CHARACTERISATION OF SPECTRAL SIGNALS IN SUB-CORTICAL PATHWAYS

The key functional feature of cone-opponent neurones is that they show excitatory responses to only a restricted band of the visible spectrum. When measured with artificial stimuli, using colour contrasts close to those expected in natural scenes, the spectral selectivity of many visually responsive neurones in subcortical visual pathways can be related in a fairly straightforward way to some weighted combination of the spectral selectivity of long-wavelength sensitive (L or ‘red’), medium-wavelength sensitive (M or ‘green’), and short-wavelength sensitive (S or ‘blue’) cone photoreceptors. For example, the spectral selectivity of many neurones in the macaque LGN is consistent with additive combination of M and L cones in a ratio close to 1:2, with very little (say, one per cent) input from S cones.\(^1\)\(^-\)\(^3\) Such three-way proportions can be expressed as a set of relative weights, where the components sum to unity: for this example, \(W_{SML} = (0.01, 0.33, 0.66)\).

Responses in cone-opponent neurones can be expressed as a ratio where the weight of one or more of the cone inputs to the cell has a negative value.\(^4\) For example, spectral responses of a typical ‘blue-yellow’ cone-opponent cell might be well-described using the above scheme as \(W_{SML} = (0.7, -0.1, -0.2)\). It is well-established that subtractive interaction between M and L cone classes is the chief determinant of the chromatic response properties of red-green opponent cells in the retina and LGN.\(^5\)\(^-\)\(^7\) For example, again using the weighted sum nomenclature given above, \(W_{SML} = (0, -0.4, 0.6)\) would accurately predict the chromatic properties of a ‘red-ON’ cell.

Cone opponency is thought to arise at an early site in the visual system, by inhibitory neuronal action in the retina.\(^8\)\(^-\)\(^10\) Information theory tells us that opponent encoding should avoid the additive effects of noise, which otherwise would accumulate if the cone signals were transmitted in separate channels through the brain.\(^11\)\(^12\) Therefore, it is argued commonly that a naturally evolving visual system would introduce opponent processing at an early stage, as an efficient way to transmit the signals required for colour vision. Such arguments may be mathematically satisfying but, as pointed out elsewhere,\(^13\)\(^14\) they shed little light on the evolutionary origin of natural vision systems. The following discussion does assume that colour percep-
tion is predicated on the activity of cone-opponent cells in subcortical channels but this does not imply that such channels have evolved with the specific purpose of enabling objects to be discriminated on the basis of their spectral reflectance. Evolutionary interpretations of the origins of chromatic pathways are given elsewhere.\textsuperscript{15,15,16}

SEGREGATION IN THE LGN: ANATOMICAL ALLEGIANCE OR FUNCTIONAL FACTIONS?

The LGN of primates was described in early anatomical studies as comprising four to six main layers (parvocellular and magnocellular) separated by cell-sparse interlaminar or koniocellular zones (reviewed by Casagrande\textsuperscript{17}). Early physiological studies in the primate retina and LGN distinguished receptive fields which were classed as ‘red-green’ or ‘blue-yellow’ opponent. Such opponent receptive fields were distinguished from broad-band (‘non-opponent’) responses.\textsuperscript{18} The pioneering studies of Wiesel and Hubel\textsuperscript{19} and Dreher, Fukada and Rodieck\textsuperscript{20} ('non-opponent') responses.\textsuperscript{18} were distinguished from broad-band opponent. Such opponent receptive fields were classed as ‘red-green’ or ‘blue-yellow’ opponent. Such opponent receptive fields which were distinguished from broad-band (‘non-opponent’) responses.\textsuperscript{18} The pioneering studies of Wiesel and Hubel\textsuperscript{19} and Dreher, Fukada and Rodieck\textsuperscript{20} first linked these anatomical and physiological observations by showing functional segregation of response properties within the LGN, with the majority of opponent responses recorded in the dorsal parvocellular layers. These and subsequent studies delineated the typical response characteristics for parvocellular and magnocellular cells in trichromatic primates. Typically, parvocellular cells have relatively small receptive fields, they respond tonically to high-contrast achromatic and low-contrast chromatic stimuli. Most parvocellular pathway cells show red-green opponent responses. Magnocellular cells, typically, have relatively large receptive fields with high contrast sensitivity. They show phasic responses to maintained contrast and show little sign of cone opponency.\textsuperscript{8,19-21}

