BRAIN STRATEGIES OF COLOUR PERCEPTION

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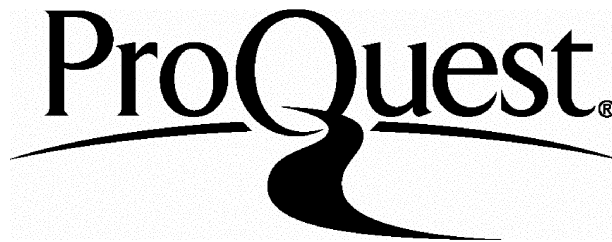## November 1996

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by K. N. Moutoussis

ABSTRACT

This thesis deals with the problem of colour vision. Part I addresses the problem of specialisation for colour, with specific reference to area V2, interposed between V1, where cells selective for the wavelength of the stimulus are found, and V4, where cells selective for the colour of the stimulus are present. The responses of cells in V2 were studied for their selectivity to the wavelength, orientation, and direction of motion of the stimulus. Cells with particular selectivities were found in clusters which were directly related to the metabolic (cytochrome oxidase, CO) architecture of V2. Orientation selective cells were mostly found in the inter and thick stripes, direction selective cells (although generally rare) in the thick stripes, and wavelength selective cells in the thin stripes. Only very few cells were selective for more than one attribute. By studying the distribution of the receptive fields of the cells in the three different stripe compartments, it became clear that the visual field is independently mapped in each set of CO stripes. The visual field is thus separately mapped for each of the different attributes of vision in V2. Wavelength selective cells in V2 were tested for colour constancy. None was found to exhibit this property, but some were selective not only to the wavelength composition of the stimulus but also to the change in the relative amount of a particular wavelength. Part II addresses the general problem of how the brain binds the different visual attributes which are processed separately, and investigates the possibility that colour, motion, form, and stereoscopic depth are not perceived in precise temporal registration with

one another. It describes the use of a psychophysical method to investigate the differences in time required to perceive colour and motion. By using a stimulus which rapidly and continuously changes in colour and direction of motion, it shows that subjects bind colour and motion incorrectly because colour is perceived before motion. The idea of functional segregation not only at the level of processing but also at the level of perception is introduced.

ACKNOWLEDGEMENTS

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INTRODUCTION

The work reported here deals with the general problem of functional specialisation in the visual brain. The concept of functional specialisation as applied to the cerebral cortex at large is not new; by contrast, it is a relatively new concept in the visual brain. Until the discovery that there are many visual areas in the brain and that the responses of cells in one area can be dramatically different from those in another was made, it used to be generally supposed that there are two visual zones in the brain, one specialised for seeing and the other for understanding what is seen (see Zeki 1993). The researches of Hubel and Wiesel in the sixties and seventies introduced a variant of this concept. The latter supposed that all the attributes of the visual scene are processed in each visual area, but at a more complex level than the antecedent areas, thus leading to the concept of a hierarchical organization in the visual brain.

The concept of functional specialisation (Zeki 1969; Zeki 1978a) in fact incorporates some of the earlier views. In particular, it recognises that there may be a hierarchy within a cortical system that is specialised for a given attribute of the visual scene, but it lays emphasis on the fundamental separation of the operations involved in processing different attributes of the visual scene. Although there is now a general consensus regarding the existence of functional specialisation, there is still a disagreement about its extent. While most agree that motion and colour are processed separately, the extent to which the processing of form is separate from colour or from depth is still a matter of debate or uncertainty. This thesis addresses this question in part, by selecting an area which is known to contain cells registering different attributes of the

visual scene, and asking whether there is any segregation or functional specialisation within that area (V2), emphasising colour very largely. In a sense, this is an arbitrary choice, since the demonstration of a specialisation for any attribute of vision would carry equal validity. In another sense the choice is deliberate, for (perhaps contrary to common belief) colour vision may yet turn out to be the simplest attribute of vision to understand. More than that, the manner in which the brain handles colour may yet prove to be the key to the way it handles other attributes as well, and any insight gained into this will undoubtedly provide insights into other aspects of vision.

The results on V2 presented here are fairly straightforward. They show that there is a local specialisation for colour even in an area which has all the attributes of vision represented in it. If so, then this implies a considerable autonomy for colour processing which, in turn, opens up the possibility that other attributes of vision may also be processed separately and at different times and that, consequently, one attribute of vision, say colour, can be perceived later or earlier than another attribute of vision. This idea is tested psychophysically, and hence remains outside the area of debate concerning the geographic details of functional specialisation, of whether entire visual areas or sub-compartments of areas are specialised for particular functions, and whether such specialisation bears any relationship to the anatomical architecture of an area. The psychophysical comparison is made with motion, since the motion system is relatively well studied and it is also well established that these two systems are separately mapped in the visual brain. The results give strong support to the notion of functional specialisation, but from an angle very different to that of the previous literature.

HISTORICAL SURVEY

Chapter 1: Concerning colour vision in general

Colour vision as a guide to the functioning of the visual brain

Colour is a product of the brain, the result of ingenious and mysterious strategies used by it in order to obtain information about the environment in which it exists. The fact that colour is not a physical reality but rather a product of our brain was realised by Newton, who wrote: "For the Rays to speak properly are not coloured. In them there is nothing else than a certain power and disposition to stir up a sensation of this or that Colour" (Newton 1704). A transformation of the colourless electromagnetic radiation takes place in the brain as a result of operations that are still only sketchily known, and immensely enriches our visual experience with both knowledge and beauty.

Studying colour vision is like studying the brain in miniature. Its role in understanding the principles of the functioning of the brain has been immense. Cells concerned with colour are not randomly distributed all over the visual brain: the concept of functional specialisation in vision is strongly demonstrated by and, in fact, conceived basically because of colour vision. Although evidence for functional specialisation in vision was available from the human brain much before the monkey studies were initiated, it was in fact disregarded for theoretical reasons (see below). This idea mainly evolved from the discovery of a colour specific (Zeki 1973; Zeki 1977) and a motion specific area (Zeki 1974) in the prestriate cortex of the monkey. Different attributes of the visual scene are thus processed simultaneously in separate parts of the visual cortex (Zeki 1978a).

Functional specialisation in the brain and in vision

The idea that each part of the brain is undertaking a different function was introduced in the beginning of the nineteenth century by Gall and became very popular mainly because of his follower Spurzheim. Gall believed that there are as many separate regions in the brain as there are psychological faculties, and that the shape of the skull was indicative of the form of the brain underneath it and therefore of the personality of the individual as well (Glickstein 1985). The lack of evidence for these claims, nevertheless, made scientists very cautious, and by 1850 the whole idea of phrenology was abandoned for the notion of a uniform, unitary nervous system, with each function being widely distributed in the cerebral cortex (Flourens 1824). It was not long, however, before the idea of localisation of function in the brain was re-initiated, based this time on correct scientific evidence. This was done by Broca's discovery of a speech centre in patient Tan in 1861 (see Von Bonin 1960), by the discovery of the motor cortical areas in the dog by Fritsch and Hitzig in 1870 (see Von Bonin 1960), and by the realisation that the cortex in the occipital lobe was visual in function by Munk (1881) in the monkey, and by Henschen (1893) and Inouye (1909) in the human (see Glickstein 1985; Glickstein 1988).

There was at that time evidence for a functional specialisation within vision as well: in 1888, Louis Verrey, a Swiss ophthalmologist, described the case of a sixty-year-old woman who had suffered a stroke affecting the occipital lobe of her left hemisphere (Verrey 1888). The result of this was that she was unable to see colour in her right visual hemifield; the rest of the visual attributes there were intact, but everything appeared in shades of grey. Eleven years later, MacKay and Dunlop described a patient with complete colour blindness in his entire visual field (MacKay and Dunlop 1899). This patient had a bilateral lesion which was smaller in

size than the one of Verrey, but in both patients the fusiform gyrus was involved. These cases, although rare, provided good evidence for a separate colour centre in the human brain. The idea of functional specialisation, though implicit in these findings, was however never established since it was against the doctrines of the time. The fact that the colour area fell outside the striate cortex, the part of the cortex responsible for "seeing", and inside the prestriate cortex where "understanding" was supposed to take place, was the principal reason against such an idea (see Zeki 1993). The evidence was dismissed and the notion of functional specialisation disregarded. It was only after anatomical and electrophysiological studies in the visual cortex of primates had established a separation of function that the idea of a functional segregation in vision was appreciated.

More recent evidence for an independent colour system in man

A functional specialisation for colour is strongly suggested by clinical studies, because colour can be more or less selectively spared or compromised following the local or diffuse damage to the brain. The syndrome of cerebral achromatopsia (inability to see colours due to a cortical misfunctioning) has been described both in a permanent (Sacks and Wasserman 1987) and transient (Lapresle *et al.* 1977) manner. In the former case, a patient lost the ability to see colours following a car accident, whereas the rest of his vision was mildly affected. In the latter case, a patient who suffered from repeated falling attacks could only see the world in shades of grey, during the period of the attack; about one minute after the attack, however, his ability to see colour was re-established. Whereas in the permanent case the effect is probably due to selective and permanent damage to the colour processing regions of the brain, a possible explanation for the transient effect is that the input to this area (by V1 and

V2, see below for monkey anatomy) is affected: both the cerebellum and occipital cortex receive their input from the vertebro-basilar artery, and it is possible that a momentary decrease in blood levels there would selectively affect the highly metabolically active colour system in areas V1 and V2, leading to a transient loss of colour vision, and the motor control centres in the cerebellum, leading to a parallel loss of motor synergy.

The high metabolic activity of the colour system (for details see below on cytochrome oxidase staining) can sometimes have the reverse result: some people suffering from carbon monoxide poisoning can still see colours, although the rest of their vision is heavily impaired (Wechsler 1933; Adler 1944; Adler 1950). It is possible that the richer vasculature of the metabolically active blobs and thin stripes in areas V1 and V2 respectively (see below) act as a protection from the effects of hypoxia in carbon monoxide poisoning (Zeki 1993). Whatever the explanation, results such as these strongly suggest a separate processing of colour by the brain.

The notion of a brain area specialised for colour is further supported by the fact that blind people can sometimes see colours which are not really there, a syndrome given the name "phantom chromatopsia" (Zeki 1990b). During these (unpleasant) "colour attacks", usually a golden or purple colour appears which then enlarges to invade their entire imaginary visual field. It is possible that this is the result of uncontrolled activity in the colour areas of the brain, since when the human colour area is stimulated magnetically in normal subjects they report seeing colours which appear in the visual field opposite to the stimulated hemisphere (G. Beckers, personal communication).

Summarising, even though it is not possible to account for all of the phenomena described above in exact neurological or anatomical terms, they nevertheless attest to the idea of a geographical specialisation for colour within a region of the human brain. With the development of human brain imaging techniques, the position of the colour centre of man in the lingual and fusiform gyri of the inferior occipital region has been confirmed (Lueck *et al.* 1989; Zeki *et al.* 1991), in agreement also with the early reports of Verrey (Verrey 1888) and MacKay and Dunlop (MacKay and Dunlop 1899).

What is colour?

Colour is, in a sense, an interpretation that the brain gives to a certain characteristic of objects in our environment. The origins of modern colour research can be traced back to Newton, who used a prism to analyse white light into its components. He discovered that white light consists of many different electromagnetic oscillations, differing from each other in wavelength and colour. Newton wrote: "Every body reflects the rays of its own Colour more copiously than the rest, and from their excess and predominance in the reflected Light has its Colour" (Newton 1704). The first part of this statement is correct, but the second one is not (see below). Nevertheless, the idea that the colour of an object is directly related to the wavelength composition of light coming from it dominated the way of thinking of scientists in this area. The equation of colour with the energy of the electromagnetic radiation in Newton's mind is also obvious in his statement that "In them [the light rays] there is nothing else than a certain power and disposition to stir up a sensation of this or that Colour". Colour research was thus for many years concentrated on studying the colours of various wavelengths, and mixtures of wavelengths, coming from a point in the field of view. However, the

wavelength composition coming from objects and surfaces is perpetually changing under different illuminations, whereas their colour may change in shade but basically remains the same. The task the brain has to achieve is thus not to simply relate each wavelength to a particular sensation, but to find a way to "discount the illuminant" (Helmholtz 1911) and obtain colour constancy, that is, colour as a constant characteristic of an object. The usefulness of colour would be much diminished if an object appeared one colour at one moment, and a different colour at another moment, depending upon the viewing condition. The brain, living under continuously changing conditions, needs to acquire knowledge which is related to the constant characteristics of objects in the environment.

Land's early experiments

The fact that the colour of a surface is not determined in a simple and obvious way by energy or wavelength has been clearly stated and beautifully demonstrated by Edwin Land (Land 1959a). Land was the first to realise that the rules for the colour at a particular point cannot give a complete picture of the more general properties of this attribute. Unlike classical colour studies which concentrated on studying the colour of a point, he chose to study natural images instead. In the scenes he photographed, Land used objects that could be of any colour (e.g. a telephone, as opposed to an orange or a banana) in order to demonstrate that the colours assigned to them were not the result of previous knowledge and memory. He used a long and a short filter to take two pictures (records) of the same scene and projected the long record through a red filter and the short record through a neutral density filter. When the red and white projectors were used without records, the colours obtained on the screen could be varied (by changing the intensities of the two projectors) from red to white, with various shades of pinks in-between.

However, when the two records were inserted, a verisimilitude picture of the photographed scene appeared, with almost all of the different colours present.

Two things astonished Land and made him pursue these studies for many years, resulting in the formation of the Retinex Theory of Colour Vision (see below). Firstly, the colour range on the screen was not limited to whites, reds, and pinks, in the way expected by the classical colour theory. Secondly, the colours on the screen were independent of the amount of energy coming from the two projectors, since using polarisers to change the relative energies from the projectors did not affect the colours at all (relative energy ratios as extreme as 100:1 were used). Furthermore, the local ratio of short to long wave light at each part of the screen was equally unimportant, since the colours did not change when a duplicate short record was used to double the contrast and therefore produce new ratios for all the objects in the picture. For the first time, the amount or ratios of the wavelengths used to illuminate a scene seemed to be the least important factor for determining its colour. The particular light sources used were also not important, and the same colours were obtained by using different filter combinations. Interestingly, some filters (e.g. narrow band yellow) could be used as either the short or the long projection light, depending on what they were paired with (see below). Land tried to account for these phenomena by proposing a new co-ordinate system, in which the percentage of available short wavelength (amount present at an area divided by the maximal amount that could be there) is plotted against the percentage of available long wavelength **(Figure 1)**. This co-ordinate system is physically dimensionless and only involves a ratio of ratios, or, in other words, a comparison of comparisons. The colour of an image is thus independent of both the

overall flux and the wavelength composition at each individual point. Black, greys, and white are points along the x=y line; points above this line represent warm colours and points below it cool colours.
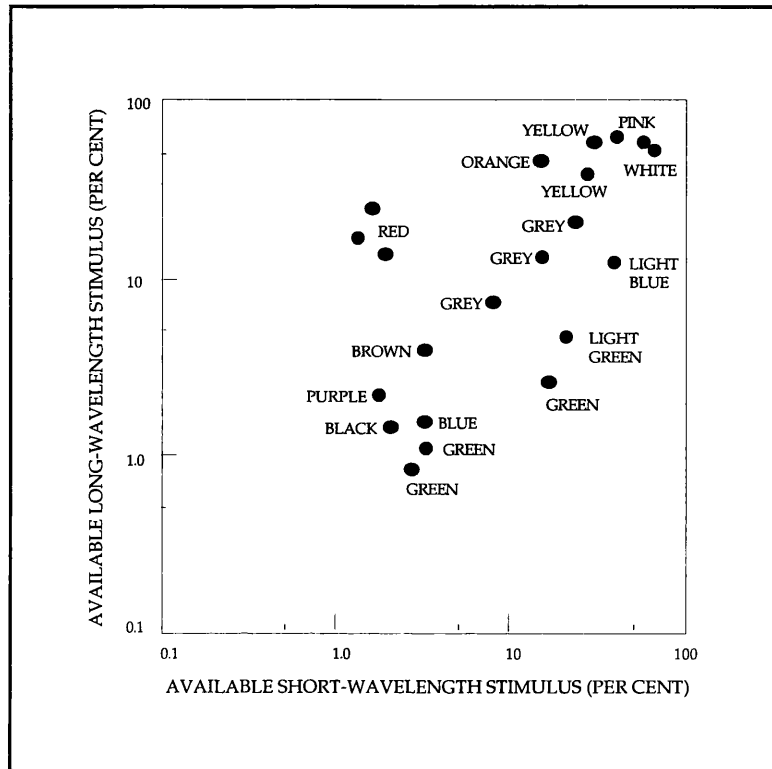
Figure 1: Land's new coordinate system to predict colours in natural images. The axes are dimensionless, each measuring the illumination at every point as a percentage of the maximum that could be there. The straight line x=y is the grey axes, the warm colours being on one side of it and the cool colours on the other.

Land used a dual monochromator to investigate the separation of wavelengths required to see the full gamut of colours, as a function of the wavelength used (Land 1959b). A pair of photographic transparencies could each be transilluminated with a narrow spectral band of light or with white light. A new graph plotting the wavelength of the stimulus used as a long record versus the wavelength of stimulus used as a short record is thus produced **(Figure 2)**. The region below the 45° line is the reversal region, where the long record is illuminated with the shorter

wavelength. No colours are seen on the 45° line and for a narrow region above it. A small reversal region also exists within the normal region,
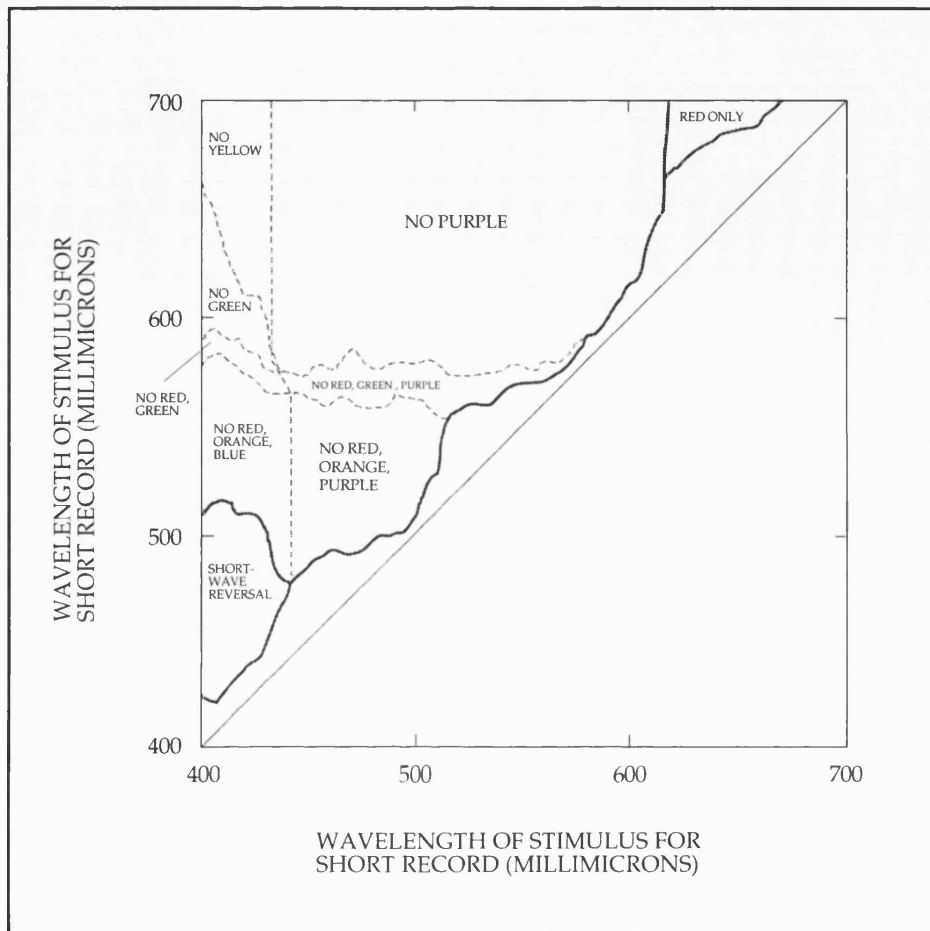
Figure 2: Land's colour map showing the colours one can obtain as a function of the wavelength of the two projection lights used. The narrow unmarked region next to and above the diagonal is an achromatic region in which wavelengths are too close together to produce any kind of colour. In the region marked "short-wave reversal" the colours are normal but the short wavelengths act as the stimulus for the long record and the long wavelengths as the stimulus for the short record. Colours are reversed in the blank area below the diagonal, where the long record is illuminated with the short wavelength and the short record with the long wavelength.

when short wavelengths are used as projecting lights. The minimum separation of wavelengths required to see colour is a function of wavelength, but is generally very small. Colours were found to be stable over a big range of relative brightnesses of the two stimuli: depending on both the illuminating wavelengths and the colour under examination,

energy ratios as high as 300:1 could be used with very little effect on the colour. In order to demonstrate that sodium light can act as the short or long stimulus simultaneously, Land used a pair of viewers to pair this with tungsten light covered with a red filter in one viewer and a green filter in the other. The sodium D-line could also be seen as green or red respectively and, if the room lights were switched on, the colours of each of the three (two viewers and the room) "colour universes" remained unchanged.

Were the phenomena described by Land new?

Induction phenomena in colour vision have been very well known for many years. When a colour is used as an annulus, it always induces its opponent colour in the centre (Hurvich 1981). This is, for example, the base for the acquisition of coloured shadows (double shadow effect) produced with a white light, a coloured light, and the shadow of an object illuminated by both. Two shadows are produced: the one where white light is absent has the colour of the coloured light, and the one where the coloured light is absent is not white but has the opponent colour of the light used, induced there by the surround. It is possible that colour induction, also known as "simultaneous colour contrast", can explain some of Land's "unexpected" phenomena. Land was surprised that, instead of his two projection lights mixing in a "classical sense", there was a variety of colours present in his projections. Nevertheless, Land was not the first to produce colours others than red, white, and pinks using a white and a red projector. The "extra" colours seen were all induced by the lack or excess of the colours of the projection lights in different parts of the scene; the colours of the projection lights, the colours these induce, and finally colours which are a result of the mixture of the two are all present in such situations (Walls 1960).

A further phenomenon known as colour (and also light) adaptation refers to a change in sensitivity of the eye, after exposure to a particular light (Hurvich 1981). A large non selective surface initially takes the colour of its illuminant but eventually becomes white or neutral. The sensitivities of each of the types of cones change independently, in an inverse relation to their stimulation. The effect of this is an approximate colour constancy, so that objects do not change their colour much under different illuminations. These phenomena are continuously active, since action/reaction equilibrium on different parts of the retina changes all the time due to eye movements; if not for eye movements, we would not be able to see anything, the system would reach equilibrium and everything would disappear. Although Land states in his papers that he obtained identical results using very short presentations, it is possible that some adaptation mechanisms are instant and therefore able to account for the phenomenon of colour constancy (Walls 1960).

Concluding, although it is possible that Land's results can be partly or fully explained in terms of isolated observations made in colour science before him, none of these observations was as clear and obvious as Land's demonstrations. Furthermore, whether "simultaneous colour contrast" and "instant adaptation" can explain the phenomena described by Land is of secondary importance. The fact is that Land's observations led him to formulate a powerful theory, which could for the first time relate wavelength and colour in a clear and unambiguous way.

The Retinex Theory of Colour Vision

A few years after his early observations, Land came up with what he called the "Retinex Theory of Colour Vision" (Land 1974). This was a theory about the nature of colour and the mechanisms necessary for it to

be established by the visual system; Land coined this name for it because he did not know where the essential comparisons are made (retina+cortex= Retinex). In order to illustrate his theory, Land used large displays consisting of many squares and rectangles of different colours which he called "Mondrians", due to their resemblance to the work of the known painter. Initially he used a black and white Mondrian, consisting of white, black and greys. He defined the reflectance at each part of the Mondrian as the percentage of available wavelength, i.e. the amount of light coming from that area divided by the amount of light incident on it. If one assumes an even level of illumination across the screen, the ratio of reflectances of two areas is equal to the ratio of the amounts of light reflected from them. Land also referred to the lightness of an area as the sensation from black to white (i.e. brightness devoid of colour), and tested whether this depends on the energy coming from the area or from its reflectance. By changing the intensity of the projector or by switching the room lights on, although the energy coming from each area is changed, its lightness remains the same. Therefore the lightness (what Land called "lightness record") of an area is the result of a comparison of the amount of light coming from that area with the amount of light coming from neighbouring areas.

In order to show that two areas reflecting the same amount of energy do not necessarily have the same lightness, even if they do so at the same time, Land placed the projector in such a way so that the energies reflected from a white and a very dark grey paper were made equal. Because of a gradient in the light energy coming from the source, the two areas were reflecting the same amount of energy at the same time, but still differed very much in lightness. Land explained these phenomena by assuming that changes in reflectance are discontinuous and form edges,

whereas changes in illumination are continuous and do not. The Retinex system thus scans the visual space and takes into account only large changes in energy when calculating the energy ratios of adjacent regions, in order to assign a position in the reflectance hierarchy for each area.

Land carried out the same experiments with a coloured Mondrian. Unlike grey, coloured papers have different lightnesses for each of the long, middle, and short wavelengths, so that the comparison procedure described above has to be repeated separately three times. Land changed into using three rather than two projectors and, because of the addition of the short-wave light, a three dimensional colour space was proposed instead of the two dimensional co-ordinate system used initially (Land 1983). Each area on the Mondrian then has three lightness records as coordinates on this system, and it is the ratios of these rather than the ratios of the energies that determine its colour. Therefore, an area with a high lightness record for long wave light and a low lightness record for middle and short wavelengths will appear red, and an area with a high lightness record for all wavelengths will appear white, irrespective of the illumination of the Mondrian. Two different areas on a Mondrian reflecting the same triplet of energies at different times, or two areas on two different Mondrians reflecting the same triplet of energies at the same time, have different colours when viewed as a part of the Mondrian but the same colour when viewed in void (i.e. isolated from the rest of the Mondrian) using a black tube. The colour of an area is therefore independent of the wavelength composition reflected from it, but instead depends on the wavelength composition of the light reflected from the other areas of the Mondrian as well, since both these factors are taken into account when calculating the lightness records of an area (Land 1974).

Where is the comparison site?

