8.3 µm; n = 68 pairs). Same-mosaic cells from trichromatic marmosets showed a slightly larger degree of overlap compared to same-mosaic cells in dichromats (overlap, mean ± SD, dichromats = 1.9 ± 2.1%, n = 47 pairs; trichromats = 3.6 ± 2.3%, n = 21 pairs; p < 0.001, Wilcoxon rank-sum).

By contrast, dendritic fields of cells from different mosaics (ON/OFF pairs; n = 43 pairs) showed a progressively larger overlap with decreasing centroid separation (correlation coefficient -0.86, r² = 0.74). Because the measurements of like– and unlike–mosaic pairs were taken from comparable eccentricities (Figure 2.6B) the difference in overlap cannot be accounted for by a generalized difference in eccentricity-dependent cell spacing.

A low degree of overlap is consistent with low coverage in the mosaic. For a hexagonal mosaic with circular dendritic fields, the distance between centroids of neighbouring cells is given by

\[ a = \frac{2}{\sqrt{3D}} \]

where \( a \) is the distance between centroids, and \( D \) is the areal density. The overlap between artificial, circular, fields was measured for a range of inter-centroid distances, then used the above formula to calculate coverage (the product of density and dendritic field area) for a hexagonal mosaic of circular fields. The result (Figure 2.6C) shows that an average overlap of 2.4% would correspond to coverage of 1.06. In conclusion, in common with midget cells in human retina (Dacey, 1993b), in marmoset retina the dendritic field of each midget cell conforms to those of its neighbours to create a uniform, space-filling mosaic.
Figure 2.6. Analysis of dendritic field overlap. (A), overlap values plotted against the dendritic field separation of pairs of cells (distance between centroids). Cell pairs from the same mosaic (ON/ON, open circles, and OFF/OFF, shaded circles) are characterized by low overlap values and high centroid separations. Same-mosaic cell pairs from dichromatic (Di) and trichromatic (tri) marmosets show overlapping distributions. Cell pairs from opposite mosaics (ON/OFF pairs; shaded triangles) show an approximately inverse relationship between overlap and distance between centroids for cell pairs. (B), eccentricity of sampled cell pairs. The slight increase in mean centroid separation with eccentricity is consistent with reduced average cell density with increasing eccentricity. (C), Conversion of overlap to coverage for a hexagonal mosaic of circular fields. The “flower” graphics illustrate overlap of hexagonally arranged circular fields at the indicated positions on the curve.
2.4 **Discussion**

2.4.1 Orientation of midget and parasol dendritic fields

No systematic difference was found in the dendritic field orientation of midget or parasol cells when male and female were compared. This result is consistent with the idea that the expression of trichromatic colour vision does not produce overt changes in the mosaic properties of ganglion cells. Both midget and parasol ganglion cells show a mild radial bias, with parasol cells showing a stronger bias despite having less elliptical dendritic fields than midget cells.

A previous study of cat ganglion cells by Leventhal and Schall (1983) showed a more pronounced radial bias than the present study. This discrepancy could be because their analysis omits cells with a low ‘orientation bias’ (a measure broadly equivalent to the measure of aspect ratio used in this study). The analysis was repeated, omitting cells with aspect ratio less than 1.25, but the resulting Rayleigh values (ARF: \( z = 12.0, u = 4.9; P_z < 0.001, P_u < 0.001 \)) were nevertheless still much lower than that reported for cat by Leventhal and Schall (1983) (\( z = 132.0, u = 16.2; P_z < 0.001, P_u < 0.001 \)). This suggests that the radial bias in marmoset is indeed weaker than in the cat.

The eye of the marmoset is smaller than the eye of the cat. Since the elongation of dendritic fields may be influenced by radial expansion of the retina during maturation of the eye (Eysel et al., 1985; Schall and Leventhal, 1987), it follows that the dendritic fields in a smaller eye may be subject to less distortion during eye growth. Examination of large-eyed New World species such as *Cebus apella* may resolve the question whether the reduced bias is specifically associated with eye size, or is a general feature of New World monkey eyes.

It was not possible to resolve conclusively the question of whether the origin of the radial bias in marmoset eye is the fovea or the optic disk. The statistical analysis is consistent with the former interpretation, as is the fact that the trajectories of ganglion cell axons are more clearly biased to the optic disk than is the axis of elongation of ganglion cell dendritic fields.

The dendritic morphology of retinal ganglion cells in marmosets is not clearly related to colour vision phenotype. For the female animals that were confirmed as
trichromatic, the dendritic fields of midget cells may be slightly less elliptical than in confirmed dichromats. The genotype of the other female animals used in this study is not known. Nevertheless it would be surprising if there were not at least one additional trichromatic female among the animals sampled in this study. Because approximately two thirds of female marmosets are trichromats (Jacobs, 1983), the probability that all four untested animals were dichromats is low (1:12). Any gross variation in morphology should thus have been apparent. This result supports and extends the earlier conclusions of Yamada et al., (1996), Ghosh et al., (1996) and Silveira et al., (1994): that variation in morphology of midget ganglion cells is more consistently related to eye size and to rod:cone ratios than to colour vision phenotype.

Recordings from the LGN of macaque (Lee et al., 1979; Smith et al., 1990), marmoset (Kremers and Weiss, 1997; White et al., 2001), cat (Shou and Leventhal, 1989), and bush baby (Xu et al., 2002) all reveal receptive fields that show deviations from circularity. Shou et al. (1989) showed that the anatomical bias in retinal ganglion cells could account for the receptive field anisotropy in cat alpha cells. Further, it has been proposed that the radial organization of receptive fields in the retina forms a ‘template’ for the formation of orientation columns in primary visual cortex (Leventhal, 1983; Schall et al. 1986b; Shou and Leventhal, 1989; however, see Vidyasagar & Urbas, 1982). The results of the present study suggest that the radial bias for midget cells is similar to the radial bias for parasol cells (Figure 2.3), despite the fact that midget dendritic fields are more elongated than the dendritic fields of parasol cells (Figure 2.2). This raises the possibility that distinct rules may govern the formation of midget and parasol mosaics, but the functional ramifications of such differences are not clear.

2.4.2 Midget cell coverage

Midget cell dendritic fields in both dichromatic and trichromatic retinas exhibited little to no overlap, consistent with a coverage factor of approximately 1. In association with the high sampling density of the midget mosaic, this feature could reflect some optimization for transmission of high-acuity spatial signals at the expense of contrast sensitivity. The possibility that there may have been an unlabelled midget cell that overlapped significantly with both of the labelled cells cannot be ruled out. However,
Chapter 2 – Mosaic properties of ganglion cells

this is unlikely for several reasons. First, measurements in this study were taken from patches of midget cells that were labelled by retrograde injection to the parvocellular layers. This means that the unlabeled cell bodies (such as seen in Figure 2.4) almost certainly belong to other, non-midget classes with different projection sites. Second, the maximum overlap among 68 same-mosaic pairs was 9.2% (see Figure 2.6A), but over 42% of ON/OFF pairs had overlap of greater than 20%, and there is no *a priori* reason to assume selective differences in uptake or transport of the dye label between the different mosaics. Finally, although data from only completely labelled cell pairs are presented here, a great number of incompletely labelled pairs and clusters of cells was also observed, and the results were consistent with those from the fully labelled pairs.

As reported in human retina (Dacey, 1993b), the dendrites of neighbouring cells seldom intruded across the borders of neighbouring cell’s dendritic fields. In some cases, an obvious interdigitation was observed (e.g. Figure 2.5). Normally, the dendrites of a cell would halt when confronted by a neighbouring dendritic field, but would occasionally intermingle with the most distal dendrites rather than forming exclusive territories (see Figure 2.4C, Figure 2.5).

In summary, although there are subtle differences in tiling properties and cell morphology, midget cells in dichromatic and trichromatic marmosets appear to tile the retina in a similar way to midget cells in humans. This result confirms that low coverage is a specialization of the midget ganglion cell mosaic rather than a specific feature of trichromatic retinas. Two putative mechanisms for mosaic formation are interactions between neighbouring dendritic fields, or activity-dependent regulation of dendritic growth (for recent reviews, see Wong and Ghosh, 2002; Jan and Jan, 2003). There is evidence for interactions between neighbouring dendritic fields during development from studies of the effects of retinal lesions, (Perry and Linden, 1982; Eysel et al., 1985; Leventhal et al., 1989), developmental studies (Lohmann and Wong, 2001), and cell culture studies (Montague and Friedlander, 1989). On the other hand, the recent results of Lin et al. (2004) show that regular mosaics can form without dendritic contact between neighbours, suggesting that intrinsic cell growth regulation also plays a role.

It is possible that instead of coverage being determined developmentally, the coverage factor of a ganglion cell mosaic may be specified by genetic factors; in this
case, the genetic profile for low coverage may have been retained in evolution because some individuals (namely, trichromats) benefited and this assisted in their survival and reproduction. Thus, despite our finding of low coverage in the midget cell mosaic of both dichromats and trichromats, we cannot conclusively rule out that low coverage arose as a specialisation for colour vision.

The data do not provide support for the hypothesis that low coverage in the midget array, and the distinct ‘elongated’ morphology of midget cells are a result of colour-selective wiring of midget bipolar inputs to midget ganglion cells (Dacey, 1993b; Martin et al., 2001). These mosaic properties might nevertheless be a necessary precondition for the development of colour selectivity in midget cells, because they would reduce the divergence (sharing) of bipolar cell inputs to ganglion cells. The functional isolation of cone inputs to neighbouring ganglion cells would thereby reduce the potential for mixing the spectrally specific signals in cone inputs to ganglion cells in trichromatic retinas. This leads to the speculation that, by analogy with the “random wiring” model for foveal colour specificity (Lennie et al., 1991), the evolution of trichromatic colour vision was enabled by pre-existing circuitry, which was optimized for spatial precision even outside the fovea.
Chapter 3

Projection of widefield ganglion cells to the lateral geniculate nucleus of the marmoset

3.1 INTRODUCTION

It is well recognised that the primate visual system analyses separate aspects of the visual scene in parallel, via morphologically and physiologically distinct ‘pathways’. These are the parvocellular (PC), magnocellular (MC) and koniocellular (KC) pathways, named after different functional divisions of the dorsal lateral geniculate nucleus (LGN).