Such ‘typical’ responses are thought to reflect the dominant input of parasol ganglion cells to the magnocellular layers, and midget ganglion cells to the parvocellular layers.\textsuperscript{22,23} Anatomical and physiological studies have revealed a diversity in the retinal projections to the LGN\textsuperscript{22-25} and in the response properties of LGN neurones; for example, the cone-opponent cells come in blue-yellow and red-green varieties.\textsuperscript{19,21,23,28} Recently, attention has focused on the question of whether some of this heterogeneity could be attributed to action in the koniocellular division of the retina-geniculo-cortical pathway.

The koniocellular layers (from Greek κόνις–dust, because the layers consist of small neurones) were considered to contain mainly interneurones but this opinion has changed with the demonstration (for all primate species studied) that many cells in the koniocellular layers, despite their small size, are relay neurones, which project predominantly to the supragranular layers (2, 3 and upper layer 4) including the cytochrome oxidase rich ‘blob’ regions of the primary visual cortex.\textsuperscript{27-33} This anatomical projection to regions of cortex supposedly devoted to opponent colour processing led to suggestions that cone-opponent neurones could form part of the koniocellular pathway.\textsuperscript{34} Results obtained in electrophysiological studies have shown a partial segregation of blue-yellow opponent responses to the internal parvocellular and koniocellular layers.\textsuperscript{35-37} For example, about two thirds of blue-ON type responses were located in koniocellular layers in a diurnal New World monkey, the common marmoset.\textsuperscript{38} The question now becomes one of whether partial segregation such as that seen in the blue-on population really reflects participation of blue-ON cells in two distinct processing streams or simply some ‘untidiness’ of anatomical organisation.

Figure 1 summarises the anatomical problem. In sections through the LGN of the marmoset, the koniocellular layers are visible as pale zones in Nissl stained sections (Figure 1a), and by a concentration of darkly-stained cell bodies in a section stained for calbindin-like immunoreactivity (Figure 1b). Calbindin, one of a fairly ubiquitous group of calcium binding proteins, has been proposed as a specific neurochemical marker for the koniocellular pathway.\textsuperscript{39} Figure 1c shows schematically the LGN superimposed with the encounter rate of single neurones recorded in a typical experiment using fine (‘single-unit’) extracellular recording electrodes.\textsuperscript{35,40} As encounter rates are typically very low compared with the neuronal density and the encounter position must be verified post hoc by histological reconstruction, it is difficult to attribute a given response (for example, a blue-yellow opponent cell, star in Figure 1c) to such small subdivisions of a small brain nucleus. Things get even worse when the inconvenient reality of anatomy is considered, as follows.

In creatures such as the marmoset or prosimians, such as the bushbaby Otolemur crassicaudatus, the koniocellular layers can be identified in Nissl-stained sections and are relatively well segregated from the main layers.\textsuperscript{41} However, the anatomical segregation is never complete. Figure 1d shows the distribution of all calbindin-immunoreactive cell somata in a coronal section through marmoset LGN. As in all species described, the immunoreactive cells are not restricted exclusively to the koniocellular zones but are also present in smaller numbers throughout the main layers. Furthermore, there is uncertainty about whether any neurochemical marker exists that labels completely yet exclusively the koniocellular layers. For example, Figure 1e shows a high-power view of the koniocellular layer between magnocellular and parvocellular layers in marmoset.\textsuperscript{32} Relay cells are identified by retrograde labelling from the primary visual cortex (punctate green label) and calbindin-immunoreactive cells are also identified (red label). Indeed, only about half the relay cell population shows strong calbindin immunoreactivity (Figure 1e, arrowheads), whereas other relay cells show little or no calbindin immunoreactivity (Figure 1e, arrows). This and other data imply that there is neurochemical diversity among the koniocellular population (reviewed by Hendry and Reid\textsuperscript{42}).