The name "Retinex" was chosen by Land to refer to both the retinal and cortical parts of the visual system undertaking the operations necessary for the perception of colour. In order to investigate whether the phenomena he described were retinal or cortical in origin, Land studied colour generating interactions in a patient with a split corpus callosum who could therefore not integrate information coming from the two fields of view (Land *et al.* 1983). When the corpus callosum is cut, the two hemispheres can no longer communicate with each other and thus are unable to combine information. Each visual hemisphere receives its input from the contralateral hemifield only, and thus if a single coloured area is presented in one hemifield and the surrounding Mondrian to the other, colour constancy can only be achieved if the information from the latter is combined with information from the former. If, however, the comparisons are made in the retina, it would make little difference whether the two hemispheres can communicate or not. When the wavelength composition of the single coloured area was varied, the colour perception of the patient varied with it as if the area was viewed in void. Land concluded that the phenomenon of colour constancy must be cortical, since it disappears when the colour centres in the two hemispheres are no longer able to communicate with one another.

The colour system comprises several stages in the cortex, which will be dealt with in more detail in the following chapters. It is worth mentioning here that the first possible site of the comparison postulated in the Retinex theory is area V4. The callosal fibres connecting V1 and V2 cells with their counterparts in the opposite hemisphere are limited to 1°-2° around the representation of the vertical meridian and thus cannot combine information positioned further apart than that. For colour

constancy, nevertheless, interactions of parts of the field in excess of 5° separation are necessary and, since areas V3 and V3A are not concerned with colour, area V4 is the first possible candidate for the comparison site (Zeki 1993). This conclusion is also supported by the fact that, in physiological terms, colour constancy seems to be established in this area but not earlier (see Introduction chapter on V4 and also colour constancy Results in V2).

Conclusion

To summarise, some of the phenomena described by Land had probably been discovered previously and were not as big a surprise to classical colour theory as Land initially thought. However, Land was the first one to demonstrate them as obviously and make the clear distinction between the study of a point and a natural image and, as a result of that, between wavelength and colour. The Retinex Theory of Colour Vision is powerful and can cope with the phenomenon of colour constancy in a much better and simpler way than the classical simultaneous colour contrast explanations can. It consists of a basic comparison method which, unlike the locality and complexity of the induction phenomena, can give a simple and global estimate of the colour of each part of the image. The reason for the appearance of "extra" colours in the early Land phenomena is thus not as important as the fact that his theory has a way of being always able to predict them. A very precise relationship between wavelengths and colours has been put forward: the comparison of the three lightness records of each object, i.e. a comparison of comparisons, can always assign each object with the correct colour. The question remains, nevertheless, whether the operations proposed by Land are also the ones used by our visual system. In order to answer this question, one

must look into the organisation of the visual brain and the properties of the cells processing colour information.

Chapter 2: The role of the striate cortex in colour vision

Introduction

Colour perception is a complex phenomenon. It involves essentially a comparison of the wavelength composition of the light reflected from one part of the field of view (the area one is looking at) and that coming from surrounding parts (Land 1974). It therefore entails a comparison undertaken by the cortex of signals coming from spatially distinct parts, registering the results of that comparison and thus assigning colours to the different parts of the field of view. The language that we adopt to talk about colour is, however, necessarily a relatively simple one. Phenomenologically, it is based primarily on the fact that different parts of the spectrum are recognised to have a different colour, and hence we speak of different wavelengths. Neurologically, the language we use when speaking about the brain is based on the fact that the areas involved in the perception of colour have anatomical connections between them, through which signals are exchanged. Thus a vaguely defined "processing", consisting of an equally vaguely defined "transmission of information", takes place in the brain. While I acknowledge the weakness and imprecision of these terms, they are nevertheless useful in talking about the brain in the context of our present knowledge of it.

From the thalamus to the cortex

The major input to V1 comes from the lateral geniculate nucleus (LGN) of the thalamus which can be subdivided into two sets of layers. The two most ventral ones consist of large bodied cells and hence are referred to as the magnocellular (M) cells, while the upper four layers consist of small bodied cells and hence are known as the parvocellular (P) cells. It was Henschen in 1930 (Henschen 1930), who, on the basis of not

very convincing evidence (namely cell size), suggested that the P layers are specialised for dealing with colour. On the basis of far better physiological evidence, Wiesel and Hubel (Wiesel and Hubel 1966) confirmed this inspired guess of Henschen and showed that these two different sources of thalamic input to V1 have very different properties. These properties are conferred upon the two sets of layers by the nature of the retinal input to them. Two different classes of retinal ganglion cells, differing both anatomically and physiologically, feed the LGN in a segregated manner: the M layers receive their input from the $P\alpha$ retinal ganglion cells, and the P layers from the $P\beta$ retinal ganglion cells (Leventhal et al. 1981). Rapid conduction velocities, high contrast sensitivity, transient responses, and total indifference to colour characterise the M system, which projects to layer $4C\alpha$ and via this to layer 4B in V1; by contrast the P system which projects to layer $4C\beta$ and via this to layers 2&3, is wavelength selective, has sustained responses, a lower contrast sensitivity, and slower conduction velocities (Hubel and Wiesel 1972; Lund et al. 1975; Leventhal et al. 1981; Perry et al. 1984; Fitzpatrick et al. 1985; Tootell et al. 1988a; Hubel and Livingstone 1990).

As mentioned above, when the P and M systems are relayed through to the primary visual cortex they remain largely segregated; nevertheless, this segregation is not complete and a certain degree of mixture between the two systems occurs. For example, magno-recipient layers $4C\alpha$ and 4B faithfully reflect the properties of the M cells, showing no evidence of any P input (Hubel and Livingstone 1990). However, more recent studies show that layer 4B seems to receive an equally strong projection from the parvorecipient layer $4C\beta$ as well (Sawatari and Callaway 1996). Furthermore, when area V1 is stained for the enzyme cytochrome oxidase (CO) a characteristic pattern of dark staining blob

regions appears most clearly in layers 2&3 (Horton and Hubel 1981); these blob regions receive a contribution from both the P and M systems (Lachica *et al.* 1992). Between blobs, i.e. within the pale cytochrome oxidase interblob regions of layers 2&3 (which receive most of their input from the parvocellular recipient layer 4Cβ), some cells show contrast sensitivities compatible with a predominantly P input whereas others show sensitivities inbetween P and M cells, suggesting a contribution from the M system (Hubel and Livingstone 1990). The same purity of segregation between the M and the P systems should thus not be expected in the cortex; rather, new systems are generated there.

Wavelength selective cells in V1

The most important factor for determining whether a visual area is involved with colour processing is to find in it cells which respond preferentially or exclusively to stimuli of a particular wavelength. Such cells, present also in the parvocellular layers of the LGN (Wiesel and Hubel 1966), were described in the pioneering studies of Hubel and Wiesel in the primary visual cortex (Hubel and Wiesel 1968). They have a central, usually circular, area excited by one colour, and a surround annulus area around the excitatory region which is inhibited or gives off responses to the opponent colour. When the wavelength preferences of V1 neurons are quantitatively tested by plotting their action spectra, some are found to give on responses to some wavelengths and off responses to others; however, these neurons are not any more sharply tuned than retinal ganglion cells (Gouras 1970). In addition to centre-surround opponent cells, cells with spatially co-extensive receptive fields and no inhibitory surround, as well as cells showing wavelength selectivity with a centre surround organisation but no opponency, have been also described in V1 (Dow and Gouras 1973; Dow 1974; Gouras 1974; Poggio *et al.* 1975; Thorell

et al. 1984; Ts'o and Gilbert 1988). For most cells, stimulation of the surround alone using annuli has no effect. There is thus what is called a "silent" surround, and the best way to test the surround properties is by measuring the influence of surround stimuli on the response obtained from the centre (Michael 1978b).

In addition to the types of wavelength selective cells described above, some cells do not respond to white spots at the centre of their receptive field and are also inhibited if the optimal colour stimulus is extended to the surround (Hubel and Wiesel 1968; Poggio *et al.* 1975; Michael 1978b; Thorell *et al.* 1984). These are the so-called double opponent cells, and were initially thought to have a colour opponent colour organisation which is opposite between the centre and the surround. However, it is now generally accepted that most "double opponent" cells are not actually double-opponent, since they show an opponency in the centre and a non-chromatically opponent inhibitory surround mechanism, i.e. they are inhibited by light of any wavelength (including white) in the surround (Ts'o and Gilbert 1988, Hubel personal communication). Nevertheless, red/green "double opponent" cells in V1 with non circular receptive fields have been described; these have a central rectangular strip and either one or two rectangular flanks of the opposite response (Michael 1978a). The flanks are also able to respond in isolation, giving both on and off responses; but when centre and flanks are stimulated together, the response is stronger. In addition to their chromatic properties, another peculiarity of these cells is that, because of the spatial characteristics of their receptive fields, the strength of response of these cells is also influenced by the orientation of the stimulus bar (Michael 1978a). Such "rectangular double opponent" cells have not been described by any other lab; on the contrary, most agree that colour and

orientation selectivity are two mutually exclusive properties of the cells in V1 (see below).

In a similar way, one should be sceptical about a limited number of reports of binocular cells in V1 responding only to specifically oriented colour edges moving in a particular direction (Michael 1978c). These cells were reported to usually have a square centre, and two antagonistic flanking surrounds with the same or opposite spectral sensitivities than the centre; some are also inhibited by the size of the stimulus used (Michael 1979). Many of these cells do not respond to anything else apart from two colour edges of a particular configuration and direction of movement. One should nevertheless be sceptical before characterising such cells "direction selective", and thus implying that they are concerned with the processing of motion, since their behaviour can be explained with reference to a sensitivity in wavelength composition changes alone (Zeki 1983b).

Colour versus luminance

A surface can be distinguished from other surfaces by virtue of the fact that it differs in colour or in luminance, and usually in both. It is not surprising to find, therefore, that many cells in V1 are sensitive to both luminance and wavelength. Different laboratories have used different ways to test and define colour selectivity for a cell, thus leading to differing percentages of colour cells in V1. What seems to be generally true is that luminance contrast responses are not absent from wavelength selective cells, i.e. the segregation between colour and luminance in V1 is not as clear as that between colour and orientation (see below). For example, in layers 2&3 only 3% of the cells were found to respond to colour but not to luminance contrast, whereas almost half of the V1 cells, although

showing no wavelength selectivity or opponency, were nevertheless able to respond to a border consisting of two wavelengths set at equiluminance (Gouras and Kruger 1979). Furthermore, it has been reported that the spectral response characteristics of some V1 cells changes with the presence of a luminance contrast, that is, if the contrast is strong enough the colour tuning is much less sharp (Thorell *et al.* 1984). In these studies most V1 cells were found to respond to pure colour stimuli as well as to luminance varying stimuli. Only a few were colour opponent; most responded well to any isoluminant colour change, i.e. were not selective for any particular colour. A possible role for these cells (especially the ones which are also orientation selective) is the determination of form from colour, rather than that of colour per se.

Segregation of function in V1

In addition to the signals reaching V1 being segregated by way of the thalamic input (see above), within V1 itself signals are further segregated with respect to the processing of different visual attributes, before being parcelled out to the appropriate prestriate areas for further processing (Zeki 1975; Livingstone and Hubel 1982; Livingstone and Hubel 1983; Livingstone and Hubel 1984a; Livingstone and Hubel 1984b; Livingstone and Hubel 1987a). This is a fundamental finding for the notion of functional specialisation, since it is logical to assume that V1 does not send the same information to different parts of the visual brain (Zeki 1975).

A segregation between colour, motion, and form processing in V1 has been revealed by electrophysiological studies. Large numbers of cells in cortical layers 2&3 respond to borders that differ in wavelength equally well at all relative brightnesses of the two wavelengths, including

brightnesses at which the border is distinguishable to human observers by wavelength difference alone (that is to say, at equiluminance) (Gouras and Kruger 1979; Hubel and Livingstone 1990). On the other hand, most of the direction selective and all of the disparity selective cells in V1 are located in layer 4B (Gouras 1974; Hubel and Livingstone 1990). Orientation selectivity is usually taken to indicate an elementary level of form processing, and orientation selective cells are thus regarded as the "building blocks" in the elaboration of forms (Hubel and Wiesel 1977). While there is a great deal more to form than the detection of contours, one can nevertheless accept this equation and use it as a working hypothesis to show that, at the physiological level, there is a segregation of form and colour. An inverse relationship between colour and orientation is the general rule (see also below concerning the relation of this to the CO architecture), and many studies have reported that the wavelength selective cells in V1 respond to all orientations (Dow and Gouras 1973; Dow 1974; Gouras 1974; Poggio *et al.* 1975; Livingstone and Hubel 1984a; Ts'o and Gilbert 1988; Lennie *et al.* 1990).

There have been, nevertheless, reports of orientation or even direction selective colour cells (Hubel and Wiesel 1968; Dow and Gouras 1973; Dow 1974; Poggio *et al.* 1975; Michael 1978a; Michael 1978c; Michael 1979; Vautin and Dow 1985). These isolated cells, however, seem to be the exception to the rule, and are reported to occur either in close proximity or intermixed with V1 colour clusters (Ts'o and Gilbert 1988), and also outside colour clusters in layers 2&3 but in very small numbers (Livingstone and Hubel 1984a). Furthermore, most of these cells are not as tightly tuned as the non-colour orientation ones, and it is usually their receptive field spatial organisation that makes them respond better to some orientations than to others (Dow and Gouras 1973; Michael 1978a).

Therefore, the rule seems to be that wavelength and orientation selectivity are mutually exclusive properties of cells in V1, and therefore colour and form (as well as motion, see above) processing are undertaken by separate populations of cells in this area. The segregation of these populations is clearly evident when ones considers the functional architecture of V1.

The columnar organisation of colour cells in V1

Area V1 is the most richly structured area of the cerebral cortex. When stained for its cytoarchitecture, it is found to consist of a relatively large number of layers, which are usually subdivided into six with further subdivision assigned to each. Other techniques such as staining for the enzyme cytochrome oxidase (CO) reveal that this horizontal organisation is intersected by a vertical organisation consisting of columns of cells staining heavily for this enzyme. When V1 is stained for CO it produces a pattern of dark staining barrel-shape "blobs", running perpendicular to the cortical layers and centred on the ocular dominance columns; the blobs are separated from one another by the lighter staining interblobs; this patchy CO pattern is best visualised in layers 2&3 (Horton and Hubel 1981).

A columnar organisation of colour in V1 was first proposed by Michael (Michael 1981). By making perpendicular and oblique penetrations across the layers of V1, he found colour cells to be arranged in columns 100-250 μm wide. The columns were perpendicular to the layers of the cortex and ran from cortex to white matter, cells of a particular column tending to have the same colour preference. This finding is supported by later studies using CO staining, showing that cells in layer 4C underneath the blobs have the same colour opponency as the colour cluster immediately above them (Ts'o and Gilbert 1988); however,

since layer 4B contains mainly directionally selective non-colour cells (Gouras 1974), there is an interruption of colour selectivity there and thus one cannot speak about continuous "colour columns" defined in the classical way.

On this matter, another study used perpendicular penetrations to intersect the cortical layers and related the colour preferences in the middle layers to the orientation selectivity of the upper layers (Dow and Vautin 1987). Cells preferring a particular wavelength over others were found with the same frequency in all penetrations. However, middle layer cells in penetrations that contained unoriented cells in the upper layers showed poor responses to white light and colour preferences for end-spectral wavelengths (i.e. red or blue). Some middle layer cells in other penetrations responded well to white light, but some showed a preference to mid spectral wavelengths. A colour organisation of layer 4 was proposed, with red and blue zones underneath and in register with alternate cytochrome oxidase blobs of layers 2&3, white (unselective) zones in register with interblob centres, and yellow and green zones in-between. Although no CO staining was done in this study, this model has been partly confirmed by later studies in which cell properties are related to cell position with respect to the CO architecture (see below).

Recordings from squirrel and macaque V1 show that cells in the CO blobs are unoriented, the orientation selective cells being restricted to the regions between the blobs (Livingstone and Hubel 1984a). These unoriented cells have receptive fields with circular symmetry and can have colour or spatial opponency, or both. Furthermore, each blob has either red-green or blue-yellow opponent cells, but never both, suggesting a clustering of cells coding for the same colour (Ts'o and Gilbert 1988); in

addition, red-green blobs were found to be three times more frequent than blue-yellow blobs. Bridges of unoriented colour cells are present between blobs, which can sometimes belong to the opposite eye; these bridges are also visible in the CO sections (Ts'o and Gilbert 1988). Lennie *et al.* (Lennie *et al.* 1990) have also confirmed that cells in blobs are chromatically opponent, although such cells were also reported outside the blobs; whether this is a result of the quality of staining and an ambiguity in blob demarcation remains questionable, since there is no single photomicrograph in this paper that relates electrode position to cytochrome oxidase architecture.

Results from other laboratories (see above), however, illustrated with many, very convincing photographs of penetrations in V1, suggest that the colour system is indeed segregated and directly related to the blobs of this area. Blobs receive their input from the parvocellularly derived layer 4Cβ and project to the wavelength selective regions of V2 (Livingstone and Hubel 1984a), and also to area V4 (Yukie and Iwai 1985) which is specialised for colour (Zeki 1973; Zeki 1977; Zeki 1978b; Zeki 1978a). Therefore, the notion of a separate colour system running in parallel with the processing of other attributes, is directly related to the architectonic pattern emerging in areas V1 and V2 by CO staining. This idea was questioned by Leventhal et al. (Leventhal *et al.* 1995), who claimed that colour and orientation selectivity are not segregated in V1, and the presence of selectivity for these attributes is not related to the CO pattern of the area. The reason for this controversy is not clear, but may have to do with the methodology used to examine the selectivity of cells: the authors admit that when oriented bars instead of gratings were used, colour cells were found to be less orientation selective than non colour cells, and some clustering for the two separate populations was then

observed. Concerning the relationship of this clustering to the CO pattern, no firm conclusion can be drawn from their paper since, once again, the evidence relating electrode position to CO architecture is not convincing.

A study of the internal connectivity of V1 confirms the idea of functional segregation in this area, derived from electrophysiological results. Blobs were injected with HRP and found to project to other blobs, a few hundred micrometers from the injection core, avoiding the interblobs; similarly, injections confined within the interblobs produce labelling of near interblobs only (Livingstone and Hubel 1984b). By simultaneously recording with two electrodes and correlating the responses of the two recorded cells, a high correlation was found between cells of a similar colour selectivity within a blob, or within separate blobs, and also between non-blob cells of the same orientation preference; no correlation was found between the cells in the blobs and those in the interblobs (Ts'o and Gilbert 1988). In order to investigate the connections between V1 and V2, small HRP injections in the latter have shown that blobs connect to dark CO staining thin stripes and interblobs to interstripes (see below), both connections being reciprocal; furthermore, within V2 there are stripe-to-stripe and interstripe-to-interstripe intrinsic connections (Livingstone and Hubel 1984a). The basic connectivity pattern emerging from these studies thus is that a "like connects to like" strategy is used by the brain (but see intrinsic V2 connections in next chapter).

The laminar organisation of colour in V1

Although the basic colour module in V1 is arranged in a vertical organisation, i.e. across the cortical layers, colour properties between different layers seem to differ as well. This organisation is in agreement with the magnocellular input being fed to layers 4Cα and 4B, whereas the

parvocellular input to layers 4A and 4Cβ (see above). Colour cells in V1 are frequently found in layer 4A but are absent in layer 4B; some colour cells can also be found in layers 2, 4C, and 5, but only a few in layer 6 (see Dow 1974 and Gouras 1974), where the layer terminology is nevertheless incorrect). Different types of what used to be thought of as "double opponent" cells (see above) have also been reported in different layers (Michael 1985). Concentric cells (Michael 1978b) were found in layers 4A and 4Cβ, and occasionally in the supragranular but never in the infragranular layers. Cells with a central rectangular strip and either one or two inhibitory rectangular flanks found in layers 4B and upper 4Cα were always broad band, while those few found in layers 4A and 4Cβ were usually "double opponent" colour but sometimes broad band (Michael 1978a). Colour-sensitive cells with no antagonistic surround and also cells with a sensitivity to stimulus length (Michael 1978c; Michael 1979) were found in layers 2,3,5,6; however, since these cells are orientation selective (see above) they do not seem to coincide with the unoriented colour cells of the blob system (Livingstone and Hubel 1984a; Ts'o and Gilbert 1988). These early results were nevertheless partly confirmed by more recent studies showing that cells in layer 4Cβ are not selective for the orientation of the stimulus and are either broad band or colour opponent centre-surround (e.g. red-on centre, green-off surround), whereas cells in layer 4Cα are orientation selective and not colour (Livingstone and Hubel 1984a).

Demonstration of colour domains in V1 by other methods

The existence of colour domains in monkey V1 has also been demonstrated by ways other than cell recording. One such method is to inject the animal with radioactive 2-deoxy[^{14}C]glucose (2DG) sugar and then stimulate the brain (Sokoloff 1977; Sokoloff *et al.* 1977). Brain areas

involved in the processing of the particular stimulus used will have a high metabolic activity and absorb more sugar, which will show up in autoradiography after the animal is sacrificed. The metabolic activity of different brain areas can thus be compared between different conditions of stimulation. Using a colour versus a black and white pattern to visually stimulate the striate cortex in monkeys, produces two different 2DG activation patterns (Crawford *et al.* 1982). More extensive studies which have combined 2DG and CO staining have shown that 2DG active regions after colour stimulation coincide with blobs; this is true not only in layers 2&3 where the blob pattern is clear, but also in layers 5&6 (Tootell *et al.* 1988b). Spatially diffuse colour (unlike luminance) variations produce strong uptake in striate cortex, probably due to colour opponent cells with no antagonistic surround. Colour cells with a suppressive surrounds were inferred to be present in the CO blobs of layers 2&3, where there was an increase in the 2DG uptake when coloured gratings were used, as opposed to diffuse colour stimulation; this was not observed in layer 4Cβ. Blobs of the lower, less well staining, layers were also activated in a way similar to layers 2+3, although spatially-opponent colour cells were found to be present more in layer 5. Layers 4Cα (which receives a major input from the magnocellular layer of the LGN) and 4B (which receives an input from layer 4Cα and projects to V5) were found to be insensitive to colour variations. On the other hand, layer 4Cβ (which receives its input from the parvocellular layers and projects to the blobs of layers 2&3) was found to be activated by colour variations (see Livingstone and Hubel 1987b for a review of the connections). A segregation of cells involved in colour processing is therefore evident in these studies as well, in total agreement with previous electrophysiological findings (see above).

Conclusion

When one considers the properties of the cells in V1 in relation to the colour theory of Land described above, it is clear that colour vision demands a great deal more to be accomplished than what this area has to offer. The presence of a detailed topographic map of the visual field in V1, by virtue of the small size of the receptive fields of its cells, together with the presence of multiple cell populations selective for   different visual attributes, suggests that the major role of V1 is a piecemeal analysis of the visual field, separately for colour, form, and motion. Colour vision, however, is not possible unless information coming from a large part (minimum 7°, Zeki personal communication) of the field of view is integrated, since the colour of an area cannot be determined just by the wavelength composition of the light reflected from it. In that sense, cells in V1 can act in a way similar to that determined by the Newtonian colour theory, which considers the colour at a point independently of what is happening in the surrounds. Indeed, V1 cells signal the exact wavelength composition, or changes in the wavelength composition of the (small) area inside their receptive field, as has been demonstrated by Zeki (Zeki 1983a; Zeki 1983b); this is further discussed in the chapter dealing with V4.

The fact that the responses of V1 cells alone are not always correlated with the perceived colour should not, however, be taken to imply that its role in colour vision is of lesser significance. According to Lands theory, numerous local comparisons of the amount of each of the long, middle, and short wavelengths are necessary before assigning a lightness record to each area. The spatial opponency of some of the V1 colour cells described above would make them appropriate for such a comparison. It is not clear how colour opponency of a cell would be used in Land's theory, but it is very possible that, although the theory is correct,

the brain uses its own slightly different strategy for assigning colours to objects and surfaces. One way or the other, what is clear is that V1 cells are an important part of the colour system, but cannot signal the real colour of objects; the search for answering the mystery of colour vision should be thus taken on to the "higher" visual areas.

Chapter 3: The organisation of area V2 and its relation to colour

Introduction

The second visual area, area V2, which is adjacent to and slightly smaller than area V1, has two basic characteristics. Firstly, it contains a detailed topographic map of the whole visual field, and secondly, it contains a heterogeneous population of cells with respect to selectivity for different visual attributes. Since these cell populations are segregated, it raises the problem of how the visual field is represented across this area so that each part of the visual field is mapped for every attribute. The presence of a single continuous retinotopic map would mean that each part of the visual field would be processed for a single attribute of vision only. Multiple representations are therefore necessary for each part of the visual field, one for each different visual attribute, leading to a repetitiveness in mapping when one records across different functionally specialised regions of V2.

Colour in V2

Since V2 receives its input mainly from V1, including subdivisions of V1 specialised for colour processing (Livingstone and Hubel 1984a), the presence of colour selective cells in this area should be expected. Indeed, in early studies, 16% of the cells in the posterior bank of the lunate sulcus of both the anaesthetised and the awake behaving monkey were found to respond with excitation to some colours and inhibition to others (Baizer *et al.* 1977; Zeki 1978b). All gave spatially coextensive colour opponent responses in a round or oval centre, and some had in addition suppressive surrounds which limited the size of a stimulus effective in eliciting one or both of the centre responses (Baizer *et al.* 1977). Selectivity for red and green was more frequently encountered: 2/3 of the colour cells

responded maximally to red or green and 1/3 to blue or yellow. Baizer *et al.* concluded that V2 is very similar to V1, not only with respect to colour but also to other cell properties such as orientation and direction, and that the different visual attributes are processed by different populations of neurons in V2 as well. Similar results were obtained by other studies (Hubel and Livingstone 1985; Burkhalter and Van Essen 1986; Hubel and Livingstone 1987), where an additional type of colour cell, not present in V1, was also described (Hubel and Livingstone 1985; Hubel and Livingstone 1987): unoriented cells with both a colour and spatial opponency were present mainly in layers 5&6, which responded to a spot of optimum size regardless of where it was positioned over their receptive field, an area that was itself many times larger than the optimum activating spot.

In a quantitative study of cell properties in V2, 64% of the cells in this area were found to be selective for colour, but only a few showed opponency by not responding well to white light (Burkhalter and Van Essen 1986). The reliability of this percentage is, however, questionable, since it is based on only two penetrations containing 28 cells. Furthermore, the penetrations did not sample across different CO stripes, and if one of them was in a colour cluster (see below) the 64% figure is not representative of area V2 as a whole. In the same study, preferred wavelengths were found to be evenly distributed across the spectrum for colour non-opponent cells, whereas for colour-opponent cells they were found to be concentrated at the long and short wavelengths. Cells were found which were colour selective for dim stimuli, but could lose their selectivity when brighter stimuli were used. A possible explanation for this is that some luminance cells also receive weak colour information, and thus reveal their colour properties only when the luminance

response is very low. These results are similar to the ones previously obtained in V1 (Thorell *et al.* 1984).