Primate PC cells can be electrophysiologically characterised by their small receptive fields, which are often red-green colour-opponent in trichromatic primates (Wiesel and Hubel, 1966; De Monasterio and Gouras, 1975; Shapley and Perry, 1986). The PC pathway is thought to mediate high-acuity spatial vision at high image contrast, and red-green colour vision in trichromatic primates. Primate MC cells have a larger receptive field than PC cells, which is non-colour specific; also, the retinal afferents of MC cells have an axonal conduction velocity around two times faster than PC cells (4 m/s and 2 m/s, respectively: Wiesel and Hubel, 1966; Gouras, 1969; De Monasterio and Gouras, 1975; Shapley and Perry, 1986). The MC pathway is implicated in motion detection, and also may contribute to spatial vision at low image contrast (Lee, 1993). Both of these classes of cells have receptive fields that are made up of concentric centre and surround subunits, which are spatially and chromatically antagonistic (Kuffler, 1953; Hubel and Wiesel, 1960; Wiesel and Hubel, 1966). The retinal inputs to the PC and MC layers are also well characterised: the PC laminae receive their dominant input from midget ganglion cells, while the MC laminae receive their dominant input from parasol ganglion cells (Leventhal et al., 1981; Perry and Cowey, 1984; Rodieck and Watanabe, 1993). It is, however, not certain whether the PC and MC layers receive an exclusive input from the midget/parasol ganglion cell classes.

The KC pathways are less-studied than the PC or MC pathways. Electrophysiological recordings of the KC layers of the LGN of bush baby (Galago
crassicaudatus) and marmoset (Callithrix jacchus) have revealed a population of cells with a heterogeneous physiological response, analogous to the sluggish cells found in cat LGN (see Section 1.3: Norton and Casagrande, 1982; Irvin et al., 1986; Casagrande, 1994; White et al., 2001). KC cells typically have large receptive fields, and are heterogeneous in their response type – there is a large variation in spatial frequency cutoffs and contrast sensitivities between cells, and some KC cells are orientation tuned (White et al., 2001). There may even be a heterogeneity between the different KC layers: for example, cells in the most ventral KC layer of the marmoset (K1: ventral to the magnocellular layers) show larger receptive fields and more transient responses than cells in the KC layer K3 (the KC layer between the magnocellular and parvocellular layers: Norton and Casagrande, 1982; White et al., 2001). Furthermore, it has been found that some KC cells have a blue-ON/yellow-OFF response type (Martin et al., 1997), implicating this pathway in blue-yellow colour processing.

Relatively little is known about the retinal inputs to the KC layers of the LGN. The data from electrophysiological recordings (described above) imply that there is a heterogeneous ganglion cell projection to the KC layers. Consistent with this presumed heterogeneity, Rodieck and Watanabe (1993) labelled two widefield classes of cells, the parvocellular giant and small bistratified cells, following injections including the PC and KC layers. In addition, Dacey et al. (2003) have described at least six wide-field type ganglion cells that project to the LGN. Of these cell types, the small and large bistratified cells are the most likely to project to the koniocellular layers. This is because they have a blue-ON/yellow-OFF response type (Dacey and Lee, 1994; Dacey et al., 2003), in common with some KC LGN cells (Martin et al., 1997).

The present study used injections of a retrograde tracer to determine the retinal ganglion cell projections to the KC layers of the LGN of the marmoset. In the marmoset, the koniocellular layers are well-segregated from the PC and MC layers. This segregation is most complete for K3, the KC layer ventral to the PC layers and dorsal to the MC layers. This segregation makes it easier to verify retrograde tracer injections made in the KC layers, especially those that are targeted at KC layer K3. The major finding of this study is that widefield cells form the dominant projection to the KC
layers. Two main classes of widefield ganglion cells are found to project to the KC layers: these are the small bistratified and sparse cells.

### 3.2 Methods

Data were obtained from thirteen adult marmosets (*Callithrix jacchus*), body weight 290-500 g. All the female animals, and six of the seven male animals, were genetically phenotyped to determine their medium- and/or long-wavelength cone pigment(s) (Blessing et al., 2004): these data are shown in Table 3.1. All animals possess a short-wavelength sensitive cone (peak sensitivity 425 nm) in addition to those listed. These phenotypes were later verified electrophysiologically, as described by Solomon et al. (2002).

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* d= unknown dichromatic phenotype; 543, 556, 563= peak sensitivity of medium/long wavelength cone(s) in this animal

Animals were obtained from the Australian National Health and Medical Research Council (NHMRC) combined breeding facility. All procedures used conform to the provisions of the Australian NHMRC code of practice for the use and care of animals.

#### 3.2.1 LGN Injections

The animal was first anaesthetized with isoflurane (Forthane, Abbott, Sydney, Australia; ICI, 1-2%), intramuscular ketamine (30 mg/kg) and xylazine hydrochloride (Rompun, Bayer Animal Health Australia, Pymble, NSW; 2-4 µg/kg). The animal was then prepared for retrograde tracer injections (for details, see Section 2.2). Briefly, anaesthesia was maintained using sufentanil citrate (Sufenta-Forte, Janssen-Cilag, Beers, Belgium; 4-8 µg/kg), infused at a rate of 0.5-1.5 ml/h in dextrose ringer solution (Baxter, Sydney, Australia), and a 70%: 30% mixture of N₂O: carbogen (5% CO₂ in O₂). The animal was mounted in a stereotaxic frame, and a craniotomy was made over
the dorsal lateral geniculate nucleus. A microelectrode (parylene-coated tungsten or glass-coated steel, impedance 5MΩ; F. Haere Co., Bowdoinham, ME, USA) was lowered to the position of the LGN, and multiunit visually evoked activity was recorded.

The electrode was advanced through the LGN, and each recording location was classified as either the parvocellular, magnocellular or koniocellular layers, based on the contrast, movement and chromatic selectivity of the single or multi-unit visually evoked activity. In addition, the dominant eye, and position of the multiunit receptive field within the visual field was noted (Kaas et al., 1978; White et al., 1998). After a preferred injection site was located, the recording electrode was withdrawn and replaced with a micropipette (tip 25-80 µm; drawn from a glass capillary tube with 0.86 mm inner diameter, 1.5 mm outer diameter). This was filled with a solution of 2, 5 or 10% biotinylated dextran, tetramethylrhodamine conjugate (“Microruby”; Molecular Probes, Eugene, OR, USA). The micropipette was lowered to the target region, and multi-unit activity was recorded to confirm the visual field location. Injections were made by iontophoresis, using a positive pulsed current (6 s on, 6 s off; 3-15 µA) for 30 to 120 minutes.

Following these injections, 50 to 90 hours were allowed for retrograde transport of the tracer. During this time, unrelated electrophysiological experiments were carried out. The animal was then overdosed with sodium pentobarbitone (80-150 mg/kg, i.v.). The eyes were removed and processed as described below. The animal was perfused through the aorta with 0.25 l of saline (0.9% NaCl) followed by 0.3 l of freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer (PB), then 0.25 l of 10% sucrose in PB. The brain was removed, and postfixed in 4% paraformaldehyde in PB for 12 hours, before being transferred to 30% sucrose for 24-72 hours. In some animals, 10% and 20% glycerol in PB was substituted for the sucrose. The brain was sectioned in the coronal plane using a freezing microtome. The sections were processed alternately, either for Nissl substance, for visualization of diaminobenzidine hydrochloride (DAB; Sigma, St Louis, MO), or mounted in Mowiol with no histochemical processing (Harlow & Lane, 1988; Hoechst, Sydney, Australia). The LGN injection site was reconstructed from analysis of these sections, as described below.
DAB staining used the avidin-biotin method. Brain sections were rinsed several times in phosphate-buffered saline (PBS), then incubated in 0.2% Triton X-100 in 0.1 M PB for 1.5 hours. The tissue was then incubated in the avidin-biotin complex (ABC Elite; Vector Laboratories, Burlingame, CA, USA) overnight, and then rinsed again in PBS. The sections were then incubated, on ice, in 0.02% DAB-Nickel and 0.003% hydrogen peroxide in 0.1 M Tris HCL (pH 7.6).

3.2.2 In vitro photofilling

The retina was removed from the eye and placed in carboxygenated Ames’ tissue culture medium (Sigma). The pH of the Ames’ medium was monitored using phenol red indicator dye (Sigma; target pH was 7.4). Each retina was cut into 2-4 segments. Each segment was placed, photoreceptor side down, onto a piece of Millipore filter (AABP 02500, 0.8 µm pore size; Millipore Co., Bedford, MA, USA), then transferred to a superfusion chamber (Warner Instruments, Hamden, CT, USA). The chamber was maintained at 38°C and the tissue was superfused with carboxygenated Ames’ medium, at a rate of 1-2 ml/min. The chamber was mounted on the stage of a Zeiss (Oberkochen, Germany) Axioskop 2 fixed stage microscope and viewed through a 40x water immersion objective. Cells were photofilled by illumination (normally for 1-2 minutes) with a dual-band Lucifer Yellow/Rhodamine filter (Chroma filter set 51016), as described by Dacey et al. (2003).

Following photofilling, the retinal piece was removed from the superfusion chamber and fixed for one or two hours in 4% paraformaldehyde in 0.1 M PB at 4°C, then rinsed, mounted and coverslipped in Mowiol. Alternatively, fixed pieces were processed for visualization of DAB. Retinal pieces were incubated in 0.2% Triton X-100 in 0.1 M PB for 3 hours. The tissue was rinsed in 0.1 M PB, incubated in the avidin-biotin complex (ABC Elite) overnight, and then rinsed again. Labelled cells were visualized by incubation in 0.05% DAB and 0.01% hydrogen peroxide in 0.1 M PB and subsequently intensified with nitro blue tetrazolium (Vaney, 1992). Following this reaction, pieces were rinsed in phosphate-buffered saline, and then mounted in Mowiol.