In summary, single-neurone electrophysiology can provide only a rough guide to anatomical location and histology is only a rough predictor of functional segregation in the LGN.

How are we to interpret the result that blue-ON responses are predominantly but
Figure 1. Anatomical segregation in the LGN and postulated projections to the visual cortex. A Nissl-stained coronal section through the marmoset LGN is shown in Figure 1a. PE: external parvocellular layer, PI: internal parvocellular layer, Ipm: interlaminar (konio/ocular) layer, MI: internal magnocellular layer, ME: external magnocellular layer, S: superficial (konio/ocular) layer. An adjacent section processed for calbindin-like immunoreactivity is shown in Figure 1b. The immunoreactive neurones form sparse bands within the konio/ocular zones. The main layers are shown superimposed with this section in Figure 1c, together with a schematic path of a recording electrode (red line) to show the relative encounter frequency of a blue-ON cell (star) and non-blue-ON cells (circles) in a typical recording experiment. Scale bars, 1 mm. In Figure 1d, the distribution of calbindin-labelled cells (grey) is shown superimposed on the main parvocellular and magnocellular laminae. The pattern of calbindin immunoreactivity in the konio/ocular zones is shown in Figure 1e. Some retrogradely labelled relay cells (green punctate fluorescence) are strongly labelled with calbindin (red fluorescence, arrowheads), whereas others are weakly labelled or unlabelled (arrows). Two possible patterns of connections of blue-ON cells to the cortex are shown in Figures 1f and 1g. In Figure 1f, the blue-ON cells project together with other neurones in the same layer of the LGN. In Figure 1g, the cells project according to their functional characteristics, regardless of their anatomical location in the LGN. PC, parvocellular; KC, konio/ocular; MC, magnocellular. Panels A-D modified from Goodchild and Martin. Panel E modified from Solomon.
not completely, segregated to the konio-
cellular layers? Two extreme possibilities
are given in Figures 1f and 1g. In Figure
1f the blue-ON cells, represented by solid
symbols, maintain their anatomical alle-
giance to the pathway, in which their cell
body is located and contribute to multiple
cortical processing streams (as represented
at the right hand side of Figures 1f and 1g)
according to their prevalence in that stream. By contrast, in Figure 1g all the
blue-ON cells form a coherent functional
projection, regardless of the LGN layer, in
which the relay cell soma is located, allow-
ing but not requiring their signal to remain
segregated within cortical streams.

These possibilities cannot be distin-
guished by single-neurone recordings
from the LGN, in the absence of addi-
tional information, such as the site of
axonal termination of the recorded cells
or evidence for functional subgroups
among the blue-ON population. Consis-
tent with the functional segregation shown
in Figure 1g, data from a recent experi-
ment by Chatterjee and Callaway have
shown that S-cone afferent signals are func-
tionally segregated in the primary visual
system was lack of knowl-
edge of the chromatic organisation of the
cone matrix in humans and other pri-
mates. Recent descriptions of the packing
arrangement of cones in human and
macaque eyes have removed this im-
pediment and allowed a more coherent
interpretation of the physiological
literature. These authors Williams and
colleagues confirmed the well-known
quasi-hexagonal packing arrangement of
S-cones predicted from previous anatomoi-

cal and psychophysical studies, and
showed further that the arrangement of
M- and L-cones is not distinguishable from
random. This means that, at small spatial
scales, the ML-cone mosaic contains ‘clumps’ of cones of the same spectral
type. This may give a basis for the observa-
tion of variability in the strength of cone
opponency among parvocellular cells: some cells may draw inputs from a clump
of the same spectral type of cone, others
from mixed clumps.

Figure 2 summarises the best-established
features of chromatic receptive fields and
their pathways through the LGN, using
this new data as a starting point. For sim-
plicity, the diagram shows only ‘ON’- type
receptive fields unless otherwise noted.