Using a method originally applied in the LGN (Derrington *et al.* 1984) and then in V1 as well (Lennie *et al.* 1990), Levitt *et al.* have tried to describe quantitatively the chromatic properties of neurons in V2 (Levitt *et al.* 1994a). Judging by the figures in the Levitt paper, this method does not seem very appropriate for studying V2: the responses of the neurons often sit uneasily in the sinusoids that the mathematical model describes. Nevertheless, chromatic opponent neurons that respond to isoluminant colour variations were reported in this study as well, most of which, however, also responded well to luminance modulation. Preferred modulations along the isoluminant plane were often along the red/green or blue/yellow axes, although many cells responded to modulation across either of these axes. In this study, almost all V2 neurons were more responsive to luminance modulation than to pure chromatic modulation. Nevertheless, it is possible that Levitt *et al.* have underestimated the responses of their cells to chromatic stimuli, since they always presented their stimuli on a white surround of equal luminosity: spatial antagonistic mechanisms are present in the vast majority of V2 cells, and therefore white stimulation of the surround would result in diminishing or even abolishing cell responses (see above).

Segregation and cytochrome oxidase architecture in V2

In addition to colour, area V2, like V1, is involved in the processing of other visual attributes as well. It is therefore natural to ask whether the segregation of function evident in V1 (see previous chapter) is also present in V2. The population of colour selective cells in V2 seems to be separate to the population of cells selective for orientation, or direction, or

disparity (Baizer *et al.* 1977; DeYoe and Van Essen 1985; Hubel and Livingstone 1985; Shipp and Zeki 1985; Hubel and Livingstone 1987; Roe and Ts'o 1995). Polyselective neurons, i.e. neurons for which the probability of being selective for one attribute is independent of the probability of being selective for another, have however been described in V2, the incidence of which was nevertheless low (Burkhalter and Van Essen 1986; Roe and Ts'o 1995)

When area V2 is stained for the enzyme cytochrome oxidase (CO), a characteristic pattern of alternating thin (N) and thick (K) dark staining stripes, separated by pale staining interstripes (I) appears (see **Figure 3**). The CO stripes run perpendicular to the V1/V2 border: long penetrations made almost parallel to the long axes of the lunate sulcus will intersect the stripes at right angles, allowing one to compare the different cell properties across different stripes within the same penetration (Hubel and Livingstone 1985; Shipp and Zeki 1985).

In studies where such parallel penetrations were made, long stretches of cells almost entirely lacking orientation selectivity were found in the thin stripes (Hubel and Livingstone 1985; Shipp and Zeki 1985; Roe and Ts'o 1995). In macaques, 2/3 of these unoriented cells show colour opponency. Cells in both the thick and interstripes show orientation selectivity and are, in their vast majority, not colour-coded. Many of the interstripe cells are also end-stopped, whereas many cells in the thick stripes are disparity tuned (Hubel and Livingstone 1985; Hubel and Livingstone 1987). Similar results were reported in the study of DeYoe and Van Essen, where several penetrations parallel to the CO stripes were made instead, each one centred on a particular type of stripe (DeYoe and Van Essen 1985). A difference between this and the previous studies is that

very few oriented cells where found in thin stripes and interstripes. ·
Orientation selectivity was surprisingly low overall (41%) in this study, a
result which could originate from the blind use of selectivity indexes to
quantitatively characterise a cell; this method could also explain the
equally surprising presence of colour selectivity in the interstripes.
Alternatively, an unclear CO pattern combined with the use of several
parallel rather than a single perpendicular penetration, could result in
wrongly placing cells in stripes. For example, they might have placed
some of the orientation selective cells of an interstripes to the adjacent
thick stripe and some of the colour selective cells of a thin stripe to the
adjacent interstripe, especially when the penetration was at the border
between a thin stripe and an interstripe, making it difficult to attribute
cells to one stripe or another unambiguously.

In a similar V2 study, cells that respond to illusory contours or lines
defined by coherent motion were described (Peterhans and Von der Heydt
1993). These were found in thick stripes and interstripes, but were absent
from the thin stripes. No colour stimuli were used in this study, however,
which resulted in most thin stripe neurons being unresponsive, if the
recording was made from a colour cluster within the stripe. Clusters of
end-stopped cells were found only in the interstripes, whereas the thick
stripes revealed columnar clusters of disparity tuned neurons and
neurons with common orientation preference. Orientation cells were
found everywhere, but were encountered less frequently in the thin
stripes where they were more broadly tuned. Disparity tuning was also
found everywhere but was more pronounced in the thick stripes, whereas
direction selectivity was equally distributed in all stripes; finally, all
neuronal properties were distributed evenly across cortical laminae.

A number of more recent V2 studies have claimed that, although there is a tendency for different populations of cells to have different properties and cluster in certain CO compartments, this tendency is not absolute and any selectivity can be found in any CO compartment (Levitt et al. 1994a; Gegenfurtner et al. 1996). In the paper by Gegenfurtner et al., colour cells were more frequently found in thin stripes, but also in thick and inter stripes. No statistically significant difference was found in the degree of selectivity for colour between the three CO compartments, and no negative correlation between the selectivity for colour and for other stimulus attributes was reported. Instead, many cells were capable of encoding information along more than one stimulus dimension, regardless of their location with respect to the CO compartments. However, when one looks at the results of this paper, a trend for segregation is evident, which is nevertheless not statistically significant, probably due to the size of the cell sample tested. Furthermore, neurons selective for more than one attribute, for example colour and direction of motion, are usually either marginally selective for direction or marginally selective for colour. Gegenfurtner et al. admit that, although given the properties of one cell you cannot assign it with certainty to a particular stripe, given the properties of a group of cells you can assign them to a stripe with a high probability of being correct. For example, a colour selective cell can be present in any CO stripe, but clusters of colour selective cells are present only in thin stripes.

Another study along the same lines has found that the clustering with respect to the CO stripes is not absolute, and is also related to the laminar position within each compartment (Levitt et al. 1994a). Unoriented cells were more prominent in layers 2-4 of thin stripes, direction selective cells in layers 3B/4 of thick stripes, colour selective cells

in the upper layers of thin and pale stripes, and end-stopped cells mainly outside layer 4 in the thin stripes. Therefore, only a few cells in each stripe have the property characteristic of that stripe and, if the cell under study is found in a layer that projects to the higher visual areas, the probability that it will have the distinctive property of that stripe is high. One should note however that, in this study, penetrations were not made in a direction perpendicular to the stripes, nor was the brain sectioned in a plane parallel to the cortical layers, in which case the CO pattern is visualised in the most efficient way. It is thus possible that the assignment of cells to individual stripes is not as accurate as in previous reports (Hubel and Livingstone 1985; Shipp and Zeki 1985). Even so, a separation of function is evident in this paper as well: direction and colour cells were found to be kept segregated, both in regards to CO and to layers, and also colour selectivity and poor orientation selectivity were often associated. In the end, Levitt *et al.* conclude that there results are "broadly consistent with functional segregation across V2" (Levitt *et al.* 1994a).

In summary, a segregation of function directly related to the CO architecture in area V2 is the general finding, though it is more prominent in some studies than in others. The fact that a more or less limited (depending upon the study) number of isolated neurons selective for one or the other attribute can be found in the "wrong" place, or that a neuron can be occasionally found with a (weak) selectivity for more than one attributes, was already known from the pioneer V2 studies (DeYoe and Van Essen 1985; Hubel and Livingstone 1985; Shipp and Zeki 1985) and does not change the overall impression of segregation found in this area.

The anatomical connections of V2

The studies described above show that the functional architecture of V2 is characterised by a segregation of cells with common properties in different CO compartments. It is therefore logical to suppose that this functional architecture will also govern the inputs and outputs of this area, i.e. functionally specialised regions of V2 will send and receive information to and from cortical regions specialised in the same way.

Unlike V1 which receives its major input from the LGN, the input from the LGN to V2 is much more sparse and the input from the thalamus to this area is mainly from the pulvinar; this input is mainly into lower layer 3 and also layer 1, is patchy, and avoids pale stripes (Livingstone and Hubel 1982; Levitt *et al.* 1995). The main input to V2, however, comes from V1 and feeds into all three types of CO compartments (see below).

The functional segregation found in V2 is also implied in the way this area connects with area V1. The thick stripes receive their input from layer 4B in V1, and since this layer receives its input from the magnocellular division of the LGN by way of layer 4Cα, the thick stripes probably belong to the magnocellular subdivision of the visual pathway (Livingstone and Hubel 1987a). There is, however, some recent evidence suggesting that most layer 4B neurons in V1 receive strong input from both magnorecipient layer 4Cα and parvorecipient layer 4Cβ, and therefore the term "magnocellular subdivision of the visual pathway" loses its validity very early in cortical processing (Sawatari and Callaway 1996). Concerning the thin stripes, HRP injections have shown that they connect to blobs, whereas interstripes to interblobs, both in a sending and receiving manner; within V2, thin stripes connect to thin but also to thick

stripes, whereas interstripes only connect to interstripes (Livingstone and Hubel 1983; Livingstone and Hubel 1984a).

The intrinsic connections in V2 have also been studied with 250-300 μm diameter biocytin injections which produced similar sized patches (10-15) up to 4 mm from the injection site (Levitt *et al.* 1994b). The patches of label were distributed in an elongated field orthogonal to the stripes. CO-rich compartments were found to project mainly to other CO-rich compartments, both of the same and of different type. CO-poor compartments, however, were reported to project equally to CO-rich and CO-poor compartments. A non-patchy distribution of label after biocytin injections in V2 has been also reported by another laboratory (Rockland 1985), although the size of these injections was quite large and therefore included more than one type of stripe. Biocytin results are nevertheless in conflict with the HRP results, and therefore the matter still remains unresolved. One way or the other, the possibility of connections between different functional systems in V2 is very interesting, since it makes V2 into a site possible for some kind of integration between different attributes to take place (see discussion for more details).

Another study using biocytin injections made in the superficial layers of V2 (Malach *et al.* 1994), in regions identified to be orientation domains by the method of optical imaging, labelled a dense network of horizontally projecting fibres that were organised in columnar patches (200x340 μm) running continuously through layers 2-5; the extent of labelling was up to 4-5 mm away from the injection site. After staining for CO, these axonal patches were found to be in all CO stripes. A wide range of orientation domains was labelled, but domains with the orthogonal orientation preference to the injection site were avoided, suggesting a

further compartmentalisation within the orientation system itself. Since no injections into thin stripes were made, the label in V1 avoided the CO blobs, thus verifying negatively the connection between the two.

When visual areas V5 and V4, specialised for motion (Zeki 1974; Zeki 1978a) and colour (Zeki 1973; Zeki 1977; Zeki 1978a) respectively, are injected with different tracers in retinotopically corresponding regions, non-overlapping clusters of labelled neurons appear mainly in layers 2&3 of V2 (DeYoe and Van Essen 1985; Shipp and Zeki 1985; Shipp and Zeki 1989a; Shipp and Zeki 1989b). In these studies, a correlation of patches of label with cell properties and CO architecture reveals that: V5 projection clusters are found to occur mainly in CO thick stripes, whereas the ones from the V4 injections are mostly centred over thin stripes and interstripes. Colour selectivity is found to be high in V4 projection clusters and much lower in V5 projection clusters, where the few colour cells found never occur in clusters. Direction selective sites are rare but markedly higher for V5 than for V4 projection clusters, and all clustered direction-selective sites occur in V5 projection clusters. Strong orientation selectivity occurs in nearly half of the sites in V5 projection clusters, but is also common in V4 projection clusters. Therefore, a separation of function in V2 is also evident in the way this area connects to the "higher", functionally specialised, areas of the visual cortex.

Imaging studies in V2

The heterogeneous morphological organisation of V2, suggesting a functional compartmentalisation in this area, is also revealed by using the 2DG method (Tootell *et al.* 1983). When monkeys view a blank screen, 2DG uptake is higher in the dark staining CO stripe regions, suggesting that they are metabolically more active. By using oriented stimuli of a

high spatial frequency, high 2DG uptake in the interstripes is produced, as happens with the interblob regions in V1. Unoriented diffuse stimuli of low spatial frequency stimuli, on the other hand, produce high 2DG uptake in the dark-staining stripes. No distinction between thick and thin stripes was made in this paper; in a later study, however, spatially diffuse variations in colour were found to produce high uptake confined to the thin stripes (Tootell and Hamilton 1989). Coloured gratings of medium-high spatial frequency produced higher uptake than that of low spatial frequency colour gratings, a result which suggests the presence of cells with inhibitory surrounds in V2: when diffuse colours are used, only colour (but not spatially) opponent cells are activated. A possible predominance of red/green cells is also suggested by 2DG results, since yellowish wavelengths (mid-spectrum) produce much lower uptake than wavelengths from the end of the spectrum.

Concerning the non-colour parts of V2, general achromatic gratings of low luminance contrast produce a weak uptake which is nevertheless confined to the thick stripes; this suggests that input to V2 is mainly parvo (P cells in LGN are not stimulated by these stimuli) apart from a magno input to the thick stripes. High spatial frequency (gratings at all orientations) produces isolated columns of high uptake, confined to the interstripes, whereas low spatial frequency produces high uptake on every dark stripe. In agreement with electrophysiological results (Zeki 1979), there is no 2DG evidence for the possibility of an ocular dominance architecture in V2. There exist, however, orientation columns which are as wide as their counterparts in V1 but are spaced further apart.

V2 results similar to the ones obtained by the 2DG have also been obtained by the method of optical imaging. Regions of poor orientation

preference were localised in every other CO stripe (Ts'o *et al.* 1990), which were identified to be the thin stripes by a latter study in the squirrel monkey (Malach *et al.* 1994). Furthermore, orientation domains are found to form patches which avoid the thin but not the interstripes, with highly selective regions centred on thick stripes.

The topographic organisation of area V2

Area V2 in the macaque monkey is characterised by a distinct topographical organisation. The representation of the horizontal meridian in it forms the anterior border of this area and is split so that the representation of the lower visual field is located dorsally and that of the upper field ventrally; the representation of the vertical meridian, on the other hand, is adjacent to that of V1 and forms the posterior border of V2 (Cragg 1969; Zeki 1969; Gattass *et al.* 1981). Systematic anatomical and physiological mapping studies have shown that V2 contains a topographically organised representation of the contralateral visual field up to an eccentricity of at least 80°. As in V1, the representation of the central visual field is magnified relative to that of the periphery; at a given eccentricity, receptive field sizes in V2 are larger than those in V1, and hence the topography there is relatively coarser (Cragg 1969; Zeki 1969; Gattass *et al.* 1981).

The topographic organisation of V2 has also been thoroughly studied in the Owl monkey (Allman and Kaas 1971) and in the Cebus monkey (Rosa *et al.* 1988), with similar results. In the latter, receptive fields corresponding to recording sites separated by a cortical distance of up to 4 mm, when recording parallel to the V1/V2 border and therefore intersecting the different CO stripes at right angles, were in some cases found to represent the same portion of the visual field. The cortical

magnification factor, measured in mm of cortex per degree of receptive field centre progression, is thus greater when one records perpendicular as opposed to parallel to the CO stripes layout. This is a very interesting result which supports the idea of a re-representation of the visual field in the different types of CO stripes, due to functional segregation (see Results section in this thesis as well).

The mapping of visual space in V2

Since each different type of CO stripe in V2 is involved in the processing of a different attribute of vision, it would make sense for the visual field to be mapped separately in the thick, thin, and interstripes. In this way, every part of the visual field will be represented for each function, without the gaps that would result in a continuous, smooth map. This idea was initially introduced with respect to the ocular dominance columns in the striate cortex, where the visual field needs to be mapped separately for the two eyes (Hubel and Wiesel 1974), and raised again in V2 (Zeki and Shipp 1987; Zeki 1990c) after functional segregation was established in this area as well. Zeki and Shipp have found that, probably due to the fact that each part of the visual field has to be mapped separately for each function, there is a high overlap in receptive field representation between cells belonging to adjacent stripes of a different type, whereas the overlap in receptive field representation between cells belonging to adjacent stripes of the same type is minimal (Zeki and Shipp 1987; Zeki 1990c) . In this way, adjacent stripe cycles (a cycle consisting of 1 K, 1 N, and 2 I) seem to represent adjacent regions of visual space, a conclusion re-enforced by the observed discontinuity in receptive field progression at stripe borders, where a "jump-back" in receptive field centre position occurs in order to re-map that part of the visual field in the

next stripe, as opposed to the continuity from one stripe to the next like stripe (Zeki and Shipp 1987; Zeki 1990c).

Similar results were also obtained by a more recent, quantitative study, in which V2 was divided not in terms of CO stripes but in terms of selectivity for colour, orientation, and disparity (Roe and Ts'o 1995). Despite the deviation from the strict CO pattern in V2, the presence of multiple, interleaved visual maps, one for each of the colour, orientation, and disparity domains, was again demonstrated in this area. Furthermore, some evidence was also found for the presence of multiple maps within each domain, the clearer example being that of separate maps in the colour domains for different colours. This finding is also in agreement with other electrophysiological (Levitt *et al.* 1994a), 2DG (Tootell and Hamilton 1989), and biocytin (Levitt *et al.* 1994b; Malach *et al.* 1994) studies, which suggest that the stripes in V2 appear not to be uniform but to consist of further subdivisions.

The timing of signal arrival in V2

In order to see the sequence with which information is processed between the different areas and also between the different specialised systems within an area, the latencies to small flashing spots of light were measured in different layers of V1 and V2 (Nowak *et al.* 1995). In V1, layers $4C\alpha$ and 4B were activated 20 ms faster than layer $4C\beta$. The infragranular layers were found to be activated faster than the supragranular layers in both V1 and V2. Both these layers were activated with the same latency in V1 and V2, suggesting simultaneous processing in the two areas: by the time 50% of the neurons in V2 had responded to a particular stimulus, 43% of the neurons in V1 had not yet responded. In V1, no significant differences were found between cells selective and cells

not selective for colour, orientation, and direction. But in V2 oriented cells were activated before unoriented, and non-colour before colour selective cells, with a difference of about 10 ms. A similar study also related difference latencies to different CO stripes in V2 (Munk *et al.* 1995): Neurons in the thick and pale stripes were found to respond 20 ms earlier than those in the thin stripes, colour selective neurons were found to respond later than neurons with no colour selectivity, and direction selective neurons were found to have shorter latencies than neurons with no selectivity for the direction of movement.

Two basic conclusions can be drawn from these results. Firstly, the idea of a hierarchical organisation of the visual system based primarily on the temporal order with which stimulus-related information reaches the different visual areas is not correct. Such a model would predict, for example, that processing of information at a certain level of complexity would be first terminated in V1, after which the end result of this processing would be transferred to V2 for further processing, and so on. The results described above, however, suggest that visual perception is rather the result of simultaneous processing within the different visual areas. Secondly, the fact that, within a single area characterised by local functional specialisation, information concerning different attributes of vision arrive at separate times, suggests a separation of function not only in the geographical but also in the temporal domain. This notion, in turn, creates the very interesting question of how the different attributes belonging to the same object are related to each other in the correct temporal manner; this is the question which the Psychophysical part of this thesis has tried to address.

Chapter 4: Area V4 and its role in colour vision

The colour area of the brain

Area V4 was at first defined anatomically by virtue of the input it receives from V2 (Zeki 1971). The basic characteristic of the majority of cells in this area was found to be selectivity for the stimulus wavelength, and both opponent and non opponent colour cells were recorded from it (Zeki 1973; Zeki 1977). The abundance of colour cells in this area, together with the absence of cells selective for the motion of the stimulus, a result reverse to the one found in area V5 (Zeki 1974), led to the characterisation of V4 as the colour emphasising area of the brain; however, the possibility that V4 was not solely involved with colour was pointed out early on (Zeki 1973). After the discovery of area V4, systematic recordings from it undoubtedly verified its involvement with the processing of colour. Selectivity for the wavelength of the stimulus is the basic property of cells found in this area; all V4 studies agree on this, although the percentage of colour cells and the specificity of their tuning varies from one study to another. Some cells are colour biased, if they respond poorly or not at all to some wavelengths, or colour biased with opponency, if they also do not respond well to white light, or clearly colour opponent, when excited by some wavelengths and inhibited by some others (Kruger and Gouras 1980; De Monasterio and Schein 1982; Schein *et al.* 1982; Desimone *et al.* 1985; Tanaka *et al.* 1986; Desimone and Schein 1987; Schein and Desimone 1990).

The properties and frequency of colour cells in V4

Since colour is one of the chief functions of V4, one might expect wavelength selective cells there to be more tightly tuned than at earlier stages in cortical processing. However, spectral band-widths of V4 cells are

narrower than the ones of cones but comparable to those of the colour opponent cells in the retina and LGN (Kruger and Gouras 1980; De Monasterio and Schein 1982; Schein *et al.* 1982; Desimone *et al.* 1985; Schein and Desimone 1990). Some studies have suggested that colour selective and opponent responses of cells are less pronounced in area V4: across cells, the average response of the least effective colour stimulus tested was 20% of the response to the best stimulus, and the average response to white light was 60% of the response to the best colour stimulus (Schein *et al.* 1982; Desimone *et al.* 1985; Schein and Desimone 1990). Most V4 cells would thus seem to act as a broadband colour filter, which lets energy at some wavelength pass more efficiently than at others and always lets some energy through from white light. Furthermore, although the initial papers on V4 report a very high incidence of colour selective cells, some later studies have resulted in substantially smaller numbers in their physiology, suggesting that the role of V4 in colour vision is not as overwhelming as initially thought: the percentage of colour biased or colour opponent cells in V4 was found to be as low as 20-30%, whereas 50-60% of the cells were found to lack chromatic selectivity (either bias or opponency) and to respond equally well to chromatic and achromatic lights (Schein *et al.* 1982; Tanaka *et al.* 1986).

One should in many ways be critical in accepting the facts enumerated above. Differences in the percentages of colour cells in V4 can be explained by the use of different criteria to characterise a cell as colour selective by different laboratories. If, for example, one were to exclude from the colour selective category cells which respond to white light, or cells selective for an extra-spectral colour which was not used to test this cell, the percentage of colour cells would be greatly decreased. A second explanation is that, in some of the previous studies, recordings were

mainly done from regions in V4 which are indeed not concerned with colour (Zeki 1978a; Zeki 1983c). The presence of such regions in V4 is also strongly suggested by the patchy connections of this area (see below).

A more important criticism concerning these studies, however, is the fact that they did not invent any new ways of testing area V4, but rather repeated in this area the experiments previously done in striate cortex. In general, it is not a good idea to study different visual areas (which might have very different properties) in an identical way. The conclusions concerning a new area should give new insights about brain functioning, rather than simply comparative results between this and a previous area. Area V4 is a good example of this, since the responses of many of its cells are related to real colour rather than wavelength (see below), and therefore its involvement in colour processing is manifest in a way more sophisticated than a simple increase in the percentage of colour cells, tightening of tuning curves, etc.

Is V4 involved in form processing?

The fact that some cells in V4 are not selective for the colour of the stimulus together with the fact that this area is also the major input to the inferotemporal cortex (IT), which is related to form processing (Desimone et al. 1984; Tanaka et al. 1991; Kobatake and Tanaka 1994), suggests that, in addition to colour, V4 itself should also be a part of the processing stream involved with form (Schein and Desimone 1990). The involvement of V4 in form perception is further strengthened by the fact that many cells in it are orientation selective (Zeki 1975; Desimone et al. 1985; Desimone and Schein 1987; Schein and Desimone 1990), a characteristic which is thought to be strongly related to form processing (Hubel and Wiesel 1977). Most of these cells, however, have a wider bandwidth than orientation cells in V1,

although a few are as sharply tuned (Desimone and Schein 1987). Cells selective for the length, width, direction of motion, and spatial frequency of the stimulus can also be found in V4 (Tanaka *et al.* 1986; Desimone and Schein 1987). It is not clear, however, if these cells are also colour selective, i.e. whether different cell properties are segregated in V4 in a way similar to V1 and V2. Some studies report that there seems to be no difference in colour selectivity between orientation and non-orientation cells, although this has not been extensively studied (Schein *et al.* 1982; Schein and Desimone 1990). Furthermore, since there is no clear-cut definition of what form is, and since form can be the result of different causes (e.g. form defined from luminance, or from colour, or from motion), it is possible that there are more than one form systems in the brain and thus V4 might be part of one but not another of them (see also below, concerning the effect of V4 lesions in form discrimination).

The responses of V4 cells to wavelength versus colour

A big difference between V4 cells and cells in previous stages is that the former have much larger suppressive surrounds (Zeki 1983c; Desimone *et al.* 1985). Surround stimuli have no effect when used alone but, when flashed in the surround outside of, but not continuous with, the receptive field, can suppress or enhance the response to the preferred wavelength in the centre, depending on whether they are of a near or removed wavelength respectively (Schein and Desimone 1990). The influence of a large surround on the responses of V4 cells is in agreement with the involvement of this area in colour vision, since the colour of an area can only be determined if wavelength information from both the area and its surrounds is compared (see above).

The term "colour cell" has been used loosely up to this point, and no distinction has been made between wavelength and colour. The distinction should be made, however, since the colour of an object remains constant, whereas the wavelength composition reflected from it does not (see above). In order to test whether a cell is responding to colour as we perceive it, or just to the amount of the various wavelengths presented to it, one has to use a method similar to the one used by Land (to demonstrate his Retinex theory). In other words, one needs to vary the wavelength composition reflected from an area of the appropriate colour, while keeping the colour constant, and see whether the cell continues to respond to it or not. In addition, areas of the Mondrian having different colour can be made to reflect the same triplet of long, middle, and short wavelength light, and put in a cell's receptive field in order to see whether the cell can differentiate between different coloured areas when they are reflecting the same triplet of light. When this is done in V4, the responses of some cells correlate with the colour of the Mondrian area positioned inside their receptive field, irrespective of the wavelength combination reflected by it (Zeki 1980; Zeki 1983a). These are the real colour cells, as opposed to the wavelength selective cells which will respond to any area of the Mondrian as long as there is a predominance of their preferred wavelength reflected from it. In addition, real colour cells are not sensitive to the sequence with which the area in their receptive field is illuminated by lights of different wavelengths, but require that the area of their preferred colour placed inside their receptive field be trichromatically illuminated (Zeki 1983b). Wavelength selective cells, on the other hand, do not require the Mondrian to be illuminated by all three projectors, and will respond if the projector of their preferred wavelength is the only one switched on; furthermore, when the Mondrian is trichromatically illuminated, they are sensitive in the order with which

this is done (see Results for more details). Wavelength selective cells are present both in V1 and V4, whereas real colour cells have only been reported in V4. The fact that responses of V4 cells are directly related to the colour of the object of interest as we perceive it, makes this area a very likely candidate for one of the most important characteristics of colour vision, namely colour constancy (see more in V4 lesions section).