Some retinal pieces were double-labelled using an antibody specific for the carbohydrate epitope CD15 (antibody provided by Prof J. Mai, University of
Düsseldorf, Germany). In the marmoset, this antibody labels flat midget bipolar, and diffuse bipolar type 6 cells (Chan et al., 2001b). Staining protocol is as per Jusuf et al. (2006b). Briefly, retinal pieces were freeze-cracked, rinsed in PB, then preincubated for 60 minutes in PBS containing 5% bovine serum albumin (BSA), and 0.5% Triton X-100. The sections were then preincubated for 30 minutes in 5% Chemiblocker (Chemicon International, Temecula, CA, USA) in PBS containing 5% BSA and 0.5% Triton X-100. The retinal pieces were then incubated for 14 days in the primary antibody, which was diluted in either 5% Chemiblocker in PBS containing 0.5% Triton X-100, or in PBS containing 1% BSA, 0.5% Triton X-100, and 0.05% sodium azide. Retinal pieces were rinsed in PBS then incubated in secondary antibodies overnight (goat antimouse IgG coupled to Alexa 488; Molecular Probes). The secondary antibody was diluted 1:500 in PBS containing 0.5% Triton X-100 or PBS containing 1% BSA and 0.5% Triton X-100. Following immunostaining, the retinal piece was mounted as stated above.

3.2.3 Analysis

The injection site was reconstructed, as detailed in Section 2.2. The proportion of the injection site contained within each LGN layer (PC/KC/MC) was then quantified: for each section that contained tracer, the area of tracer contained within each LGN division (PC, MC or KC) was measured. The summed area of injection site contained within each division (PC, MC or KC) was then divided by the total area (PC area + MC area + KC area).

Cell counts were made within the patch of retrogradely labelled retinal ganglion cells. The majority of cells in each experiment could not be classified due to insufficient labelling (typically, only the cell soma and one or two primary dendrites were labelled). Fully- and (where possible) partially-filled cells were classified as parasol, midget or widefield cells, using their morphology, somal and dendritic size as criteria. These cell counts were used to elucidate the proportion of labelled cells that were midget, widefield, or parasol cells. See Section 2.3 for descriptions of the morphological identification of midget and parasol cells. Any non-midget, non-parasol cells were classified as widefield cells.
Of these cells, only the fully-filled widefield cells (56/182) were used for further morphological analysis. Photographs of widefield cells were taken using a Zeiss Axiocam HRc colour digital camera mounted on a Zeiss Axioplan 2 microscope, under oil immersion with a 40x or 63x objective. A sample of midget (n=48) and parasol cells (n=59) was also photographed, for comparison. A Z-stack of 40-100 digital images at 0.3 µm spacing was acquired, under both fluorescent illumination using a rhodamine filter block, and bright-field illumination using differential interference contrast (DIC) optics. Custom software written in MatLAB (Image processing toolbox, Mathworks, Natick, MA, USA) was used to analyse each stack of images, as follows.

For each cell, the dendritic field diameter was measured by tracing a convex polygon around the outermost tips of the dendritic field. An ellipse with the same area and orientation as the polygon was calculated. The dendritic field diameter is the average diameter of this ellipse. Dendritic branch density (DBD) is a new measure, designed to estimate efficiently the density of dendrites within the dendritic field of a cell. Dendritic branch density was measured by centering a sampling circle close to the centroid of the cell's dendritic field. A circle diameter of 75% of the minimum dendritic field diameter was chosen, in order to avoid sampling primary and secondary dendrites. All dendrites that crossed the sampling circle were counted. The resultant number was divided by the circumference of the sampling circle to give a number of intersects (or dendrites) per micron.

For the dendrites that crossed the sampling circle, the z-depth of the crossing was also recorded; this was compared to the z-depths of the borders of the inner nuclear layer and ganglion cell (measured using unlabelled somata, from the DIC images). The border of the ganglion cell layer was defined as the outermost point of the plasma membrane of all ganglion cell somata; the border of the inner nuclear layer was defined as the innermost point of the plasma membrane of all bipolar cell somata. Comparison of the dendritic depth of stratification with the nuclear borders resulted in a stratification percentage for each sample point, where 0% refers to the inner nuclear layer and 100% refers to the ganglion cell layer. The mean stratification of all dendrites was then calculated; for bistratified cells, the average is weighted by the differing dendritic field diameters of the inner and outer tiers, using this formula:
Chapter 3 – Koniocellular-projecting ganglion cells

\[
\text{mean stratification} = s_1 - \left( (s_1 - s_2) \times \frac{\text{area}(s_1)}{\text{area}(s_1) + \text{area}(s_2)} \right)
\]

Where \(s_1\) refers to the inner tier of dendrites of the small bistratified cell, and \(s_2\) refers to the outer tier of dendrites. \(\text{Area}(s_1)\) refers to the dendritic field area of \(s_1\). Thus, the mean stratification depth for all cells refers to the centroid (in the \(z\) dimension) of that particular cell.

For some widefield ganglion cells, tracings of the cell soma and dendritic tree were made using acetate sheets at a final magnification of 1,100× or 1,800×. These were then scanned using a flatbed scanner and made into vector graphics using the Adobe Illustrator graphics program.

3.3 Results

3.3.1 Retinal projections to the LGN

Figure 3.1 shows a series of Nissl-stained coronal sections through the marmoset LGN. Laminae are defined by cell body size – KC cells have the smallest cell bodies, MC cells have the largest, and PC cells have a cell body size intermediate to MC and KC cells. Numbers in the top right corner of each image indicate the approximate distance anterior to the interaural axis, calculated by reference to the marmoset brain atlas of Stephan et al. (1980).

In common with most primates (Casagrande and Norton, 1991), the marmoset LGN primarily consists of two PC and two MC layers. The two PC layers are larger, and extend further anterior than the MC layers – for example, MC layers are not present in Figure 3.1D, where there are still obvious PC layers evident. There are a number of KC (‘intercalated’) layers – one layer ventral to each PC or MC layer. There are thus four koniocellular layers in the marmoset LGN, referred to as K1-K4 hereinafter (K1 being the most ventral, K4 being the most dorsal: see Figure 3.1E, F). The most distinct KC division in the marmoset is K3, between the PC and MC layers, and for simplicity, this is the only KC layer labelled in Figure 3.1A-D and Figure 3.2.
**Figure 3.1.** Coronal sections through the marmoset lateral geniculate nucleus (LGN), showing the layered structure of the LGN at 4 different locations from posterior (A) to anterior (D). The number at the top-right of each picture shows the distance (in mm) anterior to the interaural axis. The parvocellular (PC), magnocellular (MC) and koniocellular (KC) layers of the LGN are indicated. There are no magnocellular layers in the most anterior section. (E) and (F) illustrate the KC layers in the marmoset, and the nomenclature used to refer to each intercalated layer. Scale bar: 1 mm.
Figure 3.1. Coronal sections through the marmoset lateral geniculate nucleus (LGN), showing the layered structure of the LGN at 4 different locations from posterior (A) to anterior (D). The number at the top-right of each picture is the distance (in mm) anterior to the interaural axis. The parvocellular (PC), magnocellular (MC) and koniocellular (KC) layers of the LGN are indicated. There are no magnocellular layers in the most anterior section. (E) and (F) illustrate Kaas' (1978) nomenclature for the KC layers in the marmoset. All scalebars: 1 mm.
The peripheral retinal representation is found towards the anterior lobe of the LGN, and the foveal representation is located towards the posterior lobe of the LGN (see White et al., 1998 for the full retinotopy). Most of the tracer injections made in this study were aimed away from the two extremes, in order to label retinal ganglion cells located away from the foveal region, and to have a clear reconstruction of each injection site (due to the unclear delineation of the layers in anterior LGN, see Figure 3.1). Non-foveal ganglion cells were desired since for most cell types, ganglion cell morphology has been better characterised away from the fovea.

Injections were targeted at one region of the LGN – for example, some injections aimed to label both PC layers; others, koniocellular layer K3. There was always a small amount of spread of tracer into neighbouring layers. This is largely unavoidable, considering that the KC layers are intercalated between the PC and MC layers. Figure 3.2 shows an example of an injection mostly localised to the PC layers (Figure 3.2 A-C), and an injection mostly localised to the MC layers (Figure 3.2 D-F). The left panels (A, C) show the coronal Nissl-stained LGN sections, which were used to delineate the layers of the LGN; the right panels (C, F) show the neighbouring fluorescent sections, where the injection site and the fibres leading away from it are visible; and the middle panels (B, E) show the schematic composite of the two.

A summary of all of the injection sites analysed in this study is presented in Figure 3.3. The dark grey regions indicate the area of each injection site. Numbers indicate an animal identifier that can be used for comparison with Figure 3.4, Figure 3.5, and Table 3.2. The light grey regions indicate schematic outlines of the PC and MC layers at different coronal slices, from the anterior to the posterior end of the LGN. Note that as indicated in Figure 3.1D, towards the anterior lobe of the LGN, the MC layers are not present, and the borders of the PC layers become harder to define.
Figure 3.2. Reconstruction of two injection sites: one localised to the parvocellular (PC) layers (A-C), one localised to the magnocellular (MC) layers (D-F). The Nissl stained coronal sections (A,D) and the neighbouring fluorescent sections (C,F) were used to create schematic composites (B,E). In the composites, the light grey regions indicate the PC and MC layers of the LGN, while the dark grey regions indicate the injection location. Scale: 1 mm.
Figure 3.3. Injection reconstructions for the 13 animals used in this study. There are seven schematic coronal sections of the LGN, moving from posterior (left) to anterior (right). The boxed number underneath each section indicates the approximate distance (in mm) anterior to the interaural axis. Light grey regions indicate schematic outlines of the PC and MC laminae at that anterior-posterior location. Each dark grey region indicates the injection site for one experiment, and is labelled with the experiment number. In cases where there were multiple injections in one experiment, this is indicated by a bracketed suffix – e.g. 88(2). Most injections were targeted around AP 4 - 5 mm.
Figure 3.4. Distribution of labelled retinal ganglion cells after the injection in Experiment 103. Gray lines indicate the outline of the retinal piece; the two major retinal landmarks, the fovea (F) and optic disk (OD) are also indicated. The positions of labelled midget, parasol, and widefield cells are marked (see key). In this injection, widefield and midget cells were the majority of those identified.
Figure 3.4 shows the distribution of ganglion cells in the two retinas following the LGN injection in experiment 103; this injection was mostly localised to the KC layers (see Figure 3.3). The outlines of the retinal pieces and the major retinal landmarks are shown. The cells in each retina fall in corresponding quadrants, as expected. The distribution of midget, parasol and widefield cells from this experiment is indicated. In experiment 103, mostly midget and widefield cells were labelled, with very few parasol cells.