A patch of perifoveal cone matrix is
shown in Figure 2a. At an eccentricity
of five degrees, a field with this many cones
would occupy approximately 0.25 by 0.25
degrees. The image has been blurred to
simulate some deterioration of spatial ac-
curacy as a result of pre-receptoral factors
such as optical imperfections.

The post-receptoral connectivity of M-
and L-cones to bipolar cells and ganglion
cells is relatively constant over the region
of retina corresponding to the first 10
degrees of visual angle, although the cone
density changes dramatically over this
eccentricity range. The connections of
S-cones are presumed to be constant over
this eccentricity range, with the excep-
tion of the small (less than 0.1 degree)
‘tritanopic’ zone in the centre of the fovea,
where S-cones are sparse or absent.

The sampling density of midget-
parvocellular projecting ganglion cells is
superimposed on the cone mosaic in Fig-
ure 2b. In all diurnal primates studied,
there is one-to-one connectivity between
M/L-cones, midget bipolar cells and
midget-parvocellular projecting ganglion
cells, so each cone is expected to contri-
bute the dominant excitatory input to at
least one ON-centre and one OFF-centre
parvocellular-pathway cell. As noted
above, only the ON-array is shown in
Figure 2b.

The high spatial density of the sampling
matrix and the small receptive field of
parvocellular pathway neurones, when
measured with high-contrast achromatic
stimuli, suggest that this pathway contrib-
utes to spatial vision at high contrast lev-
els as well as preserving the chromatic sig-
nature of each M and L cone in the foveal
array. The excitatory receptive fields are
shown as centred on the positions of indi-
vidual M- and L-cones, with little spatial
overlap between neighbouring fields. This
reflects the high spatial density and lack
of anatomical overlap in the midget-
parvocellular cell array. Measurements
of receptive fields made using laser inter-
ferometry to bypass the eye’s optics
revealed that in the receptive field of
foveal parvocellular cells, the centre input
is dominated by a single cone, with some
smaller contribution from neighbouring
cones. Nevertheless, it is important to
note that the exact correspondence be-
tween the cone matrix and receptive field
size and position is not known and the
exact influence of the eye’s optics on
receptive field dimensions is also incom-
pletely understood. For example, the
point-spread function due to low-order
optical aberrations would serve to enlarge
the receptive field of foveal parvocellular
cells to two to three times the diameters
shown in Figure 2.

Figure 2c shows schematically the excita-
tory receptive field of blue-ON cells,
which draw inputs from the same cone
mosaic. The small bifratstratified ganglion cell
class was identified as the morphologi-

cal substrate of blue-ON response proper-
ties by recordings made from an in vitro
preparation of macaque retina. Consist-
ent with the morphology of small-field
bifrattatified cells and the receptive field size
of blue-ON cells in recordings from retina
and LGN, the blue-ON excitatory recep-
tive fields illustrated in Figure 2c are
larger than the parvocellular receptive
fields. The receptive field is shown as con-
taining ‘hot spots’ that correspond to the
position of the sparsely-distributed
S-cones. The fine structure of blue-ON recep-
tive fields has not been measured in in vitro
recordings from LGN but such hot
spots are a feature of blue-on receptive
fields measured in in vitro recordings from
macaque retina and are predicted from
the known anatomy of bipolar cell inputs
to small bifratstratified cells. Physiologi-
cal measurements of S-cone mediated
visual acuity are likewise consistent with
the idea that the spatial precision of the
S-cone mosaic is preserved in the blue-ON
afferent channel.
Figure 2. Chromatic and spatial organisation of subcortical pathways. A patch of perifoveal cone mosaic based on data from Roorda, Metha and Lennie\(^45\) is shown in Figure 2a. At an eccentricity of 5 degrees, a field with this many cones would occupy approximately 0.25 by 0.25 degrees. The sampling density of ON-centre midget-parvocellular projecting cells is shown in Figure 2b. Each white circle shows anatomical sampling aperture of one midget cell. Note that the sampling array contains ‘gaps’ at the position of S-cones. Sampling density of blue-ON cells relative to the S-cone array is shown by white circles in Figure 2c. Sampling density of ON-centre magnocellular pathway cells is shown by grey circles in Figure 2d. The small white circle at position of S-cone represents the size of the postulated midget-blue-OFF ganglion cell. Retino-geniculo-cortical connectivity is shown in Figure 2f. Left, schematic view of retina. Centre, schematic of LGN layers seen in coronal section. Right, schematic coronal section of primary visual cortex (V1), showing terminations of relay cell afferents. As indicated by the question marks, the subcortical route taken by yellow-ON/blue-OFF signals is unknown.
The centres of the hot spots are shown at the position of the S-cones in the mosaic; their size is shown as larger than that of M- and L-cones to indicate that chromatic aberration in the intact eye would introduce spatial smearing of the S-cone signal above that for the M- and L-cones. This is because the emmetropic eye is optimally focused for wavelengths, which maximally activate the M- and L-cones.