The anatomical connections and extent of V4

Area V4 receives its input from the blobs of V1, either directly or via the thin stripes of V2 (see Zeki 1992). Studies in V2 (see above) have revealed the thin cytochrome oxidase stripes to be the colour processing units in V2 and also, together with the interstripes, to project to area V4 (DeYoe and Van Essen 1985; Hubel and Livingstone 1985; Shipp and Zeki 1985). After HRP injections in area V4, labelled cells in V2 were found to be distributed in two ways: either one band of label per CO cycle was found, centred on the thin stripes but also extending to the neighbouring interstripes, or two bands per cycle were found, centred over the interstripes alone (Zeki and Shipp 1989). In both cases, the thick cytochrome oxidase stripes in V2, which contain direction selective cells (Shipp and Zeki 1985) and project to the motion area V5 (Shipp and Zeki 1989b), were avoided. These results suggest a compartmentalisation in V4 itself (see also Zeki 1983c): one possibility is that one type of V4 compartment receives input from both the thin and interstripes, whereas the second type from the interstripes only. Another possibility is that one compartment projects to the thin stripes only and the other to the interstripes only, but the authors failed to make an injection restricted to one of the former compartments alone. The latter is probably the case, since after injecting two separate tracers into area V4 of the same animal the interstripes and thin stripes were found to be labelled by each tracer

separately (DeYoe *et al.* 1994); segregated clusters of label were also found in V3, V4, and part of the inferotemporal cortex after the V4 injections, and also in V4 after inferotemporal injections.

Area V4 projects to a zone lying just anterior to it and extending to the lateral part of the superior temporal sulcus (Zeki 1977). Because of the more anterior position of this zone and also because it has its own callosal connections, it can be considered to be a separate area. Since, however, it receives an input from area V2 (Zeki 1971) and contains also colour cells, it was called V4A (Shipp and Zeki 1995) and grouped together with V4 to form the "V4 complex". Another area has been described near that region, at the temporo-occipital part of the monkey brain, and named TEO by one laboratory (Boussaoud *et al.* 1991) and PIT by another (Felleman and Van Essen 1991), although it is not clear if these two coincide exactly. In a study where two different retrograde tracers were injected into V4 and TEO (Nakamura *et al.* 1993), V4 but not TEO projecting neurons were found in V1, both in blobs and interblobs. In V2, however, TEO-projecting neurons were intermingled with V4-projecting neurons, although the former were much sparser than the latter. Both V4- and TEO-projecting neurons formed bands that ran orthogonal to the V1/V2 border, and both were located in thin stripes and interstripes. Furthermore, across the cases, 6-19% of the TEO-projecting neurons were double labelled, that is, also projected to area V4. The similarities between area TEO and the area described in 1977 by Zeki as part of the V4 complex, indicate that at least the posterior part of TEO does not consist of a new area but is a part of the V4 complex. The status of the anterior part of TEO, however, remains unclear, and more detailed studies are necessary to clearly map the separate areas in that not so well understood part of the brain.

Since V4 receives both a magno and a parvocellular input (Ferrera *et al.* 1992) it is interesting to see whether the function of these two inputs remains segregated in this area. If GABA is injected to selectively block the magno- or parvo- LGN input to V4, a reduction in visual responses of around 40% is seen in both cases (Ferrera *et al.* 1994). This is unlike what happens in V5, where a magno but not a parvo blockage greatly diminishes the responses of cells (Maunsell *et al.* 1990); however, the parvo blockage in the V4 study was more extensive than the one in the V5 study, so there is a possibility that the parvo contribution to V4 has been underestimated. In V4, however, a blockage of either the M or the P layers of the LGN can diminish the responses of the same cell in V4, suggesting that the magno and parvo inputs are not segregated within this area.

The effect of V4 lesions

There is little doubt that the final test of a functional specialisation of an area is the demonstration that, without it, the organism is not able to undertake the task(s) that physiological studies have attributed to it. Unfortunately, such a demonstration is not always easy, especially in sub-human species. For example, no one would today seriously question the role of V1 in vision, in man and monkey; nevertheless, this was a matter of debate well into this century because behavioural (lesion) studies had not resolved the issue (Glickstein 1985). This is to be contrasted with the studies of the human brain, where Henschen in Sweden had already determined the essentially retinotopic projection from retina to V1 and had shown that the scotomas produced there can be related directly to the size and position of the lesion in V1. In fact, it was not until Talbot and Marshall conducted their physiological studies in macaque monkey (Talbot and Marshall 1941) that evidence was obtained for a similar retinotopic organisation of V1, in spite of the fact that Talbot and

Marshall's study had been preceded by several decades of behavioural studies involving lesions of V1. Similarly, decades of lesion studies in macaque monkey had given no hint of a functional specialisation for different attributes of vision, which physiological studies undertaken later have shown (see Zeki 1993 for a review). Arguably, therefore, lesion studies in sub-human species have not been nearly as good a guide to the functional organisation of the visual brain as have electrophysiological studies. Furthermore, a patient who suffers from an inability to perceive colours can communicate to us his defect, which we can then study in more relevant detail; this is far from true for the monkey, and these cautionary remarks should be kept in mind when exploring the effects of lesions in area V4.

Large V4 lesions in the monkey, including parts of what might or might not be a separate more anterior area (area TEO, see above), have been found to result in permanent impairment in hue discrimination (increased thresholds) but not brightness discrimination (Heywood and Cowey 1987). However, the coloured papers used in this study were seen entirely by reflected light, the composition of which changes as the monkeys moves around. Therefore, it is possible that the hue discrimination impairment reported is not a wavelength but rather a colour constancy discrimination deficit. In a later study with the same animals but using hues which were luminous in dark surroundings, with negligible reflected light, and also not as finely separated as in the previous study, the animals could perform well in what was really a wavelength discrimination task (Heywood *et al.* 1992). This is in agreement with other studies, which report that bilateral ablation of the V4 complex affects colour constancy but not wavelength discrimination (Wild *et al.* 1985); similar results have been also reported in the human

(Vaina 1994; Kennard *et al.* 1995). One explanation for this might be that chromatic mechanisms operating before V4 can construct the four primary perceptual categories of colour (red, green, blue, yellow), since V4 lesioned monkeys discriminate the colour spectrum according to the same fundamental colour categories as normal monkeys and humans (Walsh *et al.* 1992b). This is also in agreement with the fact that occipital visual evoked potentials using chromatic stimuli are the same between normal monkeys and monkeys with V4 lesions, suggesting that colour processing in areas V1 and V2 is normal after V4 lesions (Kulikowski *et al.* 1994). A very interesting conclusion of this result is that the capacity of the brain reflects the capacities of the visual areas that are left unaffected by the lesion. Nevertheless, although lesioned monkeys if trained post-operatively can reach pre-operative performance levels in hue but not colour constancy discrimination tasks, their performance never reaches that of normal animals (Walsh *et al.* 1993).

As mentioned earlier, the fact that orientation selective cells are present in V4 (Zeki 1975; Desimone *et al.* 1985; Desimone and Schein 1987; Schein and Desimone 1990), as well as the fact that this area receives an input from the interstripes of area V2 (DeYoe and Van Essen 1985; Shipp and Zeki 1985; Zeki and Shipp 1989; DeYoe *et al.* 1994) suggest that, in addition to colour, form information reaches V4 as well. Nevertheless, the effect of V4 lesions in form processing have not yielded as clear results as they have with respect to colour. An impairment in orientation, pattern and alphanumeric character (whatever the significance of this for the monkey) discrimination has been reported after lesions which included part of V4 but also parts of both dorsally and ventro-anteriorly neighbouring regions as well (Heywood and Cowey 1987; Heywood *et al.* 1992; Walsh *et al.* 1992a). However, the impairment was only a temporary

deficit in the ability of the V4 operated animals (compared to animals which had no operation at all) to reach pre-operative criteria. Furthermore, the phenomenal "severity" (Heywood and Cowey 1987) of the effect depends largely on the criteria used: in another similar study where the criterion for recovery was 90% correct in any 30 (as opposed to 100 in the studies cited above) consecutive trials, no difference was found between operated and unoperated animals (Wild *et al.* 1985). Looking at human studies, while in many cases of achromatopsia there is a reduction in form acuity, there are also cases where the sense of form is not (or is very mildly) affected; the possibility of the existence of several, rather than a single, form systems is strongly suggested by these findings (see Zeki 1990a).

An area involved in colour perception should be expected, in addition to colour selectivity, to have properties necessary for the construction of this attribute by the brain. One of these is probably the ability to emphasise particular parts of the visual field, in order to compare them with the remaining of the environment. Indeed, an involvement of area V4 in selecting objects of interest within the visual field has been suggested (Schiller and Lee 1991; Schiller 1993): V4 lesioned monkeys were found to have mild to moderate discrimination deficits, and also increased response latencies in tasks involving colour and pattern. In addition to that, monkeys had particularly severe deficits in selecting a target stimulus which was smaller, or of a lower contrast, or of a slower rate of motion, than the array of comparison stimuli from which the discrimination was to be made. It is thus the "lesser" stimulus discrimination which is affected, which the authors suggest is a more difficult task, since in nature it is the more intense stimulus that normally pops out. Furthermore, although these animals recovered their

percentage correct performance (but not latencies) with extensive training, they could not generalise the specific task to new stimulus configurations and to new spatial locations. Separation of the environment into objects of interest and "background" objects might be an important strategy for the generations of colours by the brain. Although the exact relation between the two is far from clear, the involvement of area V4 in such "higher" functions is reinforced by electrophysiological studies as well (see next).

Attentional effects on the responses of V4 neurons

In a study where the effect of attention on the firing of neurons was examined, V4 but not V1 cells were found to respond differentially depending on whether attention was towards the preferred or the non-preferred (in terms of colour) of two stimuli placed inside their receptive field at the same time (Moran and Desimone 1985). If attention is directed towards the non-preferred stimulus, the cell will not respond, although the preferred stimulus is still in the cell's receptive field; if, however, attention is focused outside the receptive field, the cell responds to the preferred stimulus inside the receptive field. The fact that V4 neurons fire not only with respect to whether the stimulus configuration is optimal, but also in relation to the context of the stimulus has been also tested in studies where monkeys had to respond when two successive gratings were identical either in colour or orientation (Haenny and Schiller 1988): the firing of 72% of the V4 cells studied (but also of 31% of the V1 cells similarly tested) to the same stimulus was different, depending on whether it was a reward stimulus or not.

Similar results are obtained if monkeys are trained to cue the colour or luminance of the stimulus, rather than its orientation: cells in V4 were

shown to differentially fire according to whether the stimulus in their RF was of the colour or luminance of the fixation point, which they had to remember in order to perform a discrimination task (Motter 1994a). The firing rate was always greater (about twice) for matching than for mismatching cases, and this was true not only for the optimal stimulus but also for any other stimulus which gave a response. It was thus the cued feature and not simply the physical colour or luminance, which determined the response for 74% of the cells studied. Furthermore, if the cued feature was switched from one alternative to another in the middle of a trial, the firing of V4 neurons reversed (Motter 1994b).

In such experiments, the cued orientation for V4 neurons need not necessarily be presented in a visual way: similar differential firing has been observed when a match to sample task for orientation discrimination is used, and the cue orientation can be either visual or somatosensory (Haenny *et al.* 1988). In the study by Haenny *et al.* a variability in the types of responses between different neurons was found, but some of the responses were clearly neither visual nor somatosensory but task specific. Some V4 neurons responded stronger to all orientations presented after a particular cue (cue selective) and some were just orientation selective and unaffected by the cue. A few cells were selective for both cue and stimulus, but the orientation of the two need not necessarily be the same. The responses of such neurons were related to the orientation of the stimulus, irrespective of its modality (whether visual or somatosensory). It is possible that some neurones had a stronger response when, for example, a tactile modality is used, but the orientation preference was the same when a visual cue was used as well (Maunsell *et al.* 1991). On the other hand, in a third task where there was no cue orientation but the animal was simultaneously presented with four

oriented gratings and had to select the one which was different from the other three, very little evidence for behavioural effect in V4 responses was found (Haenny *et al.* 1988).

Differential firing of neurons to otherwise identical visual stimuli at spatially specific locations, due to directed focal attention, has been also demonstrated in areas V1 and V2, in addition to V4 (Motter 1993). Monkeys were trained to attend to one of four different locations, one of which was the receptive field of the cell under study, and make an orientation discrimination task at the particular location ignoring stimuli presented elsewhere. 30% of the neurons responded differently to the same stimulus when their receptive field was the locus of attention than when it was not (they fired in the former but not in the later case). The presence of multiple, competing stimuli (3-4 for V1, V2 and 6-8 for V4) was essential for this differential firing, and most neurons lost their differential response when single stimuli were instead presented. This implies that information derived from a relatively wide area of the visual field contributes to the selective processing of visual information in area V4, which has large receptive fields, but also in areas V1 and V2 where the receptive field sizes are much smaller.

The results described above suggest that attentional mechanisms are able to influence the responses of neurons in the visual cortex, and therefore probably visual perception as well. This is also true in humans: our environment is overloaded with visual stimuli, but we are only consciously experiencing the ones we are interested in or the ones which draw our attention. Information concerning the rest of the visual scene is all the time made available by our visual system, as if it can actually "see" things that we do not. However, some parts of our visual system

modulate their activity with respect to our perception more than others. This is supported by PET results as well, showing differential patterns of activation across the visual cortex to the same stimulus, depending on which attribute of vision attention was directed to (Corbetta *et al.* 1991). This human study, together with the monkey results described above, show that the activity in area V4 is modulated by attention and therefore correlates more with perception. With respect to colour, a correlation of the firing of V4 cells with perception was first suggested, in a very different way, by colour constancy experiments (Zeki 1980; Zeki 1983a). In these experiments, however, anaesthetised animals were used and therefore attention or conscious experience was absent. It is a puzzle how, under these conditions, V4 cells were able to signal the correct (irrespective of wavelength composition) colour of objects. A likely possibility is that attentional mechanisms are inhibitory, for example V4 activity is depressed when one attends to colour, or the response of a red V4 cell is depressed when one attends to green, and therefore absent in the case of the anaesthetised animal. One way or another, the level and extent to which attention can modulate neural activity so that visual information is at the same time "present" and "absent" in our brain, remains a very interesting and yet unresolved question, well outside the aim of this thesis.

Conclusion: The latency of perception

The evidence described in the preceding chapters leads to two basic conclusions: Firstly, the processing of the visual information resulting in the perception of colour takes place at several different stages of a specialised system, and is therefore distributed in different regions of the visual brain. Secondly, in each and every one of these regions and therefore stages of processing, the information concerning colour remains segregated from the information concerning other attributes of the visual scene, i.e. there is functional specialisation within the visual system itself, as there is functional specialisation within the brain as a whole.

The facts of functional specialisation, as presented here and elsewhere, make it reasonable, on their own, to suppose that different attributes of vision are perceived at different latencies. Since, for example, colour and motion information follow separate routes within the brain, have very different processing requirements, and are processed by separate systems, it is very possible that the time delays between the appearance of a stimulus and the perception of its colour and motion are not identical. Of course, one should not completely rule out the possibility that through the millions of years of evolution, the nervous system has evolved marvellous adaptive systems by which different attributes of vision, though processed separately, are in fact perceived at identical times, in strict temporal co-registration. Though this is plausible, it at first sight seems unlikely, for the following reasons: the entire anatomy and physiology of the visual pathways devoted to at least two attributes of vision differs significantly enough for one to have strong hints of temporal disjunction. For example, right at the retina, the conduction velocities of the axons of cells which eventually feed the magnocellular

system and its extension into the cortex, and particularly the cortex of area V5, specialised for motion, is much higher than that of cells that feed into the parvocellular system (Dreher *et al.* 1976; Schiller and Malpeli 1978). Moreover, physiological properties of the magno system differ significantly from those of the parvo system: the responses of the magno feeding cells are transient, with a high temporal sensitivity, whereas those of the parvo feeding system are sustained, with a temporal sensitivity which is lower than the magno system (see Livingstone and Hubel 1987b for a review).

The facts given above alone might suggest that motion is perceived before colour. Such a conclusion could also be reinforced by other facts as well. It has been demonstrated, by direct electro-encephalographic (EEG) recording that, following stimulation with fast moving stimuli, signals are picked up from the prestriate cortex, and more especially from the vicinity of area V5, before they reach V1, the difference being of the order of about 20 ms (ffytche *et al.* 1995). Such a result is consistent with that derived from transcranial magnetic stimulation (TMS) which has shown that, in order to produce a reversible akinetopsia (motion imperception), V5 has to be inactivated some 40 ms before V1 (Beckers and Zeki 1995). When a comparison with the equivalent colour area is made, it is concluded that signals reach V5 before they reach V4 (Buchner *et al.* 1994). Finally, the few single cell recordings that have compared the latencies of activation of cells in V1, V2 and V5 have shown that the cells of the latter respond with the shortest latencies, at about 36 ms (Raiguel *et al.* 1989). The latter figure is consistent with theoretical predictions that the minimum latency with which signals can arrive at the cortex is in fact 30 ms (see discussion in Beckers and Zeki 1995).

Although it is very possible that, since a colour and a motion signal are routed through different pathways in the brain, there must be (in the brain) a de-synchronisation of colour and motion with respect to reality, one might assume that there is a "synchroniser" somewhere in the visual system, which arranges signals into their correct temporal order. Area V2 would be a good candidate for this, since the different visual attributes are processed within it side-by-side, and interactions between them are possible via lateral interconnections between different functional CO stripes (Levitt *et al.* 1994b). However, this is not the case since electrophysiological recordings from this area have reinforced the notion of temporal differences in the processing of different attributes: colour cells there are activated after the non-colour cells (Munk *et al.* 1995; Nowak *et al.* 1995), and also thick stripes and interstripes of V2, which are relatively poor in colour cells, are activated before the thin stripes which are rich in wavelength selective cells, and finally that direction selective cells are activated earlier than cells lacking such a selectivity (Munk *et al.* 1995).

The results described in the previous paragraphs suggest that motion is processed faster than colour. It is very interesting to ask whether these time differences in processing will have a noticeable result in perception, i.e. whether one can show, for example, that the direction of movement of an object is seen before its colour. Since the relationship between signal recording and perception is far from known, one has to turn to the psychophysical literature in looking for an answer to this question. The story told by psychophysical evidence, however, is not as clear. A standard way of measuring the temporal properties of a system is the temporal integration period, which is related basically to stimulus presentation time (maximum necessary presentation at the lowest

threshold, highest alteration frequency at which discrimination is still possible etc.). Temporal integration periods for colour and motion vary, depending on the conditions and the method used. It is thus probably not correct to draw any general conclusions about a comparison between the two systems, based on integration periods (Krauskopf and Mollon 1971; Smith *et al.* 1984). Such conclusion have indeed not been drawn, except in a direct comparison study claiming that there is also psychophysical evidence for the motion system being the faster one (Livingstone and Hubel 1987b). Our view is that there is no straightforward interpretation of the results in this field, mainly because the terms "faster" and "slower" refer to a specific processing property of each system, rather than to the total time necessary for the end perceptual result to be achieved.

The physiological and psychophysical evidence are addressed in more detail in the Discussion chapter of this thesis. As a summary concluding remark here, it is enough to say that neither temporal integration periods nor signal arrival times and conduction velocities can be equated with perception. Furthermore, the important question is not whether colour is perceived first or motion first, but whether a colour and a motion which occurred together are perceived together or separately. This is the question addressed in the psychophysical part of this thesis.

MATERIALS AND METHODS

Summary-aims

The aim of these experiments was to identify and characterise cells in the visual cortex of the monkey, and explore the possibility of a difference in the processing time necessary for the perception of different visual attributes.

Part I: Physiology

Subjects

26 male juvenile macaques, *Macaca fascicularis* , between 12 and 20 months of age were used, ranging in weights between 2 and 3 Kg. The animals were housed together (maximum 8 animals) in a multi-level large cage equipped with a number of games, in a 12:12 hours light-dark schedule, and were fed once a day. Prior to an experiment a monkey was isolated and left without food for 24 hours. Electrophysiological experiments always began on a Monday morning and went on continuously (24h per day) for up to six days. The monkey was always sacrificed at the end of the experiment. All procedures described below were carried out under aseptic conditions; gloves, masks, hats, and lab coats were always used, in order to protect both the animal and the experimenters from any infections.

Preparation for surgery; anaesthesia

The selection of the drugs used when performing electrophysiological experiments in the cortex is crucial: on one hand the animal must feel no pain or be conscious, and on the other hand the

responsivity of the cortical grey matter must be as healthy as possible. We used a regime of agents that we believe satisfied both these requirements.

12 hours before surgery the animals were given an intramuscular injection of 4 mg/kg of the anti-inflammatory corticosteroid dexamethasone sodium phosphate ("Decadron", David Bull Laboratories) as a precaution against cerebral oedema; further intra-muscular injections of 2 mg/kg were given every 12 hours. At the beginning of the experiment the monkey was first sedated with 20 mg/kg ketamine ("Vetalar", Parker-Davis), weighed, shaved, and the saphenous vein cannulated. Ketamine is a glutamate antagonist specific to the NMDA receptor, which produces a marked sensory loss and analgesia, as well as amnesia and paralysis of movement, without loss of consciousness. In some animals, where the cannulation was not easy and took a longer period of time, an additional 20 mg of ketamine was given. After cannulation, intravenous injections of propofol ("Diprivan", Zeneca) were used to keep the animal anaesthetised. Propofol (2, 6-diisopropylphenol) is a short-acting general anaesthetic agent with a rapid onset of action of approximately 30 seconds, suitable for induction and maintenance of general anaesthesia. Its mechanism of action, like all general anaesthetics, is poorly understood. Induction of anaesthesia is generally smooth with minimal evidence of excitation. Because of its rapid action, it should be given slowly (no more than 5 mg per injection) until the absence of the pedal flexion reflex (and also pinching the ears, toes, etc.) indicates the onset of anaesthesia. Propofol is rapidly metabolised and thus recovery from anaesthesia is fast, so repeated injections or continuous infusion are necessary to maintain adequate anaesthesia (ICI pharmaceuticals data sheet).

After cannulation was completed and propofol anaesthesia was induced, the animal was intubated through the mouth or directly cannulated through the trachea. The former has the disadvantage of a long, inaccessible intubation tube which can easily get blocked by mucus and blood, so direct cannulation was preferred in the more recent experiments.

After surgery (see below) was completed, an initial dose of 100 µg/kg of the neuromuscular blocker pancuronium bromide ("Pavulon", Organon Technika) was given, in order to abolish eye movements and keep the cell's receptive field at a constant position on the screen. The monkey was respired using a Harvard respirator: the tidal volume was set according to the weight of the animal (11.6 cm^3 per Kg) and the respiration rate adjusted to maintain the end tidal CO_2, as measured by a "Cardiocap" ECG/gas analyser, at between 3-4%. At that point the continuous intravenous perfusion of pavulon was initiated, at a rate of 60 µg /Kg/hr. A combination of an opiate with an anaesthetic agent was then used (Jansen *et al.* 1990), which has the advantage of good analgesia and anaesthesia without the risk of consciousness. As soon as the animal was in artificial respiration, propofol was continuously perfused intravenously in order to maintain anaesthesia, at a rate of 4-12 mg/Kg/hr. The morphine analogue analgesic sufentanil citrate (Janssen), infused alongside propofol (which has no analgesic effect) at a rate of 3-8 µg/Kg/hr, was used as the primary anaesthetic. High doses of this opioid produce anaesthesia, and also provide excellent pain relief. Sufentanil has been used in other laboratories in conjunction with O_2 and N_2O , or with CO_2; the cortex is much more responsive under sufentanil than it is under other drugs like nembutal, halothane, Sagatal, or isoflurane (Knierim and Van Essen 1992, and J. Levitt, personal communication). Sufentanil has

also been used as the sole anaesthetic for human cardiac and neurological surgery, although some patients have reported awareness (but not pain) during certain types of surgery (S. Shipp, personal communication).

Monitoring anaesthesia-precautions-further drugs

To maintain adequate anaesthetic levels throughout the experiment the depth of anaesthesia was continuously monitored by examination of the ECG, rectal temperature (maintained at 38°C with the aid of an electric blanket) and exhaled CO_2. The ECG/gas analyser machine used was equipped with an alarm which sounded if any of the animal's vital signs reached unacceptable values. The experimenter could then react appropriately and, for example, increase the rate of the anaesthetic if the heart rate was above 180 beats per minute, or decrease it, if the heart rate was below 100 beats per minute. A further indication of the adequacy of anaesthesia is the CO_2 level. An increased level of CO_2, if associated with an increase in heart rate, indicates poor anaesthesia and can be cured by a supplementary dose (5-10 mg) of propofol. An increased level of CO_2 which is not associated with an increase in heart rate, indicates that the respiration rate is too low. A decreased CO_2 level, on the other hand, which cannot be cured by decreasing the respiration rate, often indicates a blockage in the intubation tube. In such a case, a vacuum pump was used to suck the debris (blood+mucus) out of the tube. Generally, it is good practise to go through this procedure once every hour, before any debris dries inside the tube.

In order to prevent the formation of saliva and mucus which may block the tracheal tube, 0.02 mg of glycopyrronium bromide ("Robinol", A. H. Robins) were given IV to the animal every 12 h. This is a synthetic quaternary ammonium anticholinergic agent which has uses similar to

atropine but with less CNS action, since it gets highly ionised at physiological pH and thus penetrates the blood-brain barrier poorly. Its effect on secretory organs is particularly marked and prolonged, and good control of salivary and pharyngeal secretions can be obtained with doses which do not produce marked changes in heart rate. Finally, 300mg intramuscularly & sub-cutaneously (half each) of chloramphenicol ("Intramycetin", Parke-Davis), an antibiotic, were given every day to prevent infection.