**Table 3.2: Distribution of LGN projection of ganglion cells**

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>109</th>
<th>107</th>
<th>108</th>
<th>88</th>
<th>89</th>
<th>97</th>
<th>96</th>
<th>113</th>
<th>115</th>
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<td>LGN injection site</td>
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<tr>
<td>Parvocellular (%)</td>
<td>96</td>
<td>85</td>
<td>79</td>
<td>76</td>
<td>74</td>
<td>72</td>
<td>67</td>
<td>34</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>n.d.</td>
</tr>
<tr>
<td>Koniocellular (%)</td>
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<td>14</td>
<td>18</td>
<td>24</td>
<td>3</td>
<td>28</td>
<td>29</td>
<td>46</td>
<td>71</td>
<td>67</td>
<td>22</td>
<td>n.d.</td>
</tr>
<tr>
<td>Magnocellular (%)</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>29</td>
<td>33</td>
<td>78</td>
<td>0</td>
</tr>
<tr>
<td>Outside LGN (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>23</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
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Labelled retinal ganglion cells - contralateral eye

| Midget (%) | 99  | 91  | n.d. | n.d. | 95  | 38  | 62  | 89  | 39  | 45  | n.d. | 60  |
| Widefield (%) | 1   | 9   | n.d. | n.d. | 0   | 57  | 35  | 6   | 50  | 53  | n.d. | 38  |
| Parasol (%) | 0   | 0   | n.d. | n.d. | 5   | 5   | 3   | 5   | 11  | 2   | n.d. | 2   |
| n | 298 | 148 | n.d. | n.d. | 21  | 21  | 69  | 101 | 28  | 38  | n.d. | 63  |

Labelled retinal ganglion cells - ipsilateral eye

| Midget (%) | 98  | 95  | 93  | 96  | 90  | 97  | n.d. | 90  | 51  | 53  | 15  | 70  |
| Widefield (%) | 2   | 3   | 3   | 2   | 5   | 3   | n.d. | 4   | 38  | 40  | 6   | 18  |
| Parasol (%) | 0   | 2   | 4   | 2   | 5   | 0   | n.d. | 6   | 11  | 7   | 79  | 12  |
| n | 123 | 205 | 285 | 750 | 37  | 108 | n.d. | 210 | 84  | 30  | 47  | 17  |

n.d. = not determined

In Table 3.2 and Figure 3.5A, the proportion of the injection site that was localised to each LGN division is quantified. Figure 3.5B shows the corresponding proportions of retinal ganglion cell classes labelled following each injection. Two points are immediately apparent: first, there are always midget cells labelled after injections in the LGN, even for injections wholly localised to the MC and KC layers. This will be discussed below (Section 3.4.1). Secondly, there is correspondence between the region labelled by the injection site, and the classes of ganglion cells that were labelled. In other words, after injections localised to the PC layers (e.g. experiment 109, 89), almost all labelled retinal ganglion cells are midget cells; after injections localised to the MC...
layers (e.g. experiment 111), a great proportion of the labelled cells are parasol cells. For those injections that were largely localised to the KC layers (e.g. experiment 103, 115) a significantly higher proportion of widefield ganglion cells were labelled. A linear regression comparing the proportion of injection localised to the KC layers, versus the proportion of widefield cells labelled, shows a high correlation (correlation coefficient 0.81, $r^2 = 0.66$). Furthermore, following 5 injections that were more than 25% localised to the KC layers, $27.8 \pm 18.7\%$ of labelled ganglion cells were widefield cells; from the remaining 6 injections, $3.57 \pm 2.1\%$ of labelled ganglion cells were widefield cells ($p=0.0173$, Wilcoxon rank-sum).

These data suggest that there is a bias in projection of widefield ganglion cells to the KC layers. Further evidence for this bias can be determined by considering the relationship of the injection site in each animal, compared to the eye-dominant layers of the LGN. To explain: it is well known that each PC and MC layer is dominated by a monocular input (Kaas et al., 1978; Spatz, 1978). Thus, in the marmoset, there is one contralateral and one ipsilateral PC layer, and one contralateral and one ipsilateral MC layer, as shown in Figure 3.5C. K3 is thought to either receive a dominant projection from the contralateral eye (Kaas et al., 1978; Spatz, 1978), or a projection from both eyes (Fitzpatrick et al., 1983). Since K3 is flanked by two ipsilateral layers (one PC, one MC), it is possible to make localised injections that label only (contralateral) layer K3 and an ipsilateral PC or MC layer. If widefield cells are biased towards projecting to the KC layers, such an injection (Figure 3.5C) should, therefore, predominantly label PC or MC ganglion cells in the ipsilateral eye, and KC ganglion cells in the contralateral eye.

As predicted, following the injection shown in Figure 3.5C, there was a higher proportion of widefield ganglion cells in the contralateral eye, and conversely, a higher proportion of midget cells in the ipsilateral eye. Furthermore, this trend in ganglion cell labelling was consistently recorded following all injections that labelled only the ipsilateral PC/MC layers, and K3 (see Table 3.2: experiments 97, 103, 107 and 113). Thus, these data supports the earlier conclusion: there is a bias in the projections of widefield cells to the KC layers.
Figure 3.5. Quantification of LGN injection sites, and retinal ganglion cell counts. A shows the proportions of each injection site in each LGN division: parvocellular (PC, cyan bars), koniocellular (KC, open bars) and magnocellular (MC, purple bars). The distribution of labelled retinal ganglion cells, following these injections, is shown in B: midget cells (cyan bars), widefield cells (open bars) and parasol cells (purple bars). Midget cells were labelled after all injections. Injections more confined to the koniocellular layers yield a higher proportion of widefield cells. C shows a sample injection site from experiment 97 (with the injection site shown in red). Following this injection, there was a higher resultant proportion of widefield cells in the contralateral eye, consistent with the differing eye dominance of the LGN layers (c=contralateral input, i=ipsilateral input).
3.3.2 Morphology of labelled widefield cells

About one-third (56/182) of the identified widefield cells were sufficiently well-labelled for morphological analysis. Three separate types of widefield ganglion cell were identified – the small bistratified (n = 31), sparse (n = 13) and hedge (n = 3) ganglion cells. In addition, a number of cells (n = 9) were labelled that did not belong to the small bistratified, hedge or sparse cell classes: these cells had heterogeneous morphology, stratification, dendritic field size and density (for an example, see Figure 3.8, upper-right). These cells are referred to as “unclassified widefield cells” in the following figures.

Measurements of the dendritic field size, density, and stratification of the different cell types will be presented later in this section; first, a qualitative appraisal of the morphology of the different cell types will be outlined. Photomicrographs of labelled widefield cell types are shown in Figure 3.6; drawings of small bistratified cells are shown in Figure 3.7; and other labelled widefield cells (hedge, sparse and unclassified) appear in Figure 3.8.

The majority of the small bistratified, hedge, and sparse ganglion cells had a dendritic field diameter of the same size or larger than parasol cells at equivalent eccentricity (see Figure 3.10). Small bistratified cells (n = 31) were distinguished by their dense, spiny, inner-stratifying dendritic tier (Figure 3.6B; Figure 3.7, black dendrites), and their sparser, much smaller outer-stratifying tier (47.2 ± 15.2% of the size of the inner tier; see Figure 3.6A; Figure 3.7, red dendrites). Hedge cells (n = 3; Figure 3.6 C and D; Figure 3.8, lower left) were qualitatively identified by their dense, broadly stratifying dendritic fields, comprising very thin, highly recurving dendrites. Finally, large sparse cells (n = 12; Figure 3.6E; Figure 3.8, lower right) had very few, dispersed dendrites, which seldom branched, in a dendritic field that stratified close to the ganglion cell layer (mean stratification depth: 79 ± 4.7%). One example of a, much larger, giant sparse cell was also seen (Figure 3.6F; Figure 3.8, upper left). The large and giant sparse cells were morphologically similar to the classes of the same name described by Dacey et al. (2003) in macaque.
Figure 3.6. Photomicrographs of widefield cell classes identified in this study. Eccentricity (distance from the fovea) is indicated in the bottom left of each picture. (A, B), Small bistratified cell: (A) shows the outer tier, (B) shows the inner tier. (C), Hedge cell. The region indicated is shown at higher magnification in (D). (E), Large sparse cell. (F), Giant sparse cell. Arrowheads indicate labelled midget cells. Scale bars: B =10 µm; C, E=20 µm; D=5 µm; F=50 µm.
Figure 3.7. Small bistratified cells (inner tier, black; outer tier, red) labelled after injections in either the parvocellular layers (*), koniocellular layers (**), or mixed injections (no asterisk). Cells show common morphology despite the differing injection locations. Eccentricities are indicated beneath each cell. Arrows indicate axons.
Figure 3.8. Other labelled widefield cells showed a variety of morphologies. Clockwise from upper left: a giant sparse cell (same cell as Figure 6, panel F); an unclassified widefield cell, which resembles the thorny cell of Dacey et al. (2003); a large sparse cell; and a hedge cell. Inset (lower right) shows a midget cell from a comparable eccentricity. Eccentricities are indicated beneath each cell. Arrows indicate axons.
Previous studies using intracellular injection techniques have been unable to label small bistratified cells close to the fovea (< 2 mm eccentricity) in either the marmoset (Ghosh et al., 1996; Ghosh et al., 1997) or the macaque (Dacey, 1993a); the authors attribute this difficulty to the thickness of the nerve fibre layer close to the fovea. However, the thickness of the nerve fibre layer is not a limitation when photofilling. Thus, the present study demonstrates the morphology of foveal small bistratified cells for the first time. Drawings of these small bistratified cells can be seen in Figure 3.7. Taking into account a scaling of dendritic field size due to eccentricity, it can be seen that foveal small bistratified cells retain the characteristic morphology of their peripheral counterparts. Also note that small bistratified cells labelled following primarily PC injections (> 95% PC; indicated with an asterisk in Figure 3.7) bear no obvious differences in morphology to small bistratified cells labelled after primarily KC injections (> 65% KC; indicated with a double asterisk) or mixed injections (no asterisk).