The receptive field centre matrix of parasol-magnocellular pathway cells is shown for the same patch of cone mosaic in Figure 2d. The parasol-magnocellular pathway cells have larger receptive field centre diameter (when measured with high-contrast stimuli) and a greater degree of receptive field overlap (‘coverage’) than cells in the midget-parvocellular pathway.28,71-74 The parasol-magnocellular pathway cells show little sign of cone opponency, except at very low spatial and temporal frequencies, where an L-cone opponents, except at very low spatial and temporal frequencies, where an L-cone dominated inhibition is present.3,19,20 The question of whether the magnocellular cells receive functional input from S-cones remains controversial. One recent study suggests functional input to magnocellular cells roughly proportional to that expected on the basis of the prevalence of S-cones (five to 10 per cent) but previous and subsequent studies have suggested under-representation of functional input from S-cones.1,36

Less well understood than the three receptive field types described so far is the ‘yellow-ON, blue-OFF’ type (Figure 2e), which was described sporadically in recordings from retinal ganglion cells27,79 but more consistently in LGN recordings.5,20,75 The chromatic properties of these cells show clear signs of strong inhibitory input from S-cones.67 One anatomical study predicts that blue-OFF signals are carried through a single cone-contacting, midget bipolar-ganglion cell pathway (Figure 2e, small circle) whereas recent recordings from macaque retina in vitro showed blue-OFF type responses from a wide-field ganglion cell type (Figure 2e, large circle). The spatial properties and distribution of blue-OFF cells in the LGN are largely unmeasured. Experiments that address this issue are important because they can provide a further test of the hypothesis that S-cone pathways are part of a ‘primordial’ system for colour vision.18

CORTICAL DESTINATIONS

It has long been recognised that the parvocellular and magnocellular projections are segregated within layer IV of the primary visual cortex (striate cortex, area 17, area V1). The midget-parvocellular pathway relay cells project predominantly to layer IVCp, whereas the parasol-magnocellular pathway relay cells project to layer IVc.81 As described above, the projections of the koniocellular pathway include cytochrome-oxidase rich ‘blob’ regions of the superficial layers 2/3. This discovery led to the hypothesis that some or all of the cone-opponent signals could travel through the koniocellular layers directly to the blob regions, which in turn were associated specifically with high proportions of colour-selective neurones in some studies.82,83 However, a recent study by Chattajee and Callaway has provided some much-needed clarification and called this ‘blob’ hypothesis into question.

Chattajee and Callaway measured the cone-opponent properties ofafferent axon terminals in macaque V1. They applied muscimol, a γ-amino butyric acid ‘A’ type receptor (GABAa receptor) agonist, to the exposed surface of area V1 in concentrations sufficient to inactivate essentially all intrinsic neuronal activity. Thus, the residual visually-evoked activity, measured using coarse (‘multi-unit’) extracellular recording electrodes, should arise largely from the LGN afferents, which dominate the direct visual input to V1.