Surgery

The animal's head was rigidly placed in a head holder, utilising ear bars and a mouthpiece, and designed to offer no obstruction to the field of view. A cut was made through the skin and, after retracting the skin and muscle layers, a small hole was made in the skull using a dental drill, and enlarged with rongeurs to expose an area of dura of about 1 cm in diameter. When drilling through the bone, high temperatures can be reached due to friction, and it is thus important to cool the scull down by small squirts of saline. The exposure was made roughly 1.5 mm forward from the occipital crest, in order to reveal the lunate sulcus, a useful marker for cortical geography (see **Figure 3**). The dura mater was then cut using dural scissors to avoid damaging the cortex, and an area of cortex roughly 1 cm in diameter exposed. This exposure was covered in cooled 2% agar-in-saline solution to reduce cerebral pulsations and allow stable recordings over prolonged periods, up to six days. If the angle of the penetration permitted it, a plastic chamber with a hole was used, fixed onto the scull with dental cement. The chamber prevents the agar from drying and thus obstructing the free movement of the electrode in it. If it was not possible to use a chamber, the agar was kept moist by means of a

piece of gelfoam ("Sterispon", Allen and Handbury's) placed on it and kept wet by frequent squirts with saline.

Preparation of the eyes

The eyelids were retracted with 10% phenylephrine drops (Richard Daniel & Son), a sympathomimetic amine, and kept open with tape. The pupils were dilated using 1% atropine eye drops (Schering-Plough), an antimuscarinic amine, so that the retinas could be clearly seen, and neutral contact lenses soaked in a bicarbonate buffer were placed over the eyes to provide a moisture layer at the cornea. Drying of the eyes can sometimes ruin an otherwise successful experiment. This can be avoided by externally applying hypotonic saline 5-6 times a day. Furthermore, the eyes commonly become cloudy after the first two or three days, in which case the lenses must be removed and the eyes cleaned with hypotonic saline and given a rest until they become clear again (usually after a couple of hours). In some cases, however, the transparency of the corneas is not restored even after very long periods of eye closure; regular irrigation of the eyes is therefore of critical importance. Finally, 0.05% Chloramphenicol eye drops ("Snophenicol", Chauvin) were applied every 12 hours, to prevent infections of the cornea.

Additional auxiliary lenses, the strength of which was determined by streak retinoscopy, were used to focus the eyes on a target screen 114 cm in front of the animal. Using a reversible ophthalmoscope, the foveas were identified and, by rotating the instrument through 180°, the centre of gaze was marked on the tangent screen. Because of clouding of the eyes, the foveas can often not be plotted as accurately during the latter stages of the experiment as they can be during the first day. An appropriate landmark which is easier to plot, usually a characteristic intersection of

two or more blood vessels, was thus also plotted for each eye and its position relative to the fovea stored by the computer. These landmarks were periodically checked and, if necessary, replotted during the course of the experiment.

Recording

Extracellular recording of neuronal activity was achieved using low impedance (1Mohm at 1Khz) and 10-12 μm exposed tip length gold-platinum plated tungsten-in-glass microelectrodes (Merrill and Ainsworth 1972), mounted in an impedance matching headstage on a microdrive system. These electrodes, used together with a spike discriminator (see below) can allow groups of cells but also single spikes to be recorded from. The electrode was advanced by a Burleigh "Inchworm" microdrive attached to the micromanipulator, which can make steps as small as one micron. Such small electrode movements can sometimes be very helpful in getting the maximum response out of a cell. In moving from one cell to the next, however, the electrode must advance for a minimum of 50 microns, to make sure one does not record from the same cell twice (cell diameter=20-30 microns). The signal was fed into a "Neurolog" NL 100 headstage preamplifier, and then filtered using a notch filter set to pass 2-5 KHz (NL 104 AC preamplifier, NL 125 filters, NL 200 spike trigger, NL 606 Latch counter). Spikes were displayed on a Tektronix RM 565 oscilloscope fitted with a type 3A9 amplifier, and relayed over a loudspeaker. The signal was also used to drive a spike trigger, and the Transistor Transistor Logic (TTL) signals produced were recorded by the stimulation computer. This allowed recording from small groups of cells, or, by use of a spike discriminator after the amplification stage, from individual spikes. In order to identify individual tracks and locations within the tracks, electrolytic lesions were made by passing

current, 5-10 µA tip positive for 5-10 sec. The presence and positions of any lesions along the track and the distances between lesions, together with the angle and point of entry were crucial in separating one electrode track from another, since multiple electrode penetrations (but no more than 5-6) were often made in the same hemisphere.

Angle of penetration

When we wanted to sample cells from all the different cytochrome oxidase stripes in V2, the electrode was inserted in the part of V2 at the edge of the lunate sulcus, at an angle parallel to the lunate sulcus and the V1/V2 border, and advanced in a medial to lateral direction. When doing so, the electrode track is almost perpendicular to the CO stripes, and recording from many different stripes in a single penetration is possible. In some animals, however, several penetrations perpendicular to the cortex were made, aiming at the posterior edge of the lunate sulcus. In both cases, a dissecting microscope (Zeiss) was used to clearly visualise and magnify the cortical surface under the agar. In this way, the precision of the electrode angle and entry point were largely improved, and blood vessels avoided.

Perfusion and brain removal

The experiments were continued for as long as we were able to characterise cells adequately. If the cortical responsivity was lost at early stages, this was usually due to cortical damage during surgery; in that case, changing the recording hemisphere normally solved the problem. After 4-5 days of continuous (day and night) recording, however, the responsivity of the cortex normally deteriorated and the cells could not be driven any longer. The healthy spontaneous firing rate perceived in the first days was lost, and sometimes swelling of the cortex was also observed. The eyes

became quite cloudy after that period of time, and it was normally a combination of all these factors that made further recording impossible. In this case, the monkey was given an injection of 25,000 units of heparin ("Monoparin" CP) to prevent blood clotting, followed by a lethal overdose of propofol (over 10 ml). It was then transferred to the perfusion room where the chest cavity was exposed and, after removal of the pericardial membrane, a gauge cannula inserted into the left ventricle of the heart and held in place with a haemostat. The lower aorta was clamped to prevent the fluids circulating to the lower body, and the right atrium was cut, thus providing an open system to allow the escape of fluids.

To remove the blood from the animal's circulation, a solution of 0.9% saline containing a further 25,000 units of heparin was used, at a flow rate of 40 ml per minute, until the outcoming saline from the right atrium ran clear. The perfusion was then switched to 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4 at room temperature, for a period of about 30 minutes. The fixative was washed out by perfusing with 1.5 litres 10% phosphate buffered sucrose, followed by 1.5 litres 20% phosphate buffered sucrose, followed by 1.5 litres 30% phosphate buffered sucrose, at a flow rate of 50 ml per minute. It is important to remove the fixative completely, since it interferes with the cytochrome oxidase staining (see below). Furthermore, sucrose prevents water in the brain from forming ice when frozen, and thus destroying the tissue. All sucrose solutions were buffered to pH 7.4 and used at a temperature of 4°C. The brain was removed from the skull, and stored in 30% buffered sucrose at 4°C until it sunk.

Brain sectioning

In order to visualise the cytochrome oxidase stripes in V2, it is better to first remove the operculum from the rest of the brain and then section it in a plane parallel to the V2 surface (Livingstone and Hubel 1982). In this way one cuts parallel to the stripes, which can be seen running from surface to white matter as shown in **Figure 3**. The operculum was dissected out by cutting through the fundi of the lunate, inferior occipital, and calcarine sulci, and by making short cuts across the cortex between these sulci. It was then flat-mounted on a freezing microtome, and sectioned at 50 or 60 μm intervals. Sections were removed from the knife with a camel hair brush and placed into 0.1M phosphate buffer chilled to 4°C. The exterior surface of the removed operculum is almost entirely V1, apart from a narrow strip of V2 along its dorsal rim, and the interior surface is composed of a triangular portion of V1 from the "roof" of the calcarine sulcus, flanked by V2 from the posterior banks of the lunate and inferior occipital sulci.

Section staining: cytochrome oxidase (CO)

Sections were stained for CO in order to visualise the stripe architecture of V2 in relation to the electrode track. A benzidine derivative (diaminobenzidine DAB) becomes oxidised by the tissue and forms a coloured insoluble polymeric deposit at its site of fixation, thus staining the tissue. Molecular oxygen is the oxidative agent in the CO reaction, but a further enzyme, cytochrome C, requires to be added to couple the DAB into the redox chain of reactions. Sections reacted for the presence of CO were processed according to the procedure of Wong-Riley (Wong-Riley 1979). Incubation was allowed to proceed at room temperature for 1-4 hours, until a suitable endpoint was established.

Following four post-incubation rinses in 0.1M phosphate buffer at pH 7.4, sections were mounted from the final buffer rinse onto gelatinised slides.
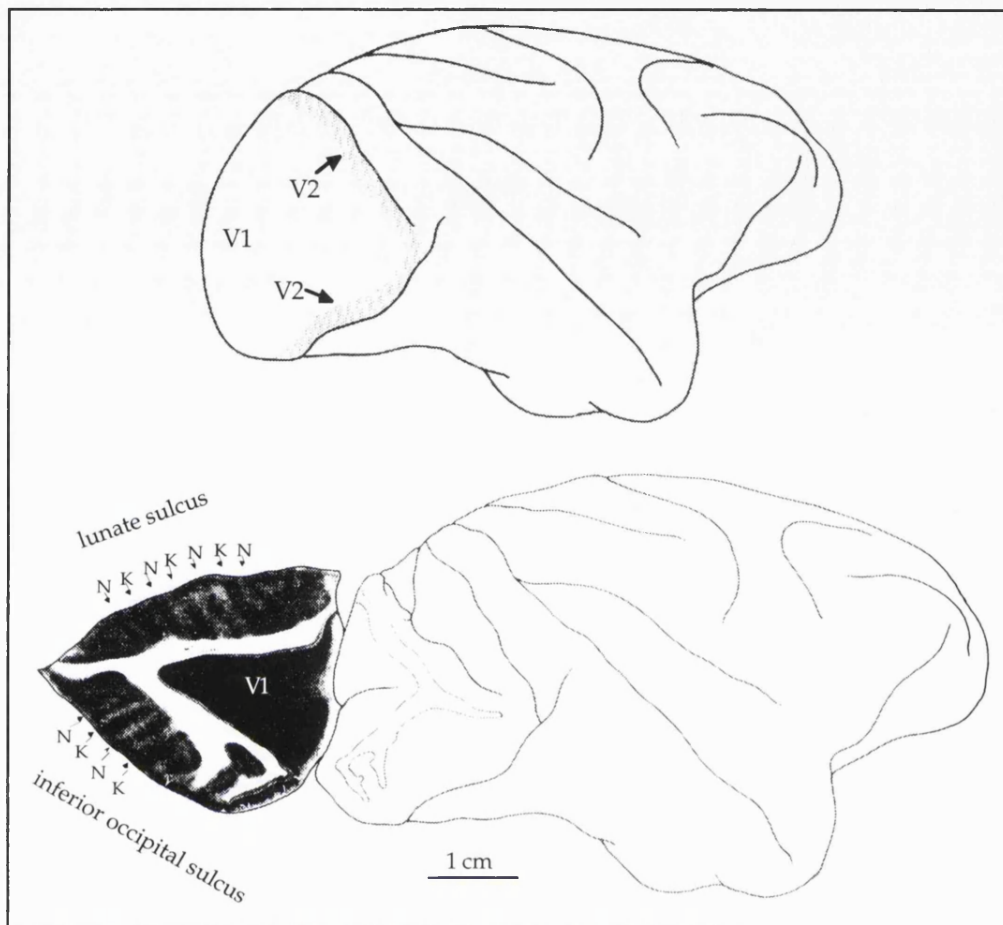
Figure 3: Area V2 of macaque monkey visual cortex surrounds area V1 and most of it lies buried within sulci; it is thus best seen when the back of the brain is opened up. Its characteristic functional architecture is revealed by staining for the metabolic enzyme cytochrome oxidase (CO): sections through the operculum (removed part of the brain containing cortex of areas V1 and V2) taken in the plane of the paper show the thick (K) and thin (N) dark staining CO stripes, separated from each other by the more lightly staining interstripes.

Sections were dried onto the slides overnight, then dehydrated slowly in graded alcohols, cleared for 30 minutes in histoclear and cover-slipped using DPX mounting medium.

The identification of cell position in relation to the CO architecture

Cells in different cytochrome oxidase stripes in V2 have been reported to have different response properties (see Introduction). When mounted onto glass slides, the stripes of V2 can be seen running from surface to white matter. The sections were examined under a microscope, and drawn using a camera lucida. Any electrode tracks and lesions were marked on the drawings. The identification of stripes was not always straightforward, however, and it was sometimes necessary to resort to reconstruction techniques involving several contiguous sections, aligned on top of each other using blood vessels as markers. Where a penetration was more or less perpendicular to the stripes and where the entire penetration could be recovered from one or a few well stained sections, there was little difficulty in assigning cells to particular stripes, especially if we had made multiple lesions to mark the positions of interesting cells. But the reconstructions were not always as straightforward as this, sometimes owing to the obliquity of the sections in relation to the electrode penetration, sometimes because the stripes were not easy to classify as belonging to one group or to another, or because of an inadequacy in staining, or because not all the lesions could be identified. We determined the position of all the cells in each penetration that belonged to a particular stripe, and separated them from the cells belonging to the adjacent stripe, and so on, until we had assigned all the cells in the penetration to their respective stripes. Overall, we were able to identify and relate the positions of 75% of our cells to the metabolic architecture of V2.

Visual stimulation and quantitative measurement of cell responses

Stimuli were generated on a microcomputer system (Amiga 2000 microcomputer, CBM Ltd.), and presented on a 19" Grundig BGC155

colour monitor. The stimuli consisted of bars of variable length, width, colour and orientation. The background colour could also be varied. The stimuli were controlled manually by use of a trackball, or automatically by the computer, in a sequence designed to test one or another attribute of the cell. The receptive field of the cell could be located using the manual control system, and the positions of the edges of the receptive field were entered into the computer; this information was used in the automatic tests to centre the stimulus on the receptive field, and to determine the period for which the stimulus was inside the cell's receptive field. The parameters of stimulus bar length, width, colour, speed and duration, as well as the background colour, intertrial period, and number of repetitions, could be entered for automatic computer controlled testing of a cell. This enabled a wide range of quantitative tests to be devised, and the operators experience was used to optimise testing for a particular cell.

Before any computerised tests were initiated, the manual control system was used to get a first impression of the cell's properties (orientation and colour preferences, directional selectivity, preferred stimulus shape and dimensions, preferred speed of stimulus movement etc.). In the visual areas we recorded from, the receptive fields of nearby cells are very close to each other, so the receptive field of the previous cell is always a good guide in trying to elicit a response from the new one. At the beginning of each penetration, however, or when big jumps were made from one recording site to the next (undriven patches of cortex can sometimes be quite large), the position of the receptive field of the cell under examination could not be as accurately predicted. In these cases, a big white tangent screen was put in front of the screen, and a hand-held projector was used to search for the receptive field location. The length and width of the white rectangle produced by the projector could be

varied, and narrow-band Nature filters (red, green, and blue) were used ·
when any colour preferences were suspected. The receptive field was then
plotted on the tangent screen using a pencil and the cell properties briefly
tested, before the television screen was re-positioned appropriately in
order to proceed with the computer-aided examination of the cell.

The cell's spikes were thresholded, converted to TTL pulses and
then fed to the computer, where they were collected in 20 ms bins and
recorded. The strength of the cell's response was thus measured according
to the frequency of its firing rate, but not its amplitude of firing (which
seemed to remain constant). The spike record was displayed during the
stimulation on another monitor, driven by a secondary processor card
fitted inside the Amiga. The average spike rate for each condition was
displayed graphically, and the period during which the stimulus was
inside the cell's receptive field was highlighted. This enabled a trained
operator to see if the receptive field of the cell had been inaccurately
plotted, in which case it was re-plotted and the test sequence began again.
The spike record, receptive field and stimulus parameters for each test
were stored on magnetic disk. After the experiment, the individual tests
were printed out on paper and analysed by the experimenter, and also
passed onto a Sun Microcomputer for long term storage and future
analysis.

In order to get a quantitative measurement of a cell's selectivity, the
following index was calculated by the computer: $I = 1 - \dfrac{(worst - base)}{(best - base)}$
(DeYoe and Van Essen 1985) This compares the best and worst responses
for each attribute, after subtraction of the baseline firing rate (average
firing rate between trials, when the screen is black). The more selective a
cell's response, the higher the index, up to an index value of 1; beyond 1,

the selectivity is accompanied by overt inhibition. An index of 0.7 or higher is taken to indicate "strong" selectivity for the attribute tested; values between 0.5 and 0.7 indicate a bias. With indices higher than 1, the ability to compare the selectivity of different cells is lost: the stronger the inhibition, the higher the index, but, in the presence of that inhibition, the stronger the response to the best stimulus, the lower the index. We used the index in our classification, leaving out of account all cells in which the standard error bars of the best and the worst responses overlapped (see Burkhalter and Van Essen 1986). The index classification system takes into account the response of the cell to only two of the many conditions tested. We thus did not blindly follow the index classification, but examined the computer-stored results of all of the cell's responses, and also the notes taken at the time of the test, before characterising the cell selective for one attribute or the other.

Orientation and direction

The orientation index was used to compare the response to the best orientation with the response to the orthogonal orientation, while the direction index to compare the best direction to the opposite direction, using the above formula. Cells were tested with moving and stationary bars of various orientations, covering the whole range of orientations in both directions of movement (0°-360°), usually in steps of 30°; the movement of the bars was always vertical to their orientation. If a cell responded well to stationary stimuli, its orientation index was calculated by the responses to the stationary test. Some cells, nevertheless, require the stimulus to be presented in motion in order to give a strong response. In such cases, the average between the response to the best direction and the direction opposite to that was compared to the average between the worst direction and the direction opposite to that, in order to calculate an

orientation selectivity index for the cell. If a cell was directionally selective we did not consider it as being neither orientation selective or not, unless it was also tested with stationary stimuli. If a cell showed a particularly tight orientation tuning curve, the step could be decreased down to 15° or 10°. Cells which did not seem to have any orientation preferences (when examined manually) were only tested at 45° intervals to save time. One should be cautious, however, since a very tightly tuned orientation selective cell might not reveal its properties in this test, and thus be regarded wrongly as an unoriented cell.

Wavelength selectivity

The television screen used to project the stimuli consisted of two, separately controllable parts: the moving or stationary stimulus (foreground), and the remainder of the screen (background). The intensity of each one of the red, green, and blue phosphors of both the stimulus and the background could be independently adjusted, so that any colour combination of the stimulus/background was possible. The properties of the cell were first tested manually using different foreground/background colour combinations and stimulus shapes, to identify preferences for stimulus colour and size, and also examine the effect of backgrounds of different colour. After a first impression was made in this way, a series of pre-specified tests were run by the computer in order to study the colour properties of the cell in more detail.

In the computer tests, six isoluminant colours were used- red, green, blue, yellow, magenta, and cyan, together with black and white. The colours were made equiluminous for two human observers through the technique of Anstis and Cavanagh (Anstis and Cavanagh 1983). The experimenter could choose either a constant colour for the background, in

which case this was used to test all 8 possible stimulus colours, or a constant colour for the stimulus, in which case this was tested against the 8 different coloured backgrounds. It was also possible for the experimenter to make any variations in the foreground or background colour in any one of the conditions of these tests. A further series of tests used isoluminant stimuli of colours which varied gradually along either the blue-green-red, or red-yellow-green, or red-magenta-blue, or green-cyan- · blue axes; the results of these tests were similar to action spectra plotted in the past using more traditional methods.

Together with the moving stimuli tests, a series of stationary ones were also made, in order to investigate the presence of any inhibitory surrounds in the cell's receptive field. The size of the stimulus was varied between different trials, and an area threshold tuning curve for the particular cell was plotted. After the excitatory and inhibitory wavelengths and spatial regions of a particular cell were plotted, we proceeded with the Mondrian experiment to test for correlation of cell responses with colour, as opposed to wavelength (see below).

Colour selectivity

The distinction between colour and wavelength selectivity in the cortex has been described by Zeki (Zeki 1983a). We used a multi-coloured display that consists of a series of squares and rectangles made of matt papers, known as a "Mondrian". The papers reflected a constant amount of light in all directions to avoid specular effects, and were assembled in such a way that none was surrounded by another shape of a single colour. The display was illuminated by three 350W Kodak Carousel projectors, each one having its own light intensity control. Different band-pass filters were used with each one, so that the first projector illuminated

the display with long-wave light only (610-700 nm, peak transmittance at 660 nm), the second one with middle-wave light only (510-570 nm, peak at 530 nm), and the third one with short-wave light only (400-480 nm, peak at 445 nm). On several occasions, narrow-band interference filters (Ditric Optics Inc.) were substituted for the band-pass filters, the three filters having peak transmittances at 630, 530, and 470 nm, and bandwidths at half maximum transmittance of 8-10 nm; the results using either sets of filters were identical. A Gamma Scientific Telephotometer, equipped with an equal energy filter, was used to measure the precise amount of light of any waveband from any area of the display, in radiometric (as opposed to photometric) units.

Different areas of the Mondrian were put in the receptive field of a cell, and each was made to reflect various combinations of long, middle, and short wave light. If the cell had shown any signs of spatial opponency, a piece of paper the size of the receptive field and of the appropriate colour was attached to the Mondrian and made to coincide with the cell's receptive field position. A wavelength selective cell will respond to any area of the Mondrian if there is enough light of its preferred wavelength coming from that area (irrespective of its colour). A colour selective cell, on the other hand, will respond only to areas of the Mondrian of a particular colour, irrespective of the wavelength combination reflected from them, and will not respond when an area is illuminated by light of the cell's preferred wavelength only. By using different wavelength combinations and putting different areas of the Mondrian in the receptive field of the cell, we were able to distinguish between the two.

In some cases, apart from the wavelength composition coming from a particular area, the sequence with which the projectors are turned

on can have an effect on the response of the cell, as is the case with cells in V1 (Zeki 1983b). The object of such a test was to show that a cell is sensitive to changes in wavelength composition as well as to the final wavelength composition. If, for example, when tested with monochromatic light a cell gave a good response to long-wave light only, the cell's receptive field was illuminated with all three projectors simultaneously, or with the long-wave one first, adding then the other two, or with the middle and short ones first, adding then the long one. The responses of the same cell to a particular triplet of energies could differ in each of the different sequences used, indicating that a change in wavelength composition was signalled by the cell, instead of just the presence and relative amount of a particular wavelength. A long-wave selective cell, for example, gave a good response when the long-wave projector was switched on, but this response was abolished when the medium and short-wave projectors were switched on as well. If, on the other hand, the medium and short-wave projectors were switched on first, adding the long-wave resulted in a response from the cell, although the stimulus was identical to the one the cell had failed to respond in the former case.

The detection of cell grouping

Our principal concern here was to learn whether cells specialised for any given attribute are grouped in any way within the cortex of area V2. As we recorded from many different stripes in long penetrations, parallel to the V1/V2 border, we characterised cells with respect to their selectivity for orientation, colour, and direction. We were interested to see, even in penetrations for which the CO staining was unsuccessful, whether the distribution of the 3 different types of cells along the penetration was random or not. We used a One Sample Runs Test to

calculate the probability of obtaining cell sequences like the ones we did (see page 52 in Siegel 1956). This is done by marking cells that are selective or biased for one of the stimulus properties we were testing (orientation, colour, or direction) with a "+" sign, and non-selective cells with a "-" sign. Each V2 penetration thus gives three series of +'s and -'s, one for each attribute tested. Within a series, a "run" is defined as a succession of identical symbols which are followed and preceded by different symbols or by no symbols at all. For each of these series we calculate the number of + and - symbols, denoted as n_1 and n_2 respectively, and the number of runs, denoted by r. In the series $\underline{+\ +}\ \underline{-\ -\ -}\ \underline{+}\ \underline{-\ -\ -\ -}\ \underline{+\ +}\ \underline{-}\ \underline{+}$, for example, n_1=6, n_2=8, and r=7. For each n_1, n_2 pair there is a range of expected values for r, if the distribution of +'s and -'s is random; if there is a grouping and the distribution is therefore not random, values of r are smaller than expected. Thus, for each n_1, n_2 pair, there exists a value R, such that $r=<R$ has a probability of $p=0.025$. This value can be found in statistical tables, if both n_1 and n_2 are not greater than 20. For values grater than 20, a z-score (significance level) can be calculated for each n_1, n_2, and r triplet.

Receptive field data statistics

The receptive field data was stored on computer as a set of four vertices for each receptive field, using a program designed by John Romaya. Each vertex is stored as an x, y co-ordinate pair. The x co-ordinate represents position right (positive) or left (negative) of the vertical meridian and the y co-ordinate represents position above (positive) or below (negative) the horizontal meridian. These data are obtained by moving a flat screen along a circular track which thus describes a cylinder. The axis of the cylinder forms a vertical line which passes through the centre of the head of the monkey. As a result a polar co-ordinate system is mapped to a Cartesian co-ordinate system and for the purposes of this

study all quantities such as receptive field size, area etc. are calculated as though the receptive vertex co-ordinates were true Cartesian co-ordinates. It is acknowledged that this process does introduce a distortion of the receptive field characteristics. This distortion is negligible at or near the horizontal meridian but increases with distance from the horizontal meridian so that at 30° above or below the horizontal meridian there is an exaggeration of up to 15% in the calculation of receptive field area, corresponding to an overestimate of 7.5% in receptive field size. This distortion is due to plotting the receptive fields on a cylinder rather than a sphere and affects the y co-ordinates only. In addition there is a local distortion caused by plotting the receptive fields on a plane surface rather than on a cylinder. Typically we use a plotting area approximately 40 cm wide at a distance of 114 cm. The maximum local distortion at the edges in this case is 1.5%.

Receptive field characteristics

1) The receptive field size is calculated as the square root of the area and is a value in degrees.

2) The receptive field area is calculated as the surface area of the polygon formed by the Cartesian co-ordinates of the four receptive field vertices. It is a value in degrees squared.

3) The receptive field eccentricity is calculated as the distance of the Cartesian co-ordinates of the centre of mass of the receptive field from the fovea.