In macaque, the two classes of sparse ganglion cells stratify in stratum 5 of the inner plexiform layer (close to the ganglion cell layer: Dacey et al., 2003). In the marmoset and macaque, the axons of the diffuse bipolar type 6 (DB6), blue cone bipolar, and rod bipolar cells terminate in stratum 5 (Mariani, 1984; Boycott and Wässle, 1991; Ghosh et al., 1997; Chan et al., 2001b; Chan et al., 2001a; Jusuf et al., 2004). Thus, an immunohistochemical stain that labels the DB6 cell was used, to confirm that the large sparse cell in the marmoset stratifies in the same sublayer of the inner plexiform layer as the large sparse cell in the macaque. Figure 3.9 shows images of a double-labelled preparation, with the large sparse ganglion cell from Figure 3.6E in red and DB6 bipolar cells axons in green. The two cell types co-stratify in stratum 5 of the inner plexiform layer. There is no apparent co-fasciculation, though the dendrites of these two cell types do cross at several points (arrows). The synaptic connectivity of the DB6 cell and large sparse cell were not quantified in this study, as at the light-microscopic level, this would require a triple-label with a synaptic marker.
Figure 3.9. The axon terminals of diffuse bipolar type 6 (DB6) bipolar cells costratify, but do not cofasciculate, with the dendrites of the large sparse ganglion cell. (A) shows an inset region of the large sparse cell from Figure 6E. The boxed region is shown in (B, C, D). (B), axon of one DB6 bipolar cell. (D), Dendrites of the large sparse ganglion cell. (C) shows the superposition of both channels. Regions where the DB6 axons cross the sparse cell dendrites are indicated by arrows. Scale bars: $A = 20 \, \mu m; \quad C = 10 \, \mu m$. Scale bar for $C$ applies to $B, D$. 
Finally, a number of morphological attributes were quantified, in an attempt to distinguish neurons of the various widefield types. Figure 3.10 shows all of the morphological parameters measured in this study. These were: the dendritic field diameter (Figure 3.10A), dendritic branch density (DBD; Figure 3.10B), and depth and breadth of stratification in the inner plexiform layer (Figure 3.10C and D). Only cells between 2 and 5 mm eccentricity were considered for statistical analysis, to minimize the effect of foveal distortions, and changes due to eccentricity. All p values referred to in the following paragraphs are from a Wilcoxon rank-sum test.

Each type of widefield cell is statistically separable from parasol cells on most of these plots. Small bistratified cells differ significantly from parasol cells in all four measures (p < 0.001 for all tests); large sparse cells have significantly different dendritic field size, DBD and stratification depth to parasol cells (p < 0.01 for all tests); and hedge cells have a significantly larger dendritic field diameter and stratification breadth, compared to parasol cells (p = 0.022 and p = 0.018 respectively).

The best test to discriminate between the various types of widefield cell is usually related to the most striking morphological feature of each widefield cell type. For example, DBD is a good test to discriminate hedge cells from small bistratified cells (p = 0.023) and large sparse cells (p = 0.044), while breadth of stratification easily discriminates small bistratified cells from large sparse cells (as the small bistratified cells have two tiers: p = 0.003). In addition, dendritic field stratification depth proved able to separate small bistratified cells from hedge cells (p = 0.023). Excluding the single giant sparse cell, none of the widefield cell types could be distinguished on the basis of dendritic field diameter alone.
Figure 3.10. Dendritic field diameter (A), dendritic branch density (DBD; B) and depth and breadth of stratification (C, D) of the cells measured in this study. With regards to stratification depth, 100% refers to the ganglion cell layer; 0% refers to the inner nuclear layer. The widefield cell types found in this study have dendritic fields that are the same size, or larger than, parasol cells of equivalent eccentricity, and have dendritic fields that are less dense than parasol or midget cells. Small bistratified (SBS) and sparse cells stratify close to the ganglion cell layer, while hedge cells straddle the ON/OFF subdivision of the inner plexiform layer (50%). Small bistratified cells have the broadest stratification (since they have two tiers), followed by hedge cells, then sparse, parasol and midget cells.
3.4 DISCUSSION

In this study, widefield ganglion cells were shown to preferentially innervate the koniocellular layers of the LGN. Two main classes of ganglion cell form this projection: small bistratified and sparse ganglion cells. These ganglion cell types are distinguishable from midget and parasol ganglion cells by a number of morphological criteria.

3.4.1 Projection of widefield ganglion cells to the LGN

The present study is the first to examine the projection of small bistratified cells directly, via tracer injections into the KC and PC layers of the LGN. Rodieck and Watanabe (1993) first labelled small bistratified cells following retrograde tracer injections, however their injections included both the PC and KC layers. Dacey and Lee (1994) showed, using intracellular recordings, that the small bistratified cell has a distinctive blue-ON/yellow-OFF response type – it responds to increments of contrast in a stimulus modulated along a tritanopic confusion line (a condition where only the S-cones are modulated). LGN neurones with the same blue-ON/yellow-OFF response type are mainly segregated to the KC layers in the marmoset (Martin et al., 1997), though one-third of the blue-ON/yellow-OFF neurons identified by Martin and colleagues (5/13) were recorded from the PC layers.

Thus, previous evidence implies that small bistratified cells preferentially innervate the KC layers, though not exclusively. The data from this study are consistent with this assertion. Small bistratified cells were labelled after injections that were largely segregated to the PC layers; they were also labelled after injections largely segregated to the KC layers (see Figure 3.7). However, there was a bias towards labelling more small bistratified cells following a KC injection. Fifteen of the small bistratified cells identified in this study were labelled following 6 injections that were > 25% localised within the KC layers (this includes 3 injections that had no tracer within the PC layers). Compared to the total number of labelled neurons in these injections, small bistratified cells comprised 2.0 % of the total neurons labelled (15/736) following KC injections. 13 small bistratified cells were labelled from the remaining 5 injections: these injections were all > 70% localised to the PC layers. Small bistratified
cells thus comprised 0.7% of the total neurons labelled (13/1886) following PC injections. Since higher proportions of small bistratified cells are seen following injections even partially localised to the KC layers, the tentative conclusion is that there is a higher proportion of small bistratified cells projecting to the KC layers.

Note that these numbers will grossly underestimate the true proportion of small bistratified cells, as only fully labelled small bistratified cells were counted; partly filled small bistratified cells would have been recognised as ‘widefield’ cells in the cell counts, but not taken for further morphological analysis, and thus not identified as small bistratified cells.

An interesting result of the tracing study was that midget ganglion cells were labelled after all injections, even including injections that had no tracer in the PC layers (experiments 103, 111, and 105: mean proportion of midget cells labelled from these three injections was 37.2 ± 19.3%). Dextran-conjugated tetramethylrhodamine is both an anterograde and retrograde tracer, and it is also taken up by broken fibers of passage (Vercelli et al., 2000) – that is, axons transected by the injection pipette, which project to more posterior regions of the LGN. Since midget cells form roughly 80% of the retinal projection to the LGN (Perry et al., 1984; Ahmad and Spear, 1993), one might assume that of these labelled fibres of passage, 80% will be fibres of midget cells (assuming an even distribution of these fibres within the fibre bundles); thus all injections would label a small proportion of midget cells, labelled from transected axons. This prediction would imply that in injections largely restricted to the KC and MC layers, most labelled midget cells should be located more foveally in the retina (since cells labelled from transected axons should arise from more posterior regions of the LGN). Analysis of the data from Figure 3.4 shows that midget cells were, on average, slightly closer to the fovea (mean eccentricity ± SD: midget cells, 3.47 ± 1.28 mm, n = 66; widefield cells, 3.892 ± 1.171 mm, n = 23) though this difference was not significant (p = 0.38, Wilcoxon rank-sum).

Alternatively, like the bias for widefield cells to project to the KC layers of the LGN, midget cells may be mostly segregated to the PC layers, but sparsely present in other layers also. To discriminate between these alternatives, an experiment like that described by FitzGibbon et al. (1983) is advised for future studies. FitzGibbon and
colleagues found that by leaving their injection micropipette in the brain for 12 hours before injecting their tracer, the labelling of broken axons of passage by HRP could be greatly reduced. The authors surmise that after this time period, damaged axons have either healed or degenerated sufficiently that most axons will not take up the tracer.

Results of the present study show a heterogeneous population of widefield ganglion cells that project to the KC layers; corroborating evidence has previously been found in the physiology of KC LGN neurones, where the diverse physiological properties are thought to imply a diversity of retinal inputs. Norton and Casagrande’s (1982) electrophysiological study of the KC layers of the bush baby (*Galago crassicaudatus*) showed that cells in the KC layers were heterogeneous in many attributes: in their receptive field structure (47% of KC cells had a typical centre-surround receptive field); in their response to standing contrast; in their response to moving targets (25% of KC cells responded to rapidly moving targets); and in their antidromic and orthodromic activation latencies (3.9 ± 1.2 ms and 4.7 ± 2.1 ms, respectively). Response properties of cells in the PC and MC layers were far more homogenous. White et al. (2001) found a similar result in the marmoset LGN – cells in the KC layers had heterogeneous receptive field dimensions, contrast sensitivity and linearity of spatial summation.

### 3.4.2 Labelled retinal ganglion cell types

Small bistratified cells were the most commonly labelled cell type among the widefield cells in this study. The morphology of marmoset small bistratified cells has been previously shown to be similar to that of the macaque (Ghosh et al., 1997), with the distinction that the marmoset small bistratified cell has a proportionately smaller outer tier than that of macaque. This smaller outer tier is thought to be due to the higher cone densities in marmoset (Ghosh et al., 1997); the hypothesis is that due to the higher cone densities, the small bistratified cell in the marmoset does not require as large a dendritic tier to receive an equivalent amount of L/M cone input to the small bistratified cells in macaque. The blue ON/yellow OFF physiology of the small bistratified cell has already been discussed (Section 3.4.1).

Ghosh et al. (1996) have previously labelled hedge cells by intracellular injection in marmoset retina. Hedge cells appear to be most similar to the T class of ganglion
cells: a similarly dense, broadly stratifying (sometimes bistratified) cell class found in macaque by Rodieck and Watanabe (1993). Rodieck and Watanabe (1993) found that T cells were labelled after injections to the superior colliculus. Assuming that hedge cells are homologous to the T cells in macaque, it is possible that the hedge cells in this study were labelled from fibres en route to the colliculus. Alternately, hedge cells may have two projection targets – the LGN and the superior colliculus. Little is known about the physiological response properties of either hedge cells in the marmoset, or T cells in macaque.