The results showed that all red-green opponent responses were restricted to layer IVCp, consistent with the position of geniculate afferents arising from the parvocellular layers. More surprising was the discovery that blue-ON and blue-OFF afferents are not organised in columnar fashion in the superficial layers 2/3, as expected for direct afferents to blob regions. Rather, the blue-ON and blue-OFF responses were segregated horizontally within lower layer 3 and upper layer 4a. This result is consistent with koniocellular afferents carrying S-cone signals (part of the koniocellular termination is to superficial layers 3/4a) but not with the idea that koniocellular afferents provide any red-green cone-opponent signals directly to blobs. Therefore, this result is consistent with other evidence from recordings of colour and spatial selectivity in cortex, and from anatomical inputs to blobs in nocturnal primates, which has cast doubt on the crucial link between the blobs and red-green colour vision.84-86 These data serve as another example of the unique anatomical character of the pathway beginning in the S-cones—a conclusion that has obvious parallels in the longstanding psychophysical evidence for the unique character of the blue system in colour vision.87

CHROMATIC PATHWAYS THROUGH THE THALAMUS: A NATURAL CONCLUSION

The result of Chattajee and Callaway is included in the anatomical scheme shown in Figure 2f. Here, three ‘natural pathways’ are delineated, each of which arises from only a single natural ganglion cell type. Following Rodieck and Brenner,66 these pathways each refer to ‘a neural circuit whose excitatory visual input arises entirely from a restricted set of ganglion cell types’.66 For example, the relay cells in the parvocellular layers of the LGN are substantially preserved for midget ganglion cells and pass them on to the primary visual cortex (V1), where their axons terminate in layer IVCp. Thus, the PC abbreviation refers exclusively to the pathway formed by relay cells, which are innervated by midget ganglion cells. Likewise, the MC pathway shown in Figure 2f is formed by relay cells, which are innervated by parasoal ganglion cells.

The pathways defined in these ways are subsets of the P and M pathways delineated by Shapley and Perry.69 There is overwhelming evidence that midget and parasol cells together form the great majority (probably at least 90 per cent) of the retino-geniculate pathway in foveate primates.25,75,96-95 The distinct layers of the
LGN are interpreted as a simple result of this numerical dominance, combined with the eye-specific segregation of distinct cell type.41

In retrospect, it seems likely that at least some of the functional heterogeneity reported in early physiological studies of the LGN may have been due to misattribution of recordings from ‘ectopic’ cells belonging to the koniocellular division of the geniculocortical pathway (see Hendry and Reid45). This is shown in Figure 2f, where a third natural pathway (BN, for ‘blue-ON’) is delineated by small bistratified ganglion cells and their relay cell targets. The blue-ON pathway is shown as part of the more sparsely distributed koniocellular projection through the LGN, consistent with the fact that the majority of blue-ON cells is recorded outside the main parvocellular and magnocellular layers. The blue-ON relay cells are shown as projecting to the upper layer 4/layer 3, where blue-ON cortical afferents were recorded.45 Although it is possible that these afferents arise from an extra-geniculate source, the weight of anatomical evidence is in favour of this functional input arising from the LGN. As indicated in Figures 2e and 2f, the origin of the blue-OFF responses recorded from layer 4a is unclear. If this pathway originates in OFF-midget ganglion cells, it would form part of the PC pathway but if it originates in a wide-field ganglion cell type, it would more likely form part of the koniocellular projection system. In summary, there are now several lines of evidence for segregation of the opponent channels for colour vision within the subcortical pathways. Signals originating in S-cones are anatomically segregated in the retina, are carried in a separate channel through the LGN and arrive in a specific sublayer of the primary visual cortex. Furthermore, the functional properties of blue-ON cells are consistent with a primary role in transmitting chromatic signals. By contrast, red-green opponent responses in the midget-parvocellular system appear as an additional response dimension on a pathway, which also transmits high-acuity spatial signals. Improved understanding of the way in which these signals from two distinct pathways are extracted by central mechanisms and used to contribute to colour perception is a clear goal for future studies.

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