Calculation of overlap in visual space representation

In order to compare the overlap in visual field representation between different combinations of stripes, a method for quantifying this

overlap was used. The area of common representation between the receptive fields of cells of any two stripes was calculated by the computer (in square degrees). This number was then divided by the average receptive field area (also in square degrees) of the cells belonging to these two stripes. The result thus was a pure number, indicating by how many receptive fields the two stripes overlapped with one another. We chose to use this method rather than expressing the overlap as a percentage of the aggregate stripe area (Roe and Ts'o 1995), so that the stripes at the beginning and end of our penetrations could be also used in our calculations. Furthermore, by expressing the overlap in terms of receptive fields, one does not need to control for changes in receptive field size at different eccentricities or cortical layers.
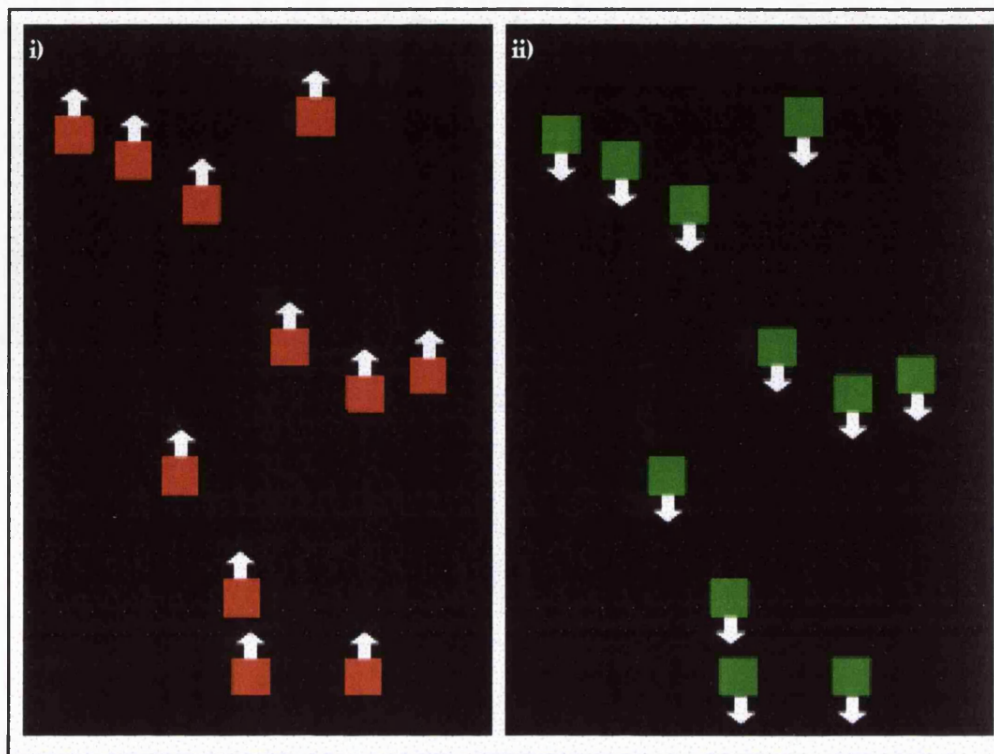
Part II: Psychophysical method

Figure 4: A schematic representation of the basic stimulus used in the psychophysical experiments. Isoluminant red and green squares were moving against a black background; at any time all the squares had the same colour and were moving in the same way. The red squares had a luminance of 14.2 cd.m^{-2} and the green squares were made isoluminant with the red for each subject. The chromaticity co-ordinates of the red and green stimuli were X= 0.601, Y =0.353, and X=0.319, Y = 0.523 respectively. Each square subtended 2.5^o and was moving at a speed of 67.6^o sec^{-1} (20 pixels per frame from a viewing distance of 30 cm). Two pictures of the TV monitor driven by a Silicon Graphics computer (refresh rate 67 Hz) are shown, in one of which the red squares are moving upwards (i), and in the other the green squares moving downwards (ii).

Stimulus

In the psychophysical study, isoluminant red and green squares that moved vertically up and down on a high resolution TV monitor were used and subjects were asked to pair each one of two colours (normally green and red) to one of two possible directions of motion (either up or down) (**Figure 4**). The background colour was black, and so the motion was defined by luminance and thus avoided the "motion slow-down" effect that is observable at equiluminance (Cavanagh *et al.* 1984). At any

one time, all the squares on the screen were either red or green and all were moving either up or down, i.e. all the squares were considered as being a single object having one of two possible colours and one of two possible directions of movement. We normally used 30 squares so that an abstract pattern, either red or green, which was moving either upwards or downwards appeared on the screen. Form and position on the screen (which is more relevant if, for example, a single square is used instead) were thus de-emphasised, whereas the emphasis was given to colour and motion. It is noteworthy, however, that we obtained results identical to the ones described below irrespective of the number of the squares used (including a single square). The changes in colour (green/red) and direction of motion (up/down) of the squares were described by two square-wave oscillations of the same period T, presented at various phase differences with respect to each other and covering the whole range of 0°- 360°. **Figure 5** (left) shows only four conditions, in which the phase differences are arranged in such a way that the upward motion can be entirely that of the green or the red squares (0° and 180°), or the colour can change midway during the motion, with the result that (at 90° and 270°) the red and the green squares move up or down for the identical times. At other phase differences, one of the colours may predominate during upward motion and the other colour during downward motion.

Prediction

If the time difference between colour and motion perception is equal to Δt, and colour is perceived first, then the colour of the squares at any time t is not perceived together with the motion of the squares at that time, but together with the motion of the squares at time $t-\Delta t$. This results in shifting the motion waveform of **Figure 5** to the right with respect to the colour waveform, by an amount equal to Δt (see **Figure 5** right). If the
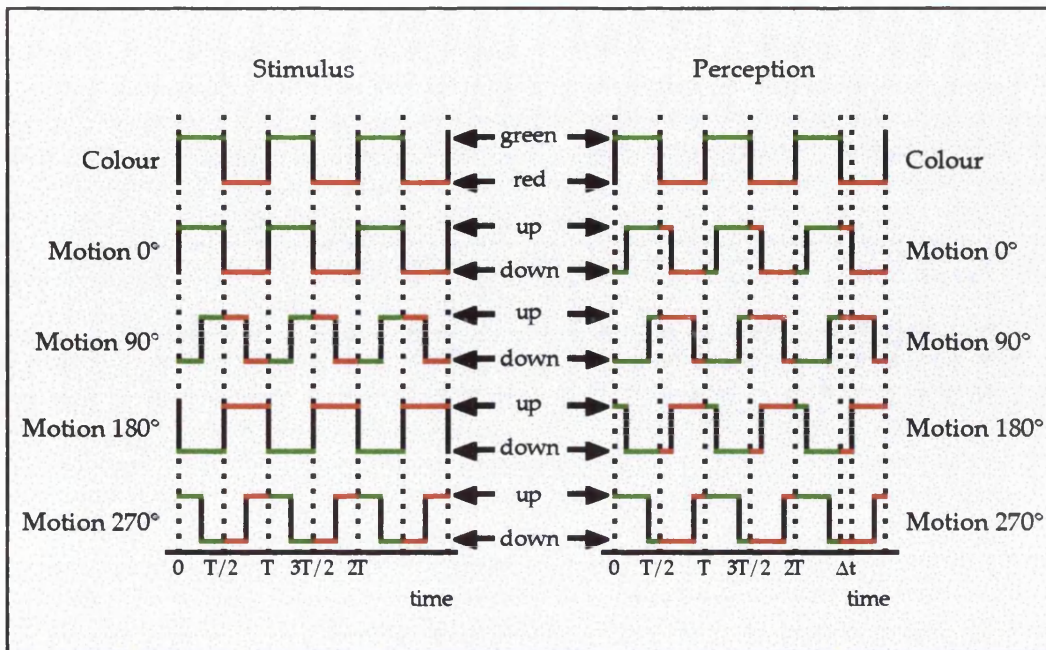
Figure 5: A representation of the colour and motion square-wave oscillations at four basic phase differences is shown to the left (T=period of colour and motion oscillations). The top row describes the colour of the squares and the four successive rows the direction of their motion. The upwards and downward motion can be of green or red squares entirely (as in 0° and 180°) or a mixture of the two. To the right is shown the perceptual effect that would result if colour is perceived Δt time before motion: all the motion waveforms are shifted to the right by an amount equal to Δt (in this example Δt=T/8) with respect to the colour waveform. This shift produces a strong perceptual effect at 90° (red seen as moving upwards and green as moving downwards) and 270° (the opposite), but little effect at 0° or 180°.

period of the colour and motion oscillation is equal to T, then this shift results in an additional phase difference equal to $(\Delta t/T) \times 360°$ between the two oscillations. The resulting perceptual effect of this shift depends on how small the period of the oscillations can be made. If, for example, $T=2\Delta t$, then the motion waveform would be displaced by half a cycle to the right and the red squares would be perceived to be moving upwards (and the green downwards) at 0°.

Testing method

Trials were presented in two blocks: in block A, the period of the colour and motion oscillations was 0.716 s and the phase difference between them was varied in steps of 15°, while in block B the period was

0.537 s and the phase difference was varied in steps of 10°. Each phase difference in both blocks was presented four times in mixed order. Subjects were asked to press one key if the colour of the upward motion was green and that of the downward motion red, and another key for the reverse. Because of the symmetrical stimulus configuration (red and green were always moving in exactly the opposite way - see below), there was never a case where one colour was seen as moving in one direction but the other colour not seen as moving in the opposite direction. All trials were forced choice and subjects were asked to indicate the confidence level in their choice after each trial, by pressing one of four keys, with 1 showing the least and 4 the most confidence. Resulting response curves are presented as polar plots in which the percentage of times that the answer was "green up, red down" is plotted for each phase difference (see **Figure 24**). If colour is perceived first, then the response curve should rotate anti-clockwise with respect to the expected veridical curve, while it should rotate clockwise if motion is perceived first. The extent of the rotation will indicate the extent of the mismatch between colour and motion. Moreover, if the perception is not veridical, the higher the frequency of the colour and motion oscillations, the larger should the resulting "mismatch", and therefore the rotation, be.

Variations

In addition to testing subjects with the basic method described above, we also introduced a few variations to the experiment. Some were clearly methodological ones, where we changed either the presentation way or the actual configuration of the stimulus, in order to see whether identical results or results altered in a predicted way would be obtained. In another variation we tried to slow down colour perception, by complicating the calculations necessary for its achievement. Finally, in

order to test the generality of our findings, we used our method to compare between more and different attributes of vision.

We have repeated the same experiment using also red and green squares moving on a Mondrian pattern background. In this case, the wavelength composition reflected from the squares was not constant but varied continuously. This was achieved by changing the illumination of the whole display, so that the wavelength composition of the light reflected from each of the many areas composing the Mondrian was also varied. In this way, due to colour constancy (see Introduction), the colour of the red and green stimulus squares did not change considerably. Isoluminance between the red and green squares was not maintained in this condition, and at different times one colour could be brighter or dimmer than the other.

Two more variations were used, as controls for the validity of the method and the results it produced. The first was a methodological one: at each phase difference, instead of all the squares having the same colour and direction of motion, we started each square at a different point on the colour/motion oscillation so that the only common thing between the squares was the way their colour changed with respect to their motion. Furthermore, the screen was split into two and reverse stimuli (differing between them by 180°) appeared in each half; the subject then had to decide at which half of the screen green was moving upwards and red downwards.

The second variation produced transient "lesions" to the motion or the colour perceptual systems, in order to test whether the results would be as predicted by our theory (see Results). We selectively replaced either

the early or the late half of the motion oscillation with stationary squares (having the same colour that they would have if moving), or the early or late half of the colour oscillation with grey squares (moving in the same direction that they would if coloured), and repeated the experiment.

As a final control on whether the results we were getting are due to a difference in the time of processing between the colour and the motion systems, we used the same method to test motion versus motion. Instead of the colour of the squares being continuously changed from red to green, we used grey squares which contained inside them texture moving either to the right or to the left, with respect to the squares (which were still moving up and down). The changes in the direction of motion of the texture in this control version were identical (described by the same oscillation) to the colour changes in the original version. Subjects were asked to pair each one of the two directions in the up/down axes with a particular direction in the right/left axes. We expected the motion/motion response curve to be veridical, since the same system is involved in both perceptions. A note of caution should be made here: although we specifically asked subjects to look separately at the motion of the texture with respect to the squares, and bind this to the motion of the squares with respect to the screen, we could not rule out the possibility of subjects looking at the motion of the texture with respect to the screen (in which case there is no binding).

In order to test possible differences in the processing times between colour and form, we used stationary gratings which were either tilted to the left or to the right, their orientation change following the same oscillation that previously described the changes in direction of motion. The colour of the gratings changed in an identical way to the colour

versus motion experiment, and subjects were asked to pair each one of the two possible orientations of the gratings to each one of the two possible colours. In a similar way, in order to test for perception time differences between motion and form, white moving gratings were used and the colour changes of the colour versus form experiment were substituted by changes in the direction of motion of the gratings (either up or down, exactly as was the case in the colour versus motion experiment).

RESULTS

Part I: Electrophysiology

Summary

We made several, normally long parallel, penetrations in dorsal V2 and tested cells for selectivity to colour, orientation, and direction; we paid particular attention to the colour properties, and in some cases also tested cells for colour constancy. We were interested in the following questions: 1) Are cells with similar properties clustered together in V2, or randomly distributed within this area? 2) Can a cell be selective both for colour and orientation or direction, or are these different attributes being processed by separate populations of cells? 3) Is there any relationship between the selectivity of a cell and the type of CO stripe it is found in? 4) Does each type of CO stripe contain within its members a complete map of the visual field and, consequently, is each function mapped to the whole visual space? In addition to our quantitatively studied cells, this thesis also includes the re-analysis of previous qualitative results obtained by S. Shipp and S. Zeki.

The grouping of V2 cells according to function

We were interested to learn whether cells in V2 sharing the same stimulus selectivity are clustered together, whether the selectivities for different attributes are mutually exclusive, and whether the different selectivities are distributed in V2 in a way related to the cytochrome oxidase stripes, which has been reported to be the best guide to the functional architecture of V2 (see Introduction). **Figure 6** is a representative example that provides answers to all three previous questions. This is a long parallel penetration through V2, i.e.

perpendicular to the stripes, where cells were quantitatively studied and assigned a selectivity index for orientation, colour, and direction (see Methods). Each index was assigned a different colour of the spectrum, on a scale running from 0 (green - unselective) to 1 (red - highly selective) (we group all cells above an index of 1 in the highly selective category and assign a single colour, red, to them). For orientation and colour, the concentrations of particular selectivities in particular parts of the penetration so reconstructed are evident at a glance, thus strongly suggesting clustering; small islands of different colour within the broad zones suggest that there is some admixture but that this is secondary to the overwhelming picture of segregation revealed by colour-coding the indexation. Much the same picture is obtained for the directional preferences where at only one small segment did the colours move to the red end (showing high selectivity for direction of motion).

The clustering pattern differs for each attribute, the clustering for cells with wavelength preferences occurring where the orientation preference is minimal; by contrast, the clustering of cells with high directional preferences occupies the same zone as that of cells with a high orientation preference and is thus also distinct from clusters with high wavelength preferences. We made detailed histological reconstructions to identify the positions of cells in this penetration with respect to the CO bands. Dark staining CO stripes are shown in dark blue, and pale staining CO stripes in light blue. The width of each stripe represents the number of cells recorded from it, and is not related to the actual histological size (thus the N stripe in this figure is, for illustrative purposes, thicker than the K stripe). The histological reconstruction shows that unoriented cells with high wavelength selectivity fell within the N bands, and those with high
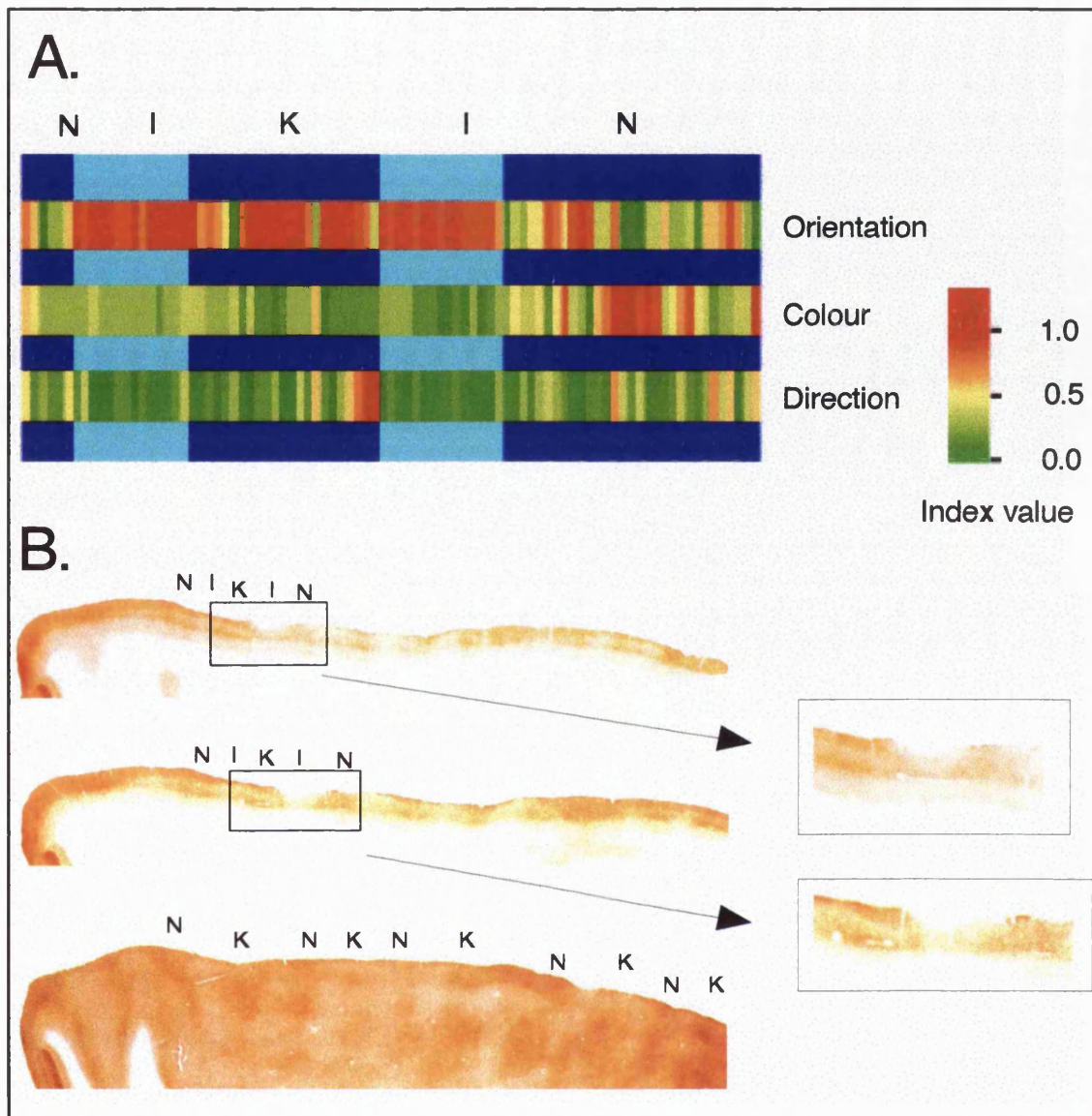
Figure 6: A. Schematic representation of the orientation, colour, and directional selectivities of cells along a single long penetration, perpendicular to the CO stripes of V2. Recordings were made from two thin (N), two inter (I), and one thick (K) stripes. Every cell was given a selectivity index (see Methods) for each one of the three visual attributes tested; each selectivity index value is represented as a colour, in the way shown to the right. The width of each stripe is representative of the number of cells recorded from it, and does not reflect the actual size of the stripe in reality. B. Cytochrome oxidase stained sections of the flat-mounted operculum reconstructed in A. The entire stripe pattern is more clearly seen in the lower section; the stripes recorded from are shown in the two upper sections, where the electrode track is present. A magnification of part of the recording sites is shown to the right, revealing parts of the electrode track and the lesions used as landmarks (see Methods).

orientation selectivity fell within the I bands and the K bands, where wavelength selectivity was absent. The small cluster of cells with high

directional selectivity fell within the K band. A small group of orientation selective cells is present in the second N stripe, but does not overlap with the larger cluster of wavelength selective cells occurring further along the penetration.

Similar results were obtained from other penetrations, and despite the occasional intrusion of a cell having a different preference to the ones in a cluster, or the few cells with the "wrong" selectivity present in a particular CO stripe, the overall impression of clustering of cells in relation to function and position with respect to the CO pattern was overwhelming. This picture was also confirmed if, instead of a single long parallel penetration intersecting several stripes, we made several short perpendicular ones, each of them sampling cells from a particular type of stripe. **Figure 7** shows the brain of a monkey in which five parallel penetrations, perpendicular to the cortical surface were made. Penetrations 1 and 6 were placed in K stripes, penetration 2 in an I stripe, penetration 5 at the border of an interstripe and a thick stripe, and penetration 3 at the edge of a thin stripe. There was a wavelength selective cluster in penetration 3, and a directionally selective cluster in penetration 6. Also, all cells were orientation selective with the exception of penetration 3 which only contained a single orientation selective cell.

Statistical tests for clustering of cells with similar properties

A summary of the clustering test results for all cells in all penetrations, studied both qualitatively and quantitatively for orientation, colour, and direction of motion, is presented in **Table 1**. A total of 720 cells (22 penetrations in 15 animals) were tested with the One Sample Runs statistical test for clustering; of these, 470 were characterised by ear and 250 by computer. In penetrations where either n_1 or n_2 was greater than 20 (see
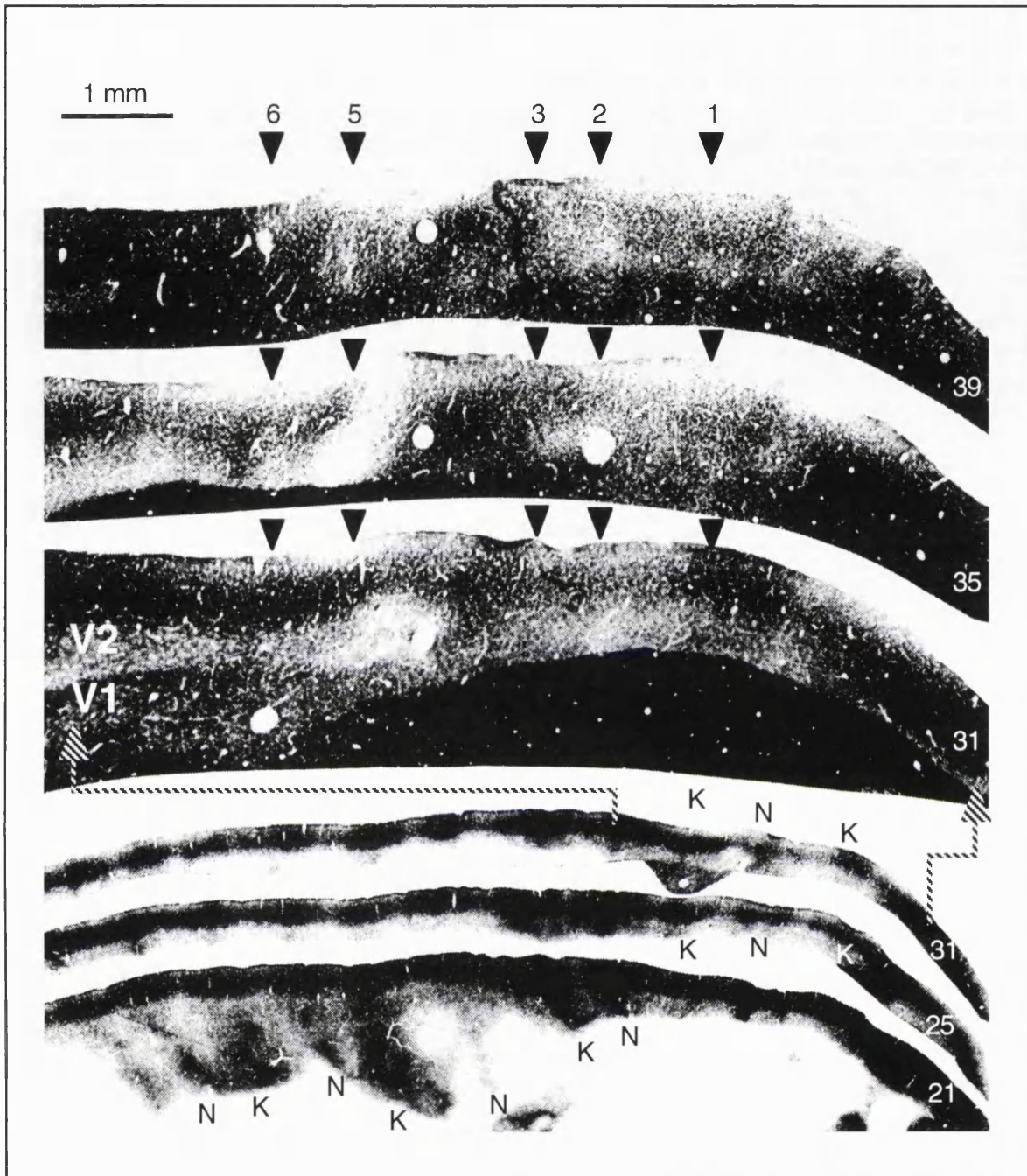
Figure 7: Multiple recordings from area V2, in which 6 penetrations parallel to the stripes were made; penetration 4 yielded no responses, but the position of the remaining penetrations is indicated by arrows. Several representative sections through the cytochrome oxidase stained flat-mounted operculum are shown (section numbers to the right) , the recording sites magnified in the upper part of the figure. Penetrations 1 and 6 are in thick (K) stripes, penetration 2 is in an interstripe, penetration 3 is at the edge of a thin (N) stripe, and penetration 5 at the border of an interstripe with a thick stripe.

Methods), a *z score* is presented, the latter being an indication of the

significance of the result; otherwise, the value of *R-r* is given for each

penetration, showing how much smaller than expected the number of runs r was. Since the direction of deviation from a random distribution is known, the values of R are significant at the $p=0.025$ level. The formula always gives negative values for z, because the deviation from randomness is due to clustering (r values smaller than expected). The critical values of z for significance are $z<-1.64$ at the $p=0.05$ level and $z<-2.81$ at the $p=0.025$ level.

	pen1	pen2	pen3	pen4	pen5	pen6	pen7	pen8	pen9	pen10	pen11
ori	3	-5.28	1	0	2	-4.55	-5.17	3	1	-5.23	-5.00
col	2	-2.59	2	1	-3.28	-1.02	-3.18	4	0	-5.59	-1.73
dir	-	-4.76	-	-	-	-2.42	-6.36	-	2	-7.69	-

	pen12	pen13	pen14	pen15	pen16	pen17
ori	4	-5.39	0	5	-5.67	-4.40
col	2	-2.03	-	-	-3.77	1
dir	-	-	-	-2.46	-4.90	-1.39

Table 1: One Sample Runs Test results for orientation, colour, and directional selectivity in V2. If either n1 or n2 was greater than 20, the value of $z=[r-2n1n2/(n1+n2)-1]/sqr[(2n1n2(2n1n2-n1-n2))/((n1+n2-1)(n1+n2)^2)]$ is given to the second decimal place. If both n1 and n2 were less than 21, the value of (R-r) is given instead. The upper table gives results for the qualitative, and the lower for the quantitative data. Black spaces indicate there were no cells selective for the particular attribute in that penetration. Penetration 2 is actually 5 parallel penetrations grouped together (shown in Figure 7), and similarly penetration 3 is 2 parallel penetrations grouped together.