The large and giant sparse cells labelled in this study are similar in morphology and stratification to the large and giant sparse cells labelled in macaque retina by Dacey et al. (2003; 2005); hence, the same naming scheme has been adopted. Assuming that these are homologous cell types, it is known that both the large and giant sparse cells in macaque exhibit a blue-OFF/yellow-ON physiology (Dacey, 2004; Dacey et al., 2005). If these widefield cells project to the KC layers, as is demonstrated here, then cells with a blue-OFF/yellow-ON receptive field type should be recorded from within the KC layers of the LGN. Cells with a blue-OFF/yellow-ON physiological signature have been seldom reported in the LGN (Wiesel and Hubel, 1966; Derrington et al., 1984; Valberg et al., 1986; De Valois et al., 2000). This question will be further addressed in Chapter 4.

In summary, projection of several different classes of widefield cells to the koniocellular layers of the LGN is consistent with the heterogeneous physiological cell types recorded from these layers. This study provides anatomical support for what was previously only inferred – that processing of blue-ON signals is mediated by the koniocellular pathway.
Chapter 4

Geniculocortical relay of blue-OFF signals in the primate visual system

4.1 Introduction

This chapter investigates the physiological properties and anatomical location of blue-OFF signals in the dorsal lateral geniculate nucleus (LGN). Single-cell recordings from the macaque retina and LGN have shown that the great majority of colour selective cells are red-green selective and are found in the dorsal (parvocellular; PC) layers. A small proportion of cells in the LGN show blue-ON responses (Wiesel and Hubel, 1966; De Monasterio and Gouras, 1975; Derrington et al., 1984). The blue-ON ganglion cells in the retina show a distinct bistratified morphology (see Figure 3.7: Rodieck and Watanabe, 1993; Dacey and Lee, 1994; Dacey and Packer, 2003), and there is evidence that blue-ON signals reach the visual cortex via the koniocellular/intercalated layers of the lateral geniculate nucleus (Martin et al., 1997; Hendry and Reid, 2000; Chatterjee and Callaway, 2003; also, see Chapter 3). Unlike cells in the parvocellular and ventral (magnocellular) layers, the koniocellular layers are comprised of cells with diverse receptive field types (Norton and Casagrande, 1982; Irvin et al., 1986; Irvin et al., 1993; Casagrande, 1994; White et al., 2001), and widespread cortical terminations (Livingstone and Hubel, 1982; Bullier and Kennedy, 1983; Fitzpatrick et al., 1983; Lysakowski et al., 1988; Rodman et al., 2001; Solomon, 2002; Chatterjee and Callaway, 2003; Sincich et al., 2004). It is thought that the koniocellular pathway arose early in the evolution of the visual system, and the blue-ON pathway has thus been considered as a primordial pathway for colour vision (Mollon, 1989; Casagrande and Norton, 1991).

Receptive fields receiving OFF– signals from S cones (blue-OFF) are rarely encountered (Hubel and Wiesel, 1960; Derrington et al., 1984; Valberg et al., 1986), and the receptive field properties and central projections of blue-OFF cells remain poorly understood. The large and giant sparse ganglion cells in macaque retina display blue-OFF type responses, and project to the LGN (Dacey, 2004; Dacey et al., 2005). As detailed in Chapter 3, these ganglion cells preferentially project to the koniocellular
layers of the LGN, meaning the koniocellular pathway could be responsible for carrying blue-ON and blue-OFF signals. Anatomical evidence for a different source of blue-OFF signals was obtained from electron microscopic reconstruction of five S cones in macaque fovea (Klug et al., 2003). These experiments revealed OFF-type connections with midget-parvocellular pathway bipolar cells, which implies that blue-OFF cells (like red-green opponent cells) could form part of the parvocellular pathway.

In this chapter, the location, and temporal and spatial properties of blue-OFF cells were measured in the lateral geniculate nucleus of marmoset monkeys. Unlike macaques and humans, in marmosets the koniocellular layers are well-segregated from the magnocellular and parvocellular layers (Kaas et al., 1978; Goodchild and Martin, 1998), enabling the location of recorded cells to be determined reliably. The results show that blue-OFF cells form part of the koniocellular pathway in marmosets.

4.2 METHODS

Adult marmosets (*Callithrix jacchus*; n=16, weight 318 g – 500 g) were obtained from the Australian National Health and Medical Research Council (NHMRC) combined breeding facility. Seven of the marmosets were female, and of these females, 6 were trichromats. The remaining animals were dichromats. All procedures used conform to the provisions of the Australian NHMRC code of practice for the use and care of animals.

4.2.1 Animal preparation

Animals were prepared for electrophysiological recordings, as detailed in Section 3.2.1. A microelectrode (parylene-coated tungsten or glass-coated steel, impedance 11 MΩ; F. Haere Co., Bowdoinham, ME, USA) was then lowered to the position of the LGN, and extracellular, single-unit recordings were made. Each visually responsive cell had its receptive field position mapped, and then the responses to test stimuli (described below) were measured. Electrolytic lesions were placed on some electrode tracks, to aid the reconstruction of the position of recorded neurones. These lesions were made by passing a current of 3-6 µA, for 3 to 6 seconds, through the recording electrode using an AM systems Isolated Pulse Stimulator (Carlsborg, WA, USA).
4.2.2 Visual Stimuli

Each cell was characterised using drifting sine-wave grating stimuli, presented in a circular 12 degree aperture, at a mean luminance of 55 cd/m². Visual stimuli were generated using a VSG Series Three video signal generator (Cambridge Research systems, Cambridge, UK), and presented on a BARCO Reference Calibrator Plus monitor at a frame refresh rate of 80 Hz. The optical path length to the monitor was 114 cm. All stimuli were surrounded by a uniform field (outer dimensions approx 20 × 15 degrees), which was composed of the mean luminance of the stimulus.

Responses were first optimised by finding the preferred orientation and contrast of the cell being recorded. Orientation tuning was ascertained by presenting drifting gratings of 16 different orientations, presented in a pseudo-random order. Contrast tuning was measured at the optimum orientation, using ten different stimulus contrasts ranging from 1.6% to 100% contrast. From this curve, a contrast was chosen that lay within the linear-responding contrast range of the cell, and which elicited robust responses (> 20 impulses/second). The spatial and temporal frequency responses of the cell were then quantified, using achromatic or S-cone selective drifting sinusoidal gratings of variable spatial or temporal frequency. Spatial frequencies measured ranged from 0.01 to 12.8 cycles/degree; temporal frequencies measured ranged from 0.5 to 32 Hz.

Peristimulus time histograms were constructed for each presentation. These responses were subjected to discrete Fourier analysis, with the amplitude and phase of the first harmonic (f1) used to characterise cell responses to the stimuli, and the null harmonic (f0) referring to the mean firing rate of the cell.

Cone-selective stimuli were predicted by convolving the spectral power distribution of the CRT phosphors with marmoset cone receptor sensitivity and lens absorption (Tovee et al., 1992) via the Judd-modified CIE 1931 color-matching functions as described (Blessing et al., 2004). Colorimetric data were verified by using a Photo Research PR-650 photometer (Photo Research, CA). The S-cone-selective grating modulated from (CIE [x, y, Y]) [0.3205, 0.2719, 32] to [0.4019, 0.4547, 32]. These values were chosen to strongly modulate the S cones (contrast 0.661) yet limit the contrast delivered to the ML opsins (peak sensitivity 543 nm, 556 nm, 563 nm).
expressed by marmosets (respective contrast 0.045, 0.041, 0.043). Background was held at [0.3612, 0.3633, 32]. To estimate cone balance and melanopsin input, the modulation vector through this background was “tilted” in 64 uniformly spaced directions in CIE chromaticity (x, y) and luminance (Y) as described (Blessing et al., 2004). Achromatic gratings were produced by in-phase phosphor modulation through the same background point or through [0.317, 0.335, 45].

4.2.3 Histological reconstruction of electrode tracks

Following these recordings, the animal was overdosed with sodium pentobarbitone (80-150 mg/kg, i.v.). The animal was then perfused (for details, see Section 3.2.1). The brain was removed, and sectioned in the coronal plane using a freezing microtome. The brain was sectioned and stained as per Section 3.2.1.

Electrode tracks were reconstructed from these sections. The DAB sections showed a dark brown peroxidase reaction along each electrode track. These were overlaid on the neighbouring Nissl sections to localise the electrode track within the layered structure of the LGN. Finally, the anatomical tracks were registered with the physiological recordings, by comparing the receptive field positions and cell recording depths with the known anatomical eye dominance and retinotopy of the layers of the LGN (Kaas et al., 1978; White et al., 1998).

4.3 Results

As expected (Hubel and Wiesel, 1960; Derrington et al., 1984; Valberg et al., 1986), blue-OFF cells were encountered only infrequently in extracellular recordings in marmoset LGN. In nine experiments, spatial and temporal response characterization of 15 blue-OFF cells was obtained. The same experiments yielded 34 blue-ON cells and 184 "non-blue" PC cells, but the true proportion of blue-ON and blue-OFF cells is probably lower than these numbers suggest. This is because attempts were made to characterize fully all blue-ON and blue-OFF cells encountered, but recordings were abandoned from most encountered PC cells after confirming that they showed no functional sign of inputs from S cones.
4.3.1 Spatial and temporal response properties

Figure 4.1A shows peristimulus time histograms of the responses of four cells to S-cone selective and achromatic gratings drifting at 4 Hz. All cells respond to achromatic modulation (BW, Figure 4.1A). The blue-OFF and blue-ON cells additionally show vigorous response to the S-cone selective grating (SWS, Figure 4.1A). The S-cone selective grating produced ~66% contrast in the S cone and less than 5% contrast in the medium/long wavelength cone class. The small residual response seen in two PC cells (tonic-ON, tonic-OFF, Figure 4.1A) can most likely be attributed to residual contrast delivered to the medium-long wavelength cones.

Blue-OFF cells, like blue-ON cells, showed relatively sluggish visually evoked responses. As expected (Yeh et al., 1995a; Solomon et al., 2005), the blue-ON cells show band-pass temporal tuning, with peak response amplitude normally close to 6 Hz. Relative to the peak, response amplitude for low (0.5 Hz) temporal frequency modulation was reduced by 45.8% (standard deviation [SD] 24.4%, n = 13) for blue-ON cells. Blue-OFF cells showed a comparable degree of low-frequency attenuation (mean response at 0.5 Hz was reduced by 36.3% relative to peak [SD 24.9%, n = 8]; p = 0.83, Wilcoxon rank-sum statistic).