Results show that there is clustering in all of the penetrations and, with the exception of two (*penetration 6* for colour and *penetration 17* for direction), all results are significant at the $p=0.05$, and most are significant at the $p=0.025$ level or even better. The lack of significance in these two penetrations is due to the fact that, in making these statistics, we cannot make allowance for the following physiological fact: within a group of selective cells, it is often possible to find also the occasional cell that is not selective for the attribute in question. The consequence of this is to artificially inflate the number of runs r and thus suggest a less pronounced

clustering than is the case. A good example is the colour selective cells in an N stripe. The sequence of wavelength selective cells in such stripes is commonly broken by an occasional cell which is not wavelength selective. Where, for example, one sequence of wavelength selective cells has inserted into it a cell which is not thus selective, the consequence would be to break it up into three runs, rather than one. A test that does not take this into account will underestimate the degree of clustering and thus yield a somewhat distorted picture, suggesting less segregation than there actually is. In *penetration 6* of **Table 1**, the *z-score* for wavelength selectivity corresponds to a probability of *p=0.1539*. In this penetration, after a sequence of orientation selective cells, the preference suddenly changed to non-selectivity for orientation (within a zone which we verified to have been in a N stripe). These unoriented cells were alternately either wavelength selective or broad band. The sequence terminated with a return to a long sequence of orientation but not wavelength selective cells, suggesting that the wavelength selective cells were actually grouped together. But, because of the alternation in selectivity from wavelength to broad-band within the N zone, this cluster of unoriented cells actually represented 5 runs rather than 1, and thus the low *z-score* for colour (but not for orientation). Nevertheless, examination of such a sequence over its entire extent leaves little doubt that the wavelength selective cells are grouped together, as indeed are the orientation selective cells. A similar picture appears in *penetration 17*, where the *z-score* for directional selectivity corresponds to a probability of *p=0.0823*: directionally selective cells are definitely clustered within a long series of non-selective cells, although within the cluster they are not all neighbouring each other but are separated into two groups by a single non-selective cell inserted in-between them. This artificially raises the number of runs from 1 to 3 and decreases the significance of the

clustering. In summary, this penetration and others like it show a significant degree of clustering of cells with common properties in area V2.
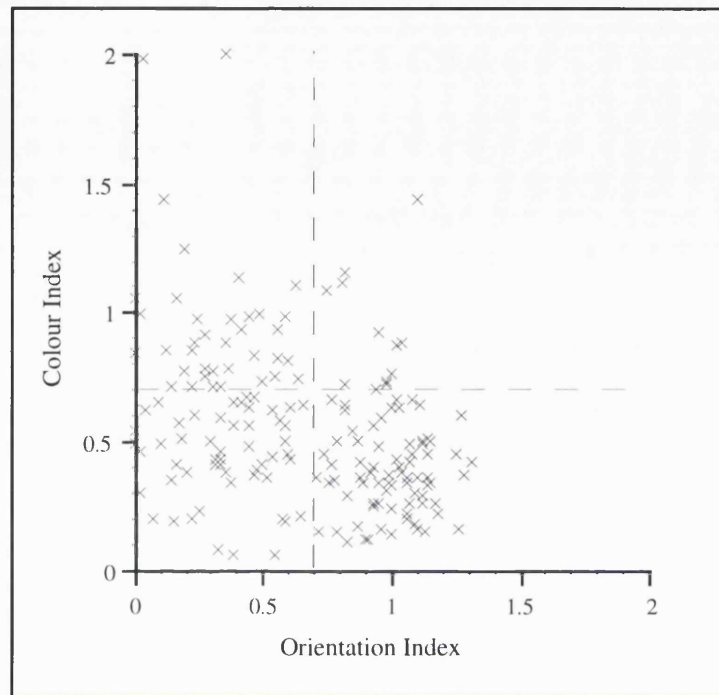

Figure 8: Plot of colour versus orientation selectivity indices; the straight lines x=0.7 and y=0.7 divide the selective from the non-selective cells. Very few cells are present in the upper right quadrant, indicating that colour and orientation selectivity are mutually exclusive.

Cells with more complex properties

It has been suggested that cells in V2 selective or biased for wavelength belong to the family of unoriented cells (Hubel and Livingstone 1987). In order to test this hypothesis, we studied the physiological properties of a total of 698 cells, 178 of which were studied by computer, and 520 of which were qualitatively classified. For both the quantitative and qualitative data, we found an inverse correlation between colour and orientation, i.e. the probability that an orientation selective cell is also colour selective is small, and vice-versa. **Figure 8** shows a graph of the relationship between the colour and orientation

indices obtained from the quantitative results. We arbitrarily chose an index value of 0.7 as indicative of specificity, with the qualifications made earlier (see Methods). The lines x=0.7 and y=0.7 divide the graph into four quadrants: the upper right quadrant is almost empty, which means there are only a few cells which are selective both for colour and orientation. **Figure 9** (a and b) shows a significant inverse correlation between colour and orientation selectivity (or bias) for the same cells. The number of cells that have both properties represents 10.1% (18/178) of the population of the quantitatively (**Figure 9a**) studied cells, and 11.9% (62/520) of the population of the qualitatively (**Figure 9b**) studied cells. The difference is probably due to the more conservative criteria of the quantitative method. This is also true for the overall percentage of wavelength selective cells in V2: quantitative results give 26.4% (47/178), whereas qualitative ones give 33.8% (176/520).

The relation between colour and directional selectivity (or bias) did not give as clear results. Because directionally selective cells are relatively rare, we took our cell population as a whole, without distinguishing between qualitatively and quantitatively determined profiles in trying to reach a conclusion as to whether directionally selective cells in V2 can also be wavelength selective. **Figure 10** shows that there is no statistically significant difference in the selectivity for direction between colour and non-colour cells and that a small proportion of either can be directionally selective. It is possible, however, that our sample is still too small to draw any firm conclusions concerning this matter.

The relationship of cell properties to CO compartments

We have been able to locate a total of 609 V2 cells derived from 20 penetrations in 13 animals within specific CO stripes, which represents

three-fourths of our total population; the remaining cells could not be allocated to any particular stripe type due to poor CO staining. Of the 609
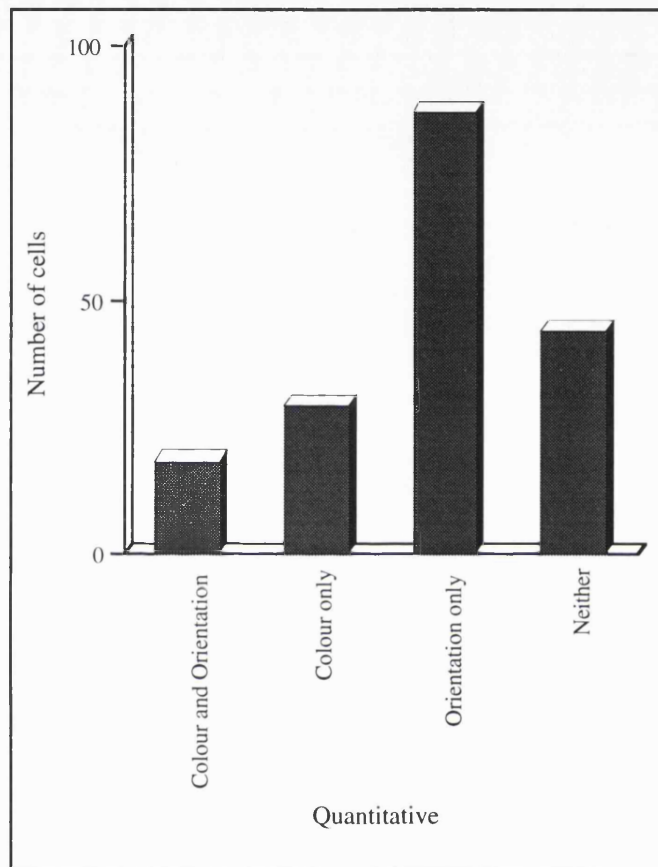

Figure 9a: Correlation between colour and orientation selectivity for the quantitatively characterised cells. The result of the chi-squared test on the number of cells selective for these two attributes (x^2=10.2) is significant at the p=0.01 level, showing that there is an inverse correlation between selectivity for colour and orientation.
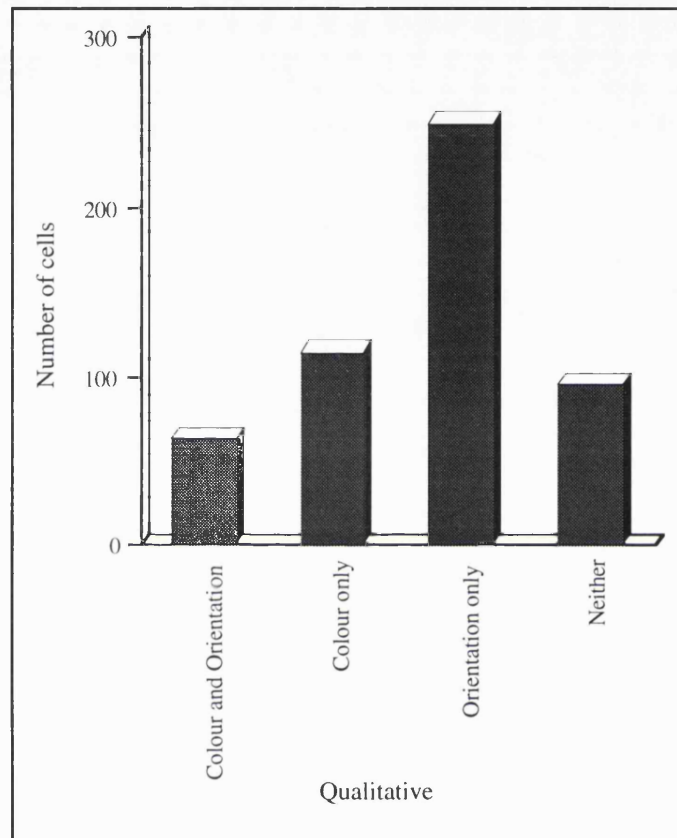
Figure 9b: Same as in figure 9a but for the qualitatively characterised cells. The result of the chi-squared test (x^2=65.3) is highly significant.
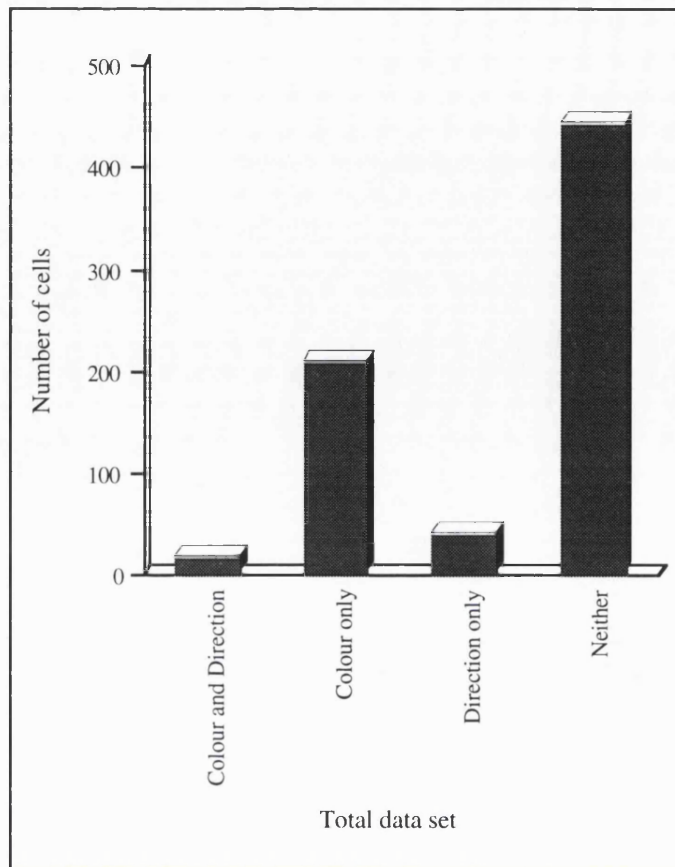
Figure 10: Correlation between colour and directional selectivity for the total number of cells (both quantitative and qualitative). The result of the chi-squared test ($x^2=0.24$) is not significant, probably due to the small number of directionally selective cells.

cells, 520 were classified for orientation, colour, and direction by ear and the remaining 89 by computer; **Table 2** shows the classification of all cells used in this study. **Figure 11** gives the percent distribution of different categories of cells within the different sets of stripes. This figure shows that orientation selective cells are found in almost equal concentrations in the K and I stripes, and in much smaller concentration in the N stripes. They also show that wavelength and unoriented cells are overwhelmingly confined to the N stripes and occur far less commonly in the K and I stripes, although they are also sporadically present in the latter. If we consider the wavelength selective cells alone, we find that they are distributed in equal, though low, proportions in both K and I stripes. If, by
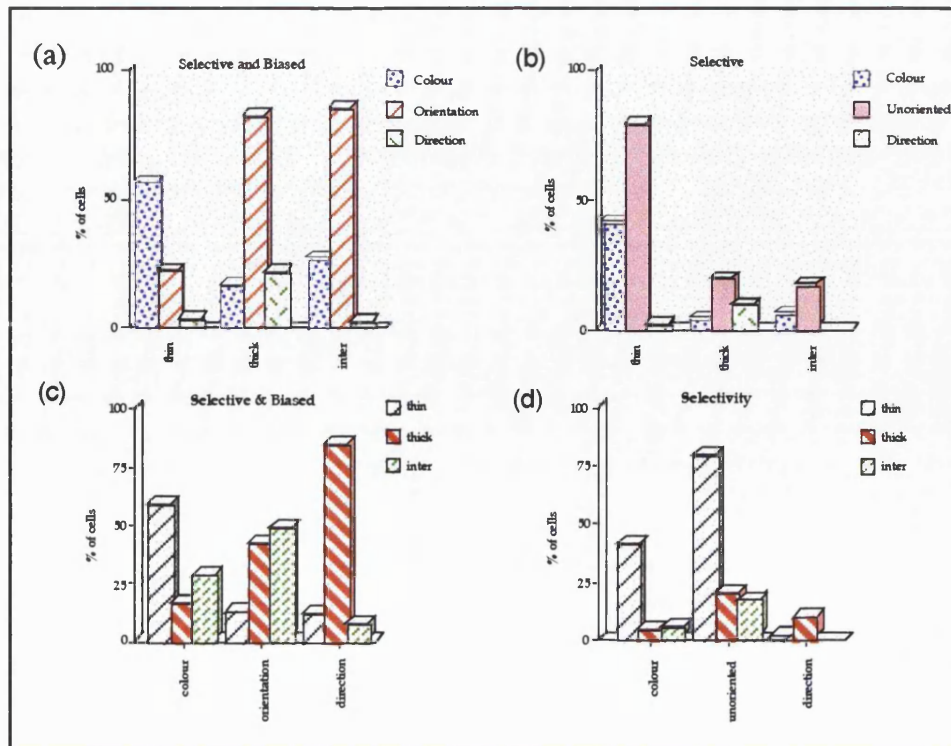
Figure 11: (a) Percentages of cells (from the total cell population) selective or biased for colour, orientation, and direction, in each of the three different cytochrome-oxidase compartments. (b) Percentages of unoriented cells, and cells selective for colour and direction (from the total cell population), in each of the three different cytochrome-oxidase compartments. (c) Percentage distribution of colour, orientation, and direction cells (selective or biased) between the three different cytochrome-oxidase compartments (from the total cell population). (d) Percentage distribution of unoriented, colour selective and directionally selective cells between the three different cytochrome-oxidase compartments (from the total cell population).

	qualitative	quantitative	total
overall cells	560	277	837
CO identified cells	520	89	609
non-identified cells	40	161	201
pen. parallel to stripes	7	8	15
pen. perp. to stripes	14	7	21
no of animals	13	13	26

Table 2: Summary of all the cells used in this study.

contrast, we group the wavelength selective and wavelength biased cells together, we find a statistically significant difference (chi-square value=4.84, significant at the p=0.05 level) in their incidence between I and K stripes, although the proportion of such cells in either stripe remains

much smaller than what is found in the N stripes. Finally, directionally selective or biased cells (the overall incidence of which is rather low) are mainly present within the K stripes. There also seems to be no difference in the incidence of unoriented cells between K and I stripes, or the incidence of directionally selective cells between N and I stripes.

The reaction of cells in V2 to wavelength and colour

The percentage of wavelength selective cells in V2 is not very high (26.4% is the value found in this study), and the responses of many of these cells are diminished when stationary stimuli are used. Therefore we only managed to further test 27 cells for colour constancy, i.e. to see whether they will respond to a surface of a certain colour irrespective of the wavelength composition reflected from it. **Figure 12a** shows the response of a wavelength selective cell which showed a clear preference for green when tested with moving bars of different colours across the spectrum; its responses to green bars of various orientations is shown in **Figure 12b**, which reveals that this cell is non-selective for orientation. **Figure 12c** shows the responses of the same cell to stationary green squares of increasing size; the cell did not respond equally well to stimuli larger than its receptive field, which means that there was a suppressive surround of the same colour preference present. For this reason, pieces of paper the size of the cell's receptive field were placed on the black area of the Mondrian and illuminated with various combinations of long, middle, and short wave light. **Figure 13** shows the responses of this cell to a blue, yellow and red coloured paper when they were made to reflect the same triplet of wavelengths, the predominant of which was middle wavelength; the cell responded to all three areas (although they were not green), and could not distinguish that they actually had a different colour. The wavelength preference, orientation tuning, and size test for another
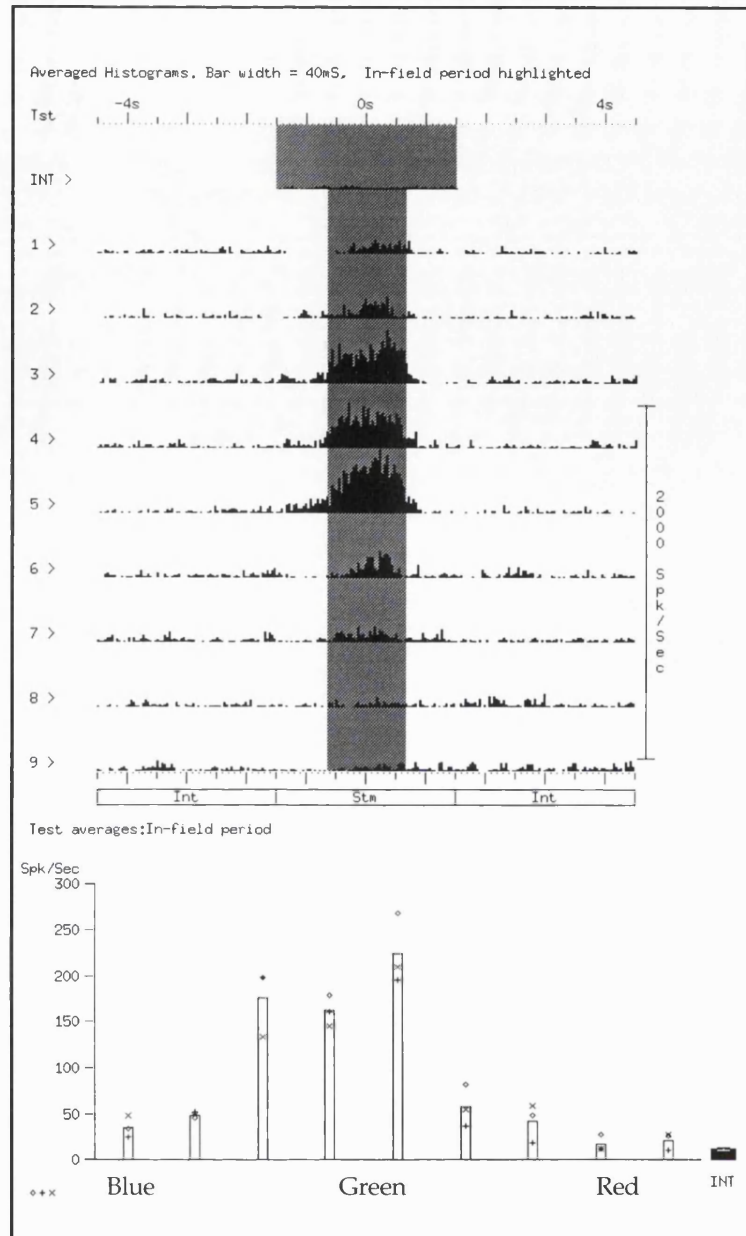
Figure 12a: The average responses of a V2 cell when stimulated with moving bars of various colours against a black background. The response of the cell when the stimulus was inside its receptive field is highlighted. Nine different colours were tested, covering the spectrum from blue, to the left, towards green, in the centre, towards red, to the right. Each colour was presented three times for 2 seconds, and between each stimulus an intertrial period of 2 seconds occurred, during which the screen was black. The cell showed a clear preference for green.

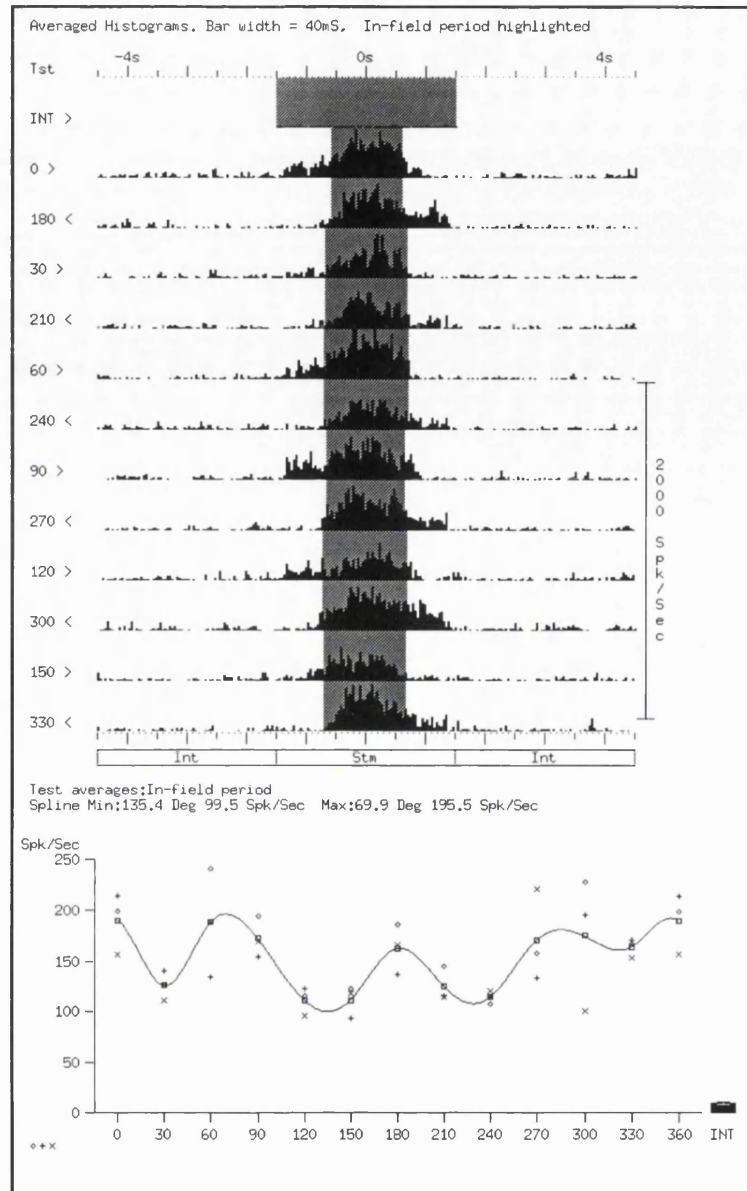**Figure 12b**: The orientation tuning curve of the cell of figure 12a is shown here, in a plot of the average response of the cell against the orientation of a green moving bar used as a stimulus. The cell was tested with orientations varying in steps of 30°, and showed no selectivity for this attribute.
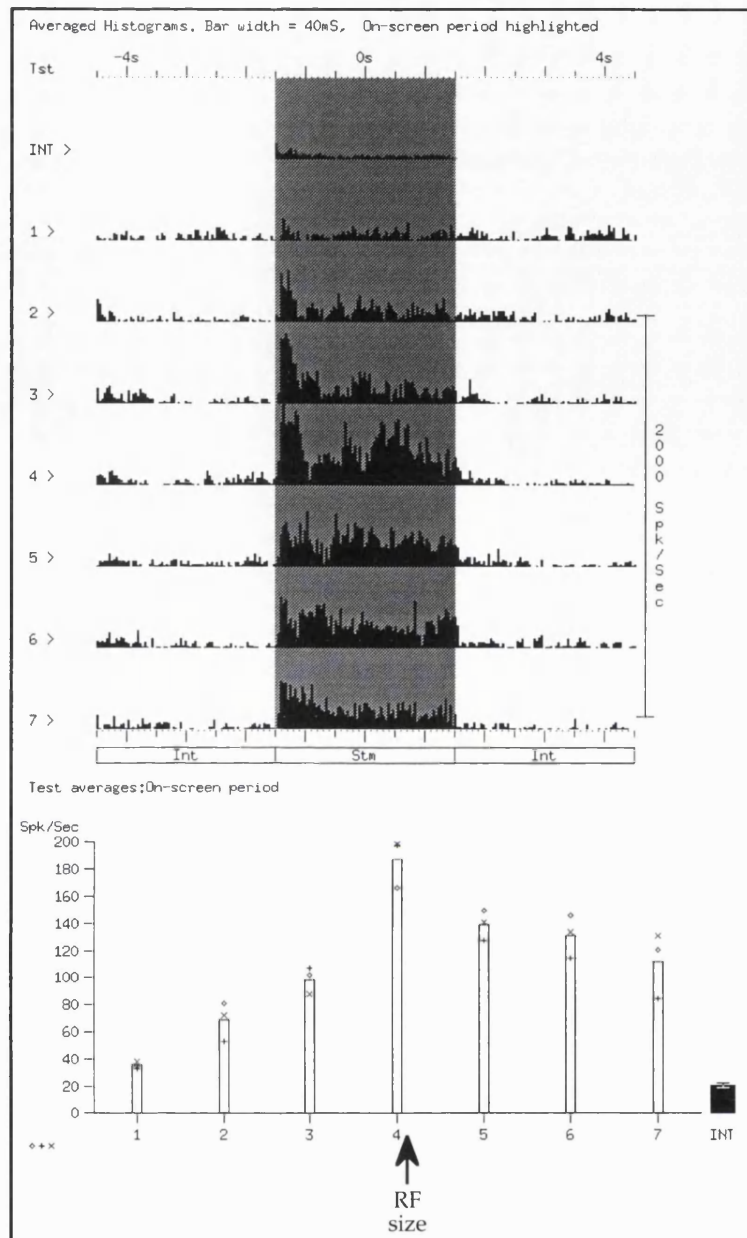
Figure 12c: The average responses of the cell of figures 12 a and b to stationary green squares of increasing size (from left to right). The cell gave an optimal response when the square was slightly smaller than its receptive field size; for larger squares the response was decreased, indicating the presence of an inhibitory surround mechanism.