Figure 4.1C shows response phase at 4 Hz for blue-ON and blue-OFF cells. Response phase for both types shows linear dependence on temporal frequency, consistent with a transport delay (response latency) of 95.9 ms (SD 33.9, n = 8) for blue-OFF cells and 71.2 ms (SD 10.3, n = 13) for blue-ON cells. The same calculation for PC cell responses to achromatic drifting gratings yielded lower equivalent latency than either blue-ON or blue-OFF cells (mean 55.2 ms, SD 7.9, n=24, p<0.001, Wilcoxon rank-sum). The longer visual-evoked latency of blue-ON and blue-OFF cells is consistent with the long orthodromic latency of other koniocellular-pathway cells (Norton and Casagrande, 1982; Irvin et al., 1986): for example, Irvin et al. (1986) found that koniocellular cells in Galago had an orthodromic latency (from stimulation at the optic chiasm) of 5.1 ± 0.6 ms; parvocellular neurons, by comparison, had a latency of 2.9 ± 0.1 ms. Visual-evoked latencies reported in this study will be longer than orthodromic latencies, as orthodromic latency only measures the conduction speed of axons. Orthodromic latency is a part of visual-evoked latency, but visual-evoked latency
Figure 4.1. Identification and temporal response properties of blue-off cells. (A) Peristimulus time histograms (PSTH) of the responses of four different LGN neurones (blue-off, blue-on, tonic off, tonic on) to achromatic (BW) and S-cone selective (SWS) stimuli. An illustration of the phase of the stimulus appears beneath each PSTH. For each PSTH, scale of the x axis is 500 ms, y axis 10 imp/s. (B) Amplitude and phase of response as a function of temporal frequency. Blue off cells (left) and blue-on cells (right) both show a band-pass temporal tuning. Grey lines indicate the spontaneous discharge rate for each cell. (C) Phase of response of blue-off and blue-on cells at a temporal frequency of 4 Hz. The blue-off and blue-on cells each form a single cluster, and these clusters are separated by 162 degrees.
is also composed of a number of other delays: for example, the delay caused by diffusion of neurotransmitter across synapses.

The spatial tuning properties of blue-OFF cells were next addressed. Figure 4.2A shows example spatial tuning curves. The fundamental Fourier component (f1) was fit with a difference-of-Gaussians (DOG) model (Rodieck, 1965; White et al., 2001). The model output is shown as a solid line. All blue-OFF cells recorded showed low-pass spatial tuning. No blue-OFF cell showed greater than 5% attenuation at 0.01 cycles/degree compared to the peak response (mean low spatial frequency attenuation 95.6%, SD 10.3, n = 15). By contrast, the low spatial frequency attenuation for blue-ON cells was 77.6%, SD 21.8 (n = 26) and for PC cells was 60.9%, SD 24.0 (n = 87); both of these are significantly different to the attenuation of blue-OFF cells (p < 0.01 for both, Wilcoxon rank-sum). The lack of low spatial frequency attenuation implies that the receptive field structure of blue-OFF cells is comprised of either a centre mechanism with no surround inhibition, or a spatially co-extensive centre-surround mechanism, similar to the Type 2 cells of Wiesel and Hubel’s (1966) original classification.

If the blue-OFF responses are carried by midget-parvocellular pathway cells, then the size of blue-OFF receptive fields should be close to that of PC cells at any given eccentricity. On the other hand, if blue-OFF responses are carried by wide-field cells then the size of blue-OFF receptive fields should be large (Dacey, 2004; Dacey et al., 2005). Figure 4.2B shows receptive field diameter of blue-ON and blue-OFF cells (measured with S-cone selective gratings), compared to PC cells (measured with achromatic gratings). Population regression coefficients for blue-ON and blue-OFF cells were significantly different to parvocellular cells (p < 0.001, Student’s t test). The blue-OFF receptive fields are always larger than those of PC cells at equivalent eccentricities, suggesting either that they do not derive one-to-one input from midget ganglion cells in the retina, or that there is spatial convergence of midget ganglion cell input at the level of the LGN.
Figure 4.2. Spatial tuning and receptive field centre size of blue-off cells. (A) Spatial tuning plots for three blue-off cells. The responses ($f_1$) and the model fit (DOG) are shown. Blue-off cells show lowpass spatial tuning. Average firing rates ($f_0$) are not consistent between cells. (B) Receptive field centre sizes of parvocellular (PC), blue-on and blue-off cells. Blue-off cells have significantly larger receptive field sizes than both blue-on and parvocellular cells, suggesting that the recorded blue-off cells are not a subclass of parvocellular cells.
The relative efficacy of S-cone selective gratings is reduced at high spatial frequencies by chromatic aberration (Flitcroft, 1989; Cottaris, 2003). This would lead to a slight overestimate of the centre diameter of blue-OFF cells. However, any such error would also apply to blue-ON cells, and as can be seen in Figure 4.2B, the great majority of blue-OFF receptive fields are nevertheless larger than blue-ON receptive fields at the same eccentricity. Thus, even allowing for the possible effects of chromatic aberration, the receptive field center diameter of blue-OFF cells is larger than expected if they derive non-convergent input from midget retinal ganglion cells. Recent recordings from the macaque LGN (Tailby et al., 2006) show mostly consistent spatial and temporal properties to those reported here.

The DOG model also allows an estimation of receptive field sensitivity. In this study, a substantially lower receptive field sensitivity was measured in blue-OFF cells, compared to blue-ON cells (blue-OFF, mean $K_c$: 0.500 ± 1.016 imp/s/deg$^2$, n = 15; blue-ON, mean $K_c$: 7.627 ± 7.941 imp/s/deg$^2$, n = 36; p < 0.01, Wilcoxon rank-sum). Note however, that systematic measurements of contrast gain were not made in this study, which would give a more precise appraisal of sensitivity. The lower sensitivity of blue-OFF cells is supported by psychophysical studies (McLellan and Eskew, 2000) and supports the retinal data that show that different cell classes are responsible for the blue-ON and blue-OFF response.

Blue-OFF cells exhibited a relatively low peak discharge rate (16.3 ± 5.67 imp/s, n = 15; cf. parvocellular cells, peak discharge: 37.79 ± 13.73 imp/s, n = 87; p < 0.001, Wilcoxon rank-sum). A high maintained discharge was recorded for four of the blue-OFF cells, and consistently, the Fourier F0 response component showed high mean amplitude (> 10 imp s$^{-1}$) and high variability in these cells (Fig. 2A, upper and lower panels). However, these cells could not be distinguished from other blue-OFF cells on the basis of their functional cone inputs or receptive field size. On average, the maintained discharge rates of the blue-OFF neurons recorded were very similar to parvocellular neurons (blue-OFF cells, mean $f_0$: 7.694 ± 9.097 imp/s, n = 13; cf. parvocellular cells, mean $f_0$: 6.763 ± 7.713 imp/s, n = 53; p = 0.90, Wilcoxon rank-sum). The spatial and temporal properties of blue-OFF cells (n = 6) recorded from
trichromatic marmosets (n = 3) were not obviously different to those recorded in dichromats.

4.3.2 Cone inputs

Dacey et al. (2005) showed that one type of blue-OFF cell projecting to the LGN in macaques has "giant" dendritic field morphology and displays intrinsic photosensitivity. The next question addressed by this study was whether a melanopsin-based functional input contributes to the modulated responses of blue-OFF cells. Figure 4.3 shows spatial frequency tuning functions for a blue-OFF cell in a dichromatic male marmoset. The figure shows the response of the blue off cell to modulation (at a temporal frequency of 4 Hz) by S-cone selective gratings, as well as gratings selective for the medium-long (ML) wavelength pigment (peak 556 nm) and the melanopsin pigment (peak 482 nm). The modulated cone contrast (the maximum achievable with the CRT monitor used in this study) was close to 13% for melanopsin, 22% for the ML cone and 66% for the S cone. S-cones dominate the response of blue-OFF cells at 4 Hz, Melanopsin and ML cone responses are feeble but appear in opposite phase to the S cone response, as shown previously in macaque (Dacey et al., 2005).

In seven other blue-OFF cells, the relative strength of cone inputs to the receptive field was analysed, by measuring responses to combined luminance and chromatic variation in 64 uniformly spaced excursions about a constant white point, as described elsewhere (Blessing et al., 2004). Responses in three of the blue-OFF cells were best accounted for by assuming functional input from S cones alone. Responses in the remaining cells were best accounted for by assuming antagonistic input from ML class cones (mean ML weight 43.1%, n = 4), but small contribution of a melanopsin-like intrinsic mechanism could not be ruled out. In summary, these data show that blue-OFF cells transmit dynamic signals from S cones and, to a variable extent, from ML cones. Responses at 4 Hz were not significantly influenced by the intrinsic photopigment melanopsin in the blue-OFF cells we sampled, either because the blue-off cells we recorded do not receive input from the giant-sparse ganglion cells or because, as hypothesized (Dacey et al., 2005), the melanopsin response is functionally uncoupled from the blue-off response by its extremely sluggish temporal characteristic. Technical limitations prevented us from exploring very low temporal frequencies.
Figure 4.3. Identifying the cone inputs to a blue-off cell. (A) Responses of one blue-off cell to S-cone selective (SWS), M/L-cone selective (MWS) and melanopsin selective (MEL) stimuli. There is a very weak response to both MWS and MEL stimuli, though this is barely above the spontaneous discharge rate of the cell (arrow). Solid lines are difference-of-gaussians (DOG) fits to the data. As shown in (B), both the MWS and MEL responses are in opposite phase to the SWS response, indicating an opponent response.
4.3.3 Anatomical reconstruction of recording sites

Finally, the location of blue-OFF cells within the LGN was measured. Figure 4.4A shows a coronal section through the marmoset LGN, reacted with DAB/HRP. The recording track can be identified by the dark peroxidase reaction. The location of recorded cells on this track is shown in Figure 4B. The position of seven blue-OFF and thirteen blue-ON cells was reconstructed in this way, as summarised in Fig. 4C. As previously described (Martin et al., 1997) most blue-ON cells are located in the koniocellular layers. Five of the seven blue-OFF cells reconstructed were located in the koniocellular layer K3 (between the parvocellular and magnocellular layers: Kaas et al., 1978; Casagrande and Norton, 1991). The position of the remaining eight blue-OFF cells was not recovered histologically. Of these remaining eight cells, the eye dominance and encounter position on the recording track of three of the cells was consistent with location in layer K3; three others were likely located in the PC layers or in layer K4 (between the two parvocellular laminae).
Figure 4.4. Anatomical reconstruction of electrode tracks. (A) DAB-labelled coronal section of marmoset LGN. The arrow indicates the electrode track. The layers of the LGN are also indicated: parvocellular (PC), koniocellular (KC) and magnocellular (MC). The schematic composite of this section and the neighbouring Nissl section are shown in (B), along with positions of all recorded cells on this track. The legend shows the physiological response class of the recorded cell. Both the blue-off (closed circle) and blue-on (open circle) cell on this track were recorded from the koniocellular layer K3 (in between the PC and MC layers). (C) shows a schematic composite of the reconstructions of 7 blue-off cells (closed circles), and 13 blue-on cells (open circles). Most reconstructed blue-off cells (5/7) were recorded from the koniocellular layers. Approximate position (in mm) anterior to the interaural axis is indicated beneath each section.
Chapter 4 – Blue-OFF signals in marmoset LGN

4.4 Discussion
The existence of blue-OFF cells as a distinct functional class was questioned by early studies (Malpeli and Schiller, 1978; Gouras and Zrenner, 1981; Zrenner and Gouras, 1981; Vingrys and Mahon, 1998), but it is now accepted that they form a small proportion of visually responsive units in the primate LGN. However, because they are very rarely encountered, the receptive field properties and subcortical pathways taken by blue-OFF signals have remained poorly understood. In this discussion, the hypothesis that blue-OFF cells form part of the koniocellular division of the afferent visual pathway is taken as a starting point. How well this hypothesis is supported by this study, and other data?

Previous studies (Norton and Casagrande, 1982; Irvin et al., 1986; White et al., 2001) have found that koniocellular cells, though heterogeneous, can be distinguished from parvocellular and magnocellular cells by a number of criteria: their sluggish temporal response properties, relatively large receptive field centres, relatively low peak discharge rate, and low maintained discharge rates. In line with these findings, the results in Section 4.3.1 show that blue-OFF cells: have a longer visual response latency than both parvocellular and blue-ON cells; have receptive field centre sizes up to ten times larger than parvocellular neurons at equivalent eccentricity (Figure 4.2); and exhibit a relatively low peak discharge rate. Thus, the bulk of the spatial and temporal data reported here supports the conclusion that blue-OFF cells are part of the koniocellular pathway. In addition, histological reconstructions showed that most blue-OFF cells were recorded from within koniocellular layer K3 (5/7; see Figure 4.4).

Recent retinal studies of blue-OFF neurones indicate two potential blue-OFF pathways: one a subset of the midget-parvocellular pathway (Klug et al., 2003), the other arising from two classes of ‘widefield’ ganglion cell (Dacey, 2004; Dacey et al., 2005). The data of this study supports a projection from ‘widefield’ type ganglion cells; the reasoning for this is as follows.

First, widefield ganglion cells preferentially project to the koniocellular layers of the LGN, as shown in Chapter 3; most of the blue-OFF cells reported in the present study were recorded from within the koniocellular layers. Second, the receptive field sizes of blue-OFF cells recorded in this study can be compared with previous studies in
macaque. The seven giant sparse (melanopsin) cells from Dacey et al.’s (2005) paper had a mean receptive field diameter of $745 \pm 306 \mu m$ (3.24 $\pm$ 1.33 degrees: Dacey and Petersen, 1992). Six of the fifteen blue-OFF cells recorded in this study fall within this size range. Dacey et al. (2003) also showed that the large sparse ganglion cell also shows blue-OFF response characteristics, which could account for the smaller blue-OFF receptive fields measured in this study.

The blue-OFF cells that were recorded here did not show functional input from melanopsin-based pigment at a stimulation frequency of 4 Hz (Figure 4.3). This is not surprising given the extremely slow time course of the intrinsic photoresponse (Berson et al., 2002; Dacey et al., 2005), and reinforces the idea that if blue-OFF cells are intrinsically photoreceptive then this may not influence their transmission of signals for colour vision (Dacey et al., 2005).

Are there midget-parvocellular blue-OFF cells that have been missed in this study? In the present study, 184 parvocellular cells were measured, and there was very little sign of strong S cone input. Assuming that S cones are even only 5% of cones, and that the sample of 184 neurons was taken randomly within the parvocellular layers, then one would expect to see at least nine tonic (parvocellular) fields with strong blue-OFF input. However, no parvocellular fields with a strong blue-OFF input were encountered in this study ($\chi$-squared test, $p < 0.01$). Thus, the findings of this study are consistent with data from Lee et al., (2005), who showed that in marmoset there are no connections between S-cones and OFF midget bipolar cells, and thus, no midget-parvocellular blue-OFF pathway in the marmoset. The weight of physiological and anatomical evidence suggests that there is a koniocellular blue-OFF pathway; thus, if there is a midget-parvocellular blue-OFF pathway in the macaque it must be additional to the widefield-koniocellular pathway.

4.5 Further work
To conclude this thesis, this section will examine three outstanding questions related to the organization of the subcortical visual pathways.
4.5.1 Random or selective wiring?

How is red-green colour opponent circuitry set up? As described in Chapters 1 and 2, there are two possible ways. First, it is possible that there is cone-selective wiring of bipolar and ganglion cells to M or L cones. Alternately, red-green colour opponency may have arisen due to chance wiring: the random wiring hypothesis (Lennie et al., 1991; Dacey and Packer, 2003) states that cone opponent wiring is assured if the receptive field centre receives pure or nearly pure L or M cone input. Since foveal midget ganglion cells have a pure L or M input by default (as they connect only one bipolar cell, which contacts only one L or M cone) this would enable red-green chromatic opponency in the fovea and perifoveal regions. Current data are unable to resolve this debate conclusively. There are many studies showing evidence in favour of each theory: for the random wiring hypothesis (Derrington et al., 1984; Mullen and Kingdom, 1996; Diller et al., 2004; Mullen et al., 2005; Jusuf et al., 2006a); and for the specific wiring hypothesis (De Monasterio and Gouras, 1975; Abramov et al., 1991; Yeh et al., 1995b; Lee et al., 1998; White et al., 1998; Martin et al., 2001; Reid and Shapley, 2002; Blessing et al., 2004; Solomon et al., 2005; Vakrou et al., 2005). It is also possible that the real answer may lie somewhere between these two extremes (Buzas et al., 2006).

4.5.2 Can the koniocellular division of the LGN be further segregated?

As described in Chapter 3, recordings from the koniocellular division of the LGN find a heterogeneous collection of cell types. In addition to V1 (primary visual cortex), koniocellular LGN cells project to a number of cortical targets, including V2 (Bullier and Kennedy, 1983), V4 (Lysakowski et al., 1988) and V5 (Sincich et al., 2004). It is thus fair to suggest that the koniocellular layers of the LGN contain cells of multiple pathways, and that labeling the koniocellular pathway as the “blue-yellow” pathway greatly understates its function. (As an example, koniocellular layers are still present in nocturnal primates like the owl monkey, and owl monkeys are monochromats: Jacobs et al., 1993). What do these other pathways signal? Can these multiple pathways be segregated to specific koniocellular layers (within the four koniocellular layers of New World primates, or the six koniocellular layers of Old World primates)?
Some evidence does exist to suggest that the more ventral koniocellular layers (K1 and K2, in the terminology of Chapter 3) are functionally and even anatomically distinct from the more dorsal koniocellular layers (K3 and K4). White et al. (2001) demonstrated that in the marmoset, cells in K1 and K2 were larger and more transiently responding than cell in the dorsal koniocellular layers. Similar results were recorded in the owl monkey (Xu et al., 2001). Finally, recent reports from Casagrande (2007) in macaque have demonstrated at least three koniocellular axonal projections to V1. One of these types of koniocellular axon originates in koniocellular K3-K6 and terminates in the cytochrome oxidase-rich ‘blobs’ of cortical layer 3Bα (see also: Livingstone and Hubel, 1982; Fitzpatrick et al., 1983; Lachica and Casagrande, 1992). The blobs have long been thought to have a role in colour processing (Livingstone and Hubel, 1984; Ts'o and Gilbert, 1988). Of the other two types of koniocellular axon reported in Casagrande (2007), one originates in koniocellular layer K1-K2, and the origin of the other axon type was not reported. The physiological roles of these remaining two koniocellular projections to V1 are uncertain.

4.5.3 Are there blue-OFF bipolar cells?

As discussed in Chapter 4, there are three potential candidates for blue-OFF retinal ganglion cells – the large sparse ganglion cell (Dacey, 2004), the giant sparse (melanopsin) ganglion cell (Dacey et al., 2005), and/or a subset of OFF midget ganglion cells (Klug et al., 2003). The data in Chapter 4, and from Lee et al. (2005), argue against a blue-OFF midget pathway in the marmoset. Thus, it would seem that the two types of sparse cell are responsible for relaying blue-OFF signals in the marmoset. It is uncertain which bipolar cell type(s) form the input to these two sparse ganglion cell types.

The two ganglion cell types are known to stratify in stratum 5 of the inner plexiform layer (IPL) – a region which usually contains ON synapses (Famiglietti and Kolb, 1976). Two types of cone bipolar cell stratify in this region: the blue-cone bipolar cell and the diffuse bipolar type 6 (DB6) cell. The blue-cone bipolar cell selectively contacts S-cones (Mariani, 1984; Kouyama and Marshak, 1992), which certainly makes it a candidate blue-OFF bipolar cell; however, this bipolar cell type relays ON signals (Kouyama and Marshak, 1992). The other alternative, the DB6 cell, seems to be biased
against connecting S-cones (Lee et al., 2004). There is thus potential that the DB6 cell could provide a yellow-OFF input to the sparse ganglion cell types; however, a number of the blue-OFF cells reported in Chapter 4 showed no substantial input from M or L cones. This leaves two possibilities: either there is a novel type of bipolar cell that contacts S-cones specifically, and carries blue-OFF signals; or alternatively, the blue-ON signal of the blue-cone bipolar cell might be inverted, perhaps via an amacrine cell that is yet to be identified.
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