cell are shown in **Figure 14a-c**: this unoriented cell also had a preference for green light, but was not inhibited if the size of the stimulus was larger than the excitatory region of its receptive field, suggesting there was no suppressive surround of the same wavelength preference present. **Figure 15** shows the responses of this cell when its receptive field was placed on
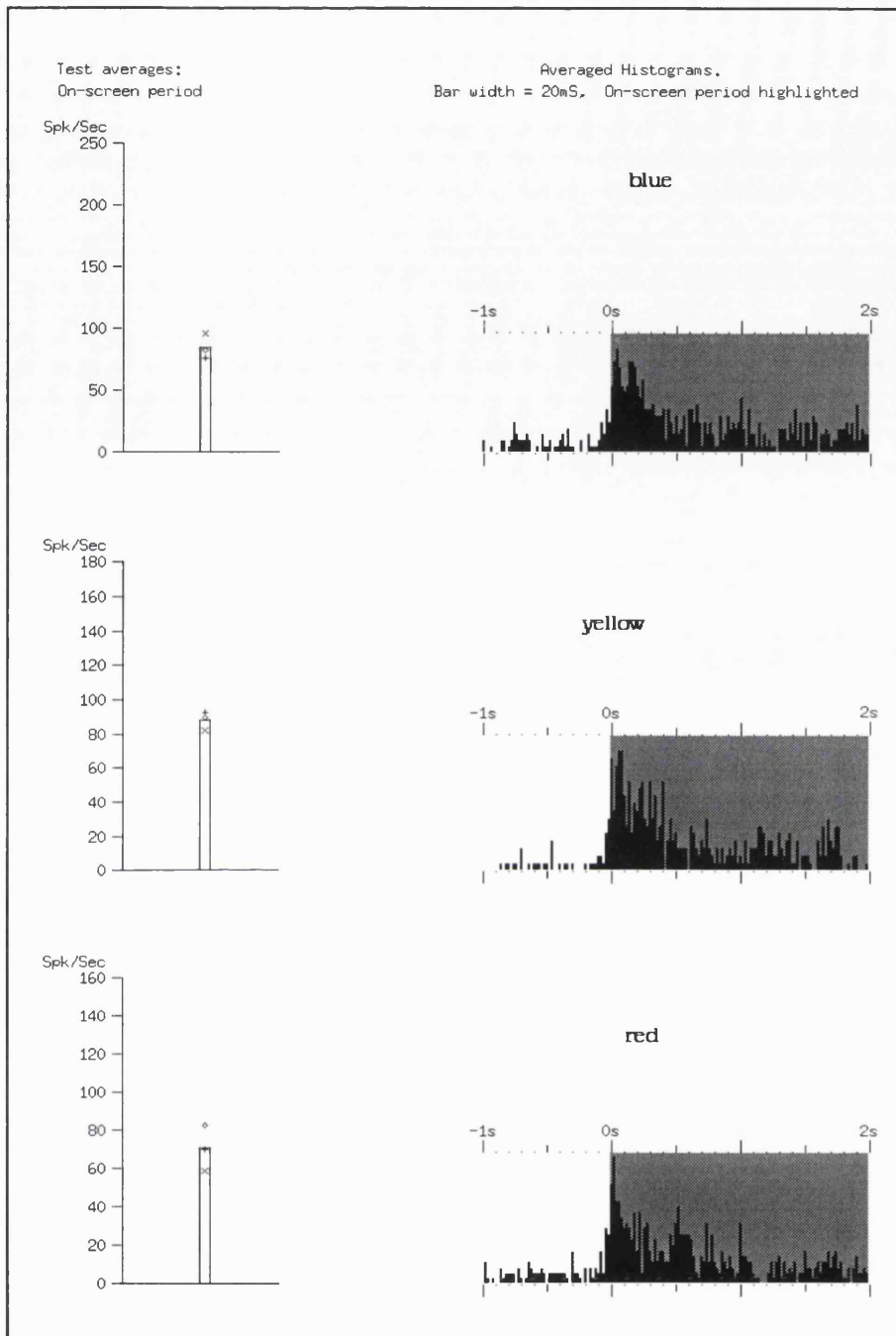
Figure 13: The average response of the cell of figure 12 to the blue, yellow, and red areas of the Mondrian, made to reflect the same triplet of long, middle, and short wave light. The cell did not distinguish between the different colours and responded equally well to all three areas of the Mondrian, since they were all made to reflect a predominant amount of the cell's preferred wavelength (middle).
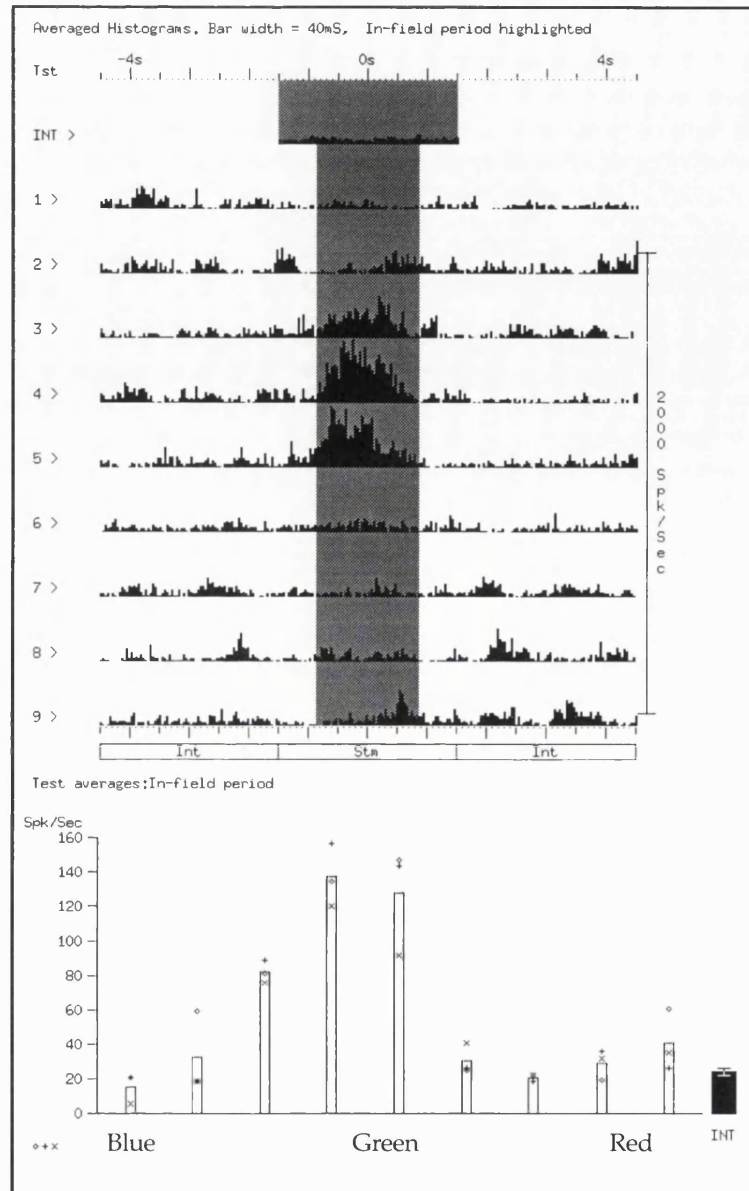
Figure 14a: The responses of another V2 cell to stimuli of different colours (as in figure 12a): this cell was selective for middle wave light.
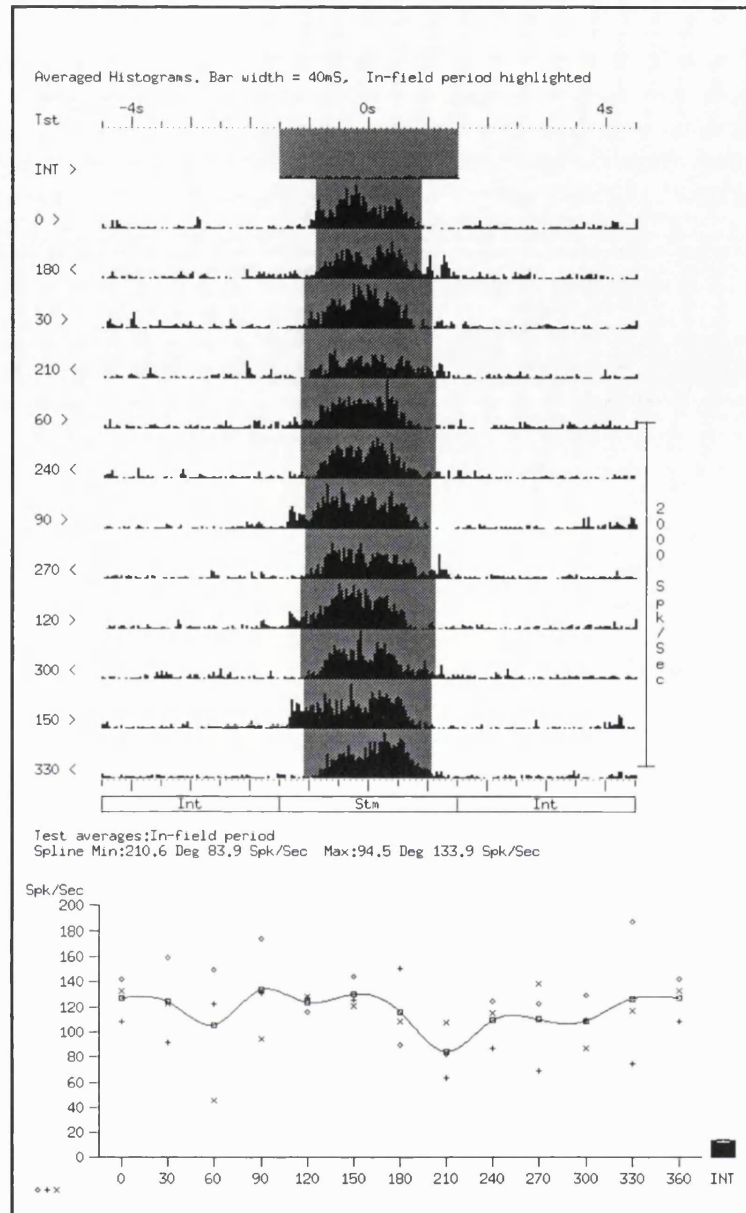
Figure 14b: The orientation tuning curve (as in figure 12b) of the cell shown in figure 14a; this wavelength selective cell was indifferent to the orientation of the stimulus.
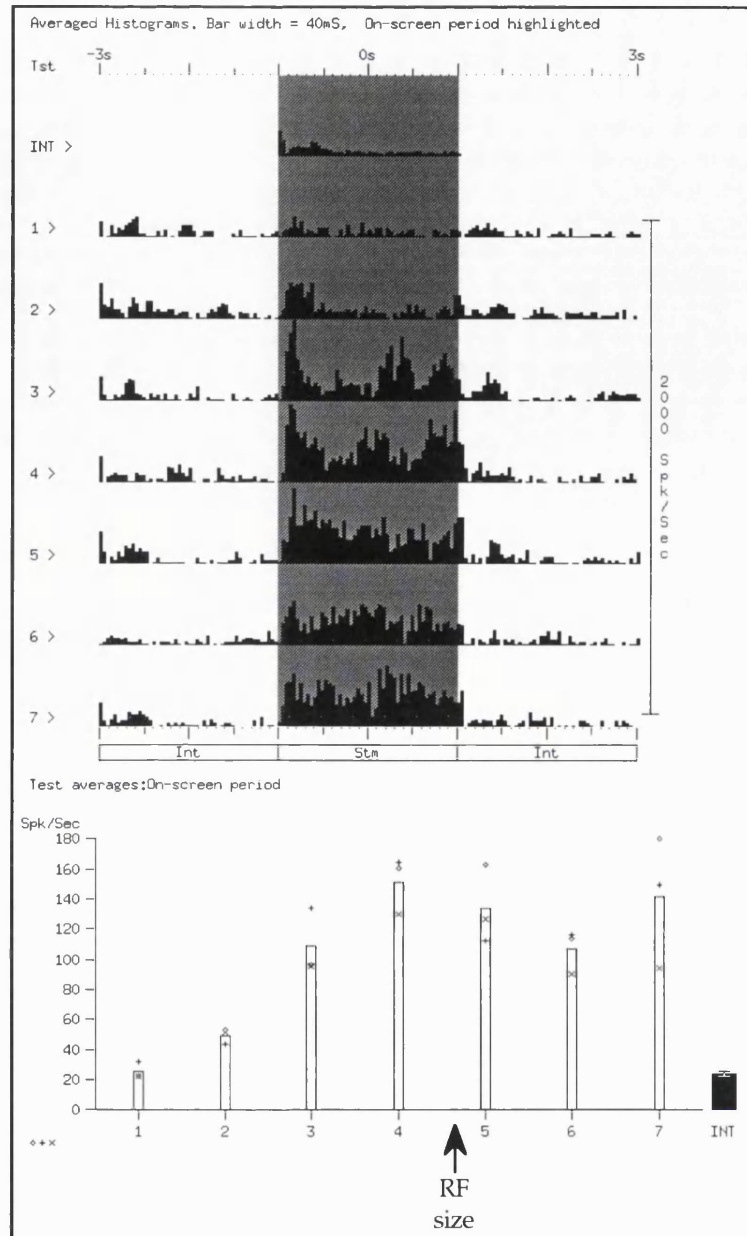
Figure 14c: A size test (as in figure 12c) for the cell of figures 14 a and b; the cell responds almost equally well to stimuli larger than its receptive field and is thus lacking a strong inhibitory surround mechanism.
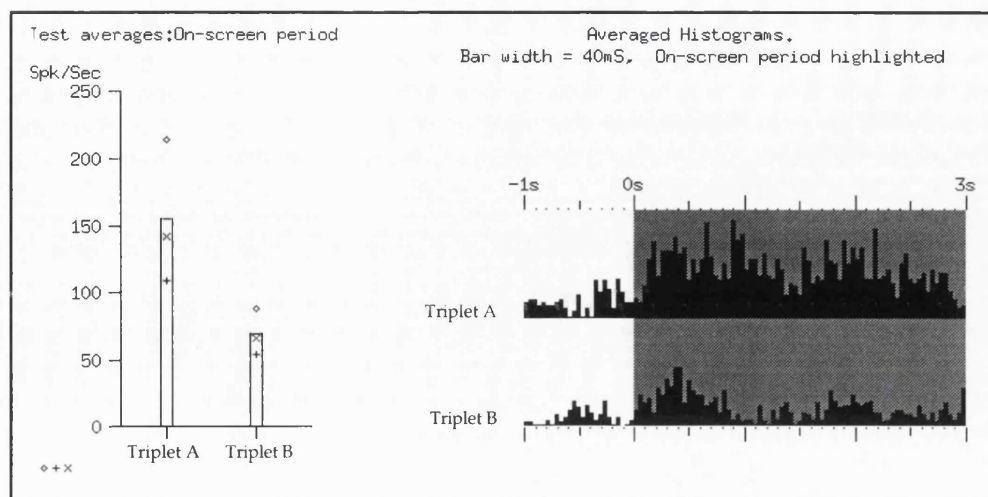
Figure 15: The responses of the cell in figure 14 to a green area of the Mondrian display, made to reflect two different energy triplets. In triplet A, mid-wave light was predominant, whereas in triplet B long and short-wave lights were predominant. Although the area looked green to a normal observer in both cases, the cell responded only when there was an abundance of middle wave light reflected from it.

the green area of the Mondrian and illuminated with different energy triplets: it responded only if the green area was made to reflect more middle than short and long wave light (triplet A), but not when it was made to reflect more short and long wave light than middle (triplet B), although it still looked green to a normal observer. The cell thus required a specific amount of middle wave light in order to respond, and could not distinguish the colour of the green area since it did not always respond to it.

All of the V2 cells tested in this way were found to be wavelength rather than colour selective, i.e. their responses did not correlate with colour but were dependent upon wavelength composition alone. Another important factor for the majority of these cells was also the sequence with which the long, middle, and short wave projectors were switched on. **Figure 16a** shows the responses of two cells, one selective for long wave

light (**Figure 16a A and B**) and the other for middle wave light (**Figure 16a C**), to different sequences of illumination of a Mondrian area of the colour corresponding to their preferred wavelengths placed inside their receptive field. When the projector of their preferred wavelength was the first one to be switched on, they gave a powerful response, which was abolished when the other two projectors were also switched on. If, on the other hand, the two other projectors were the ones to be switched on first, the cells did not respond; when, however, the projector of their preferred wavelength was added to the other two, the cells gave a powerful response. The responses of another non-orientation, short-wave selective cell to different sequences of illumination of two different areas of the Mondrian (A for the blue and B for the red area) are shown in **Figure 16b**. This cell responded to both areas of the Mondrian when they were made to reflect the same energy triplet, but did so only if the short-wave light was added to the other two. The same was true for the non-orientation, middle-wave selective cell of **Figure 16c**, in which the two conditions (preferred wavelength projected first or last) were not tested separately but in a continuous manner. Again the cell responded when the mid-wave light was added to the other two (condition 4), but not in the reverse case (condition 2). The response of the cell was therefore again not uniquely dependent upon the wavelength composition but also upon changes in wavelength composition.

Summarising, we have not found any cells in V2 showing in their responses the property of colour constancy, as previously described in V4 cells (Zeki 1980; Zeki 1983a). Our results show that wavelength selective V2 cells will respond to any area of the Mondrian, depending on whether it reflects a sufficient amount of light of their preferred wavelength and therefore irrespective of its colour. Furthermore, the
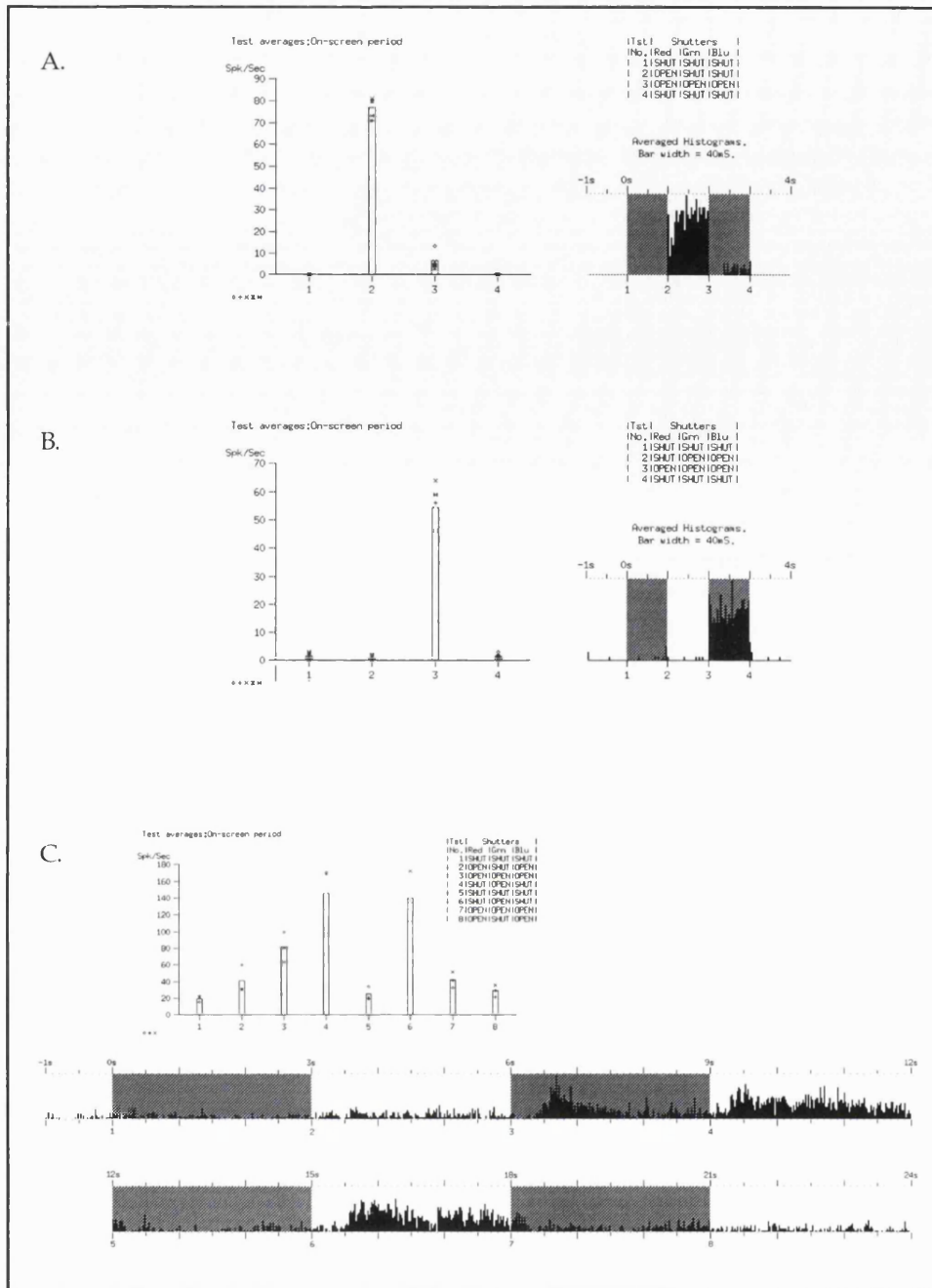
Figure 16a: The responses of two V2 cells, one selective for long wave light (A and B) and the other for short-wave light (C) to different sequences of illumination of their preferred area of the Mondrian using the same long, middle, and short wave light triplet. Both cells were activated if there was an increase in the amount of their preferred wavelength with respect to the other two, and thus gave different responses to the same energy triplets (condition 3 in A versus condition 3 in B, and condition 3 versus condition 7 in C) depending on the order with which these triplets were reached.
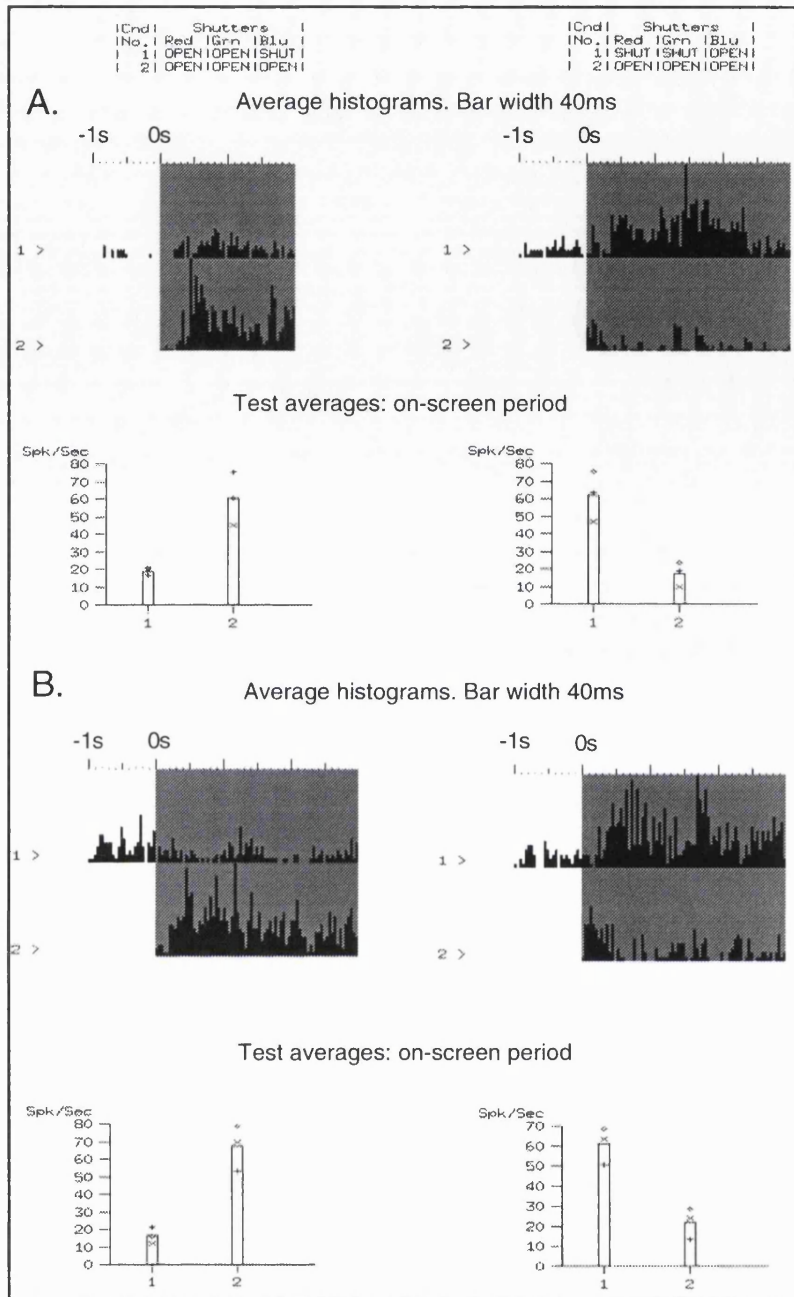
Figure 16b: The responses of a V2 cell to different sequences of illumination of the blue (A) and red (B) areas of the Mondrian, with the same triplet of energies. In the left column, the cell's preferred wavelength (short) was added to the other two, whereas the opposite is true for the right column.

```
|Cnd|   Shutters    |
|No.|Red |Grn |Blu |
| 1|SHUT|OPEN|SHUT |
| 2|OPEN|OPEN|OPEN |
| 3|OPEN|SHUT|OPEN |
| 4|OPEN|OPEN|OPEN |
```

Averaged Histograms. Bar width = 40mS

Test averages:On-screen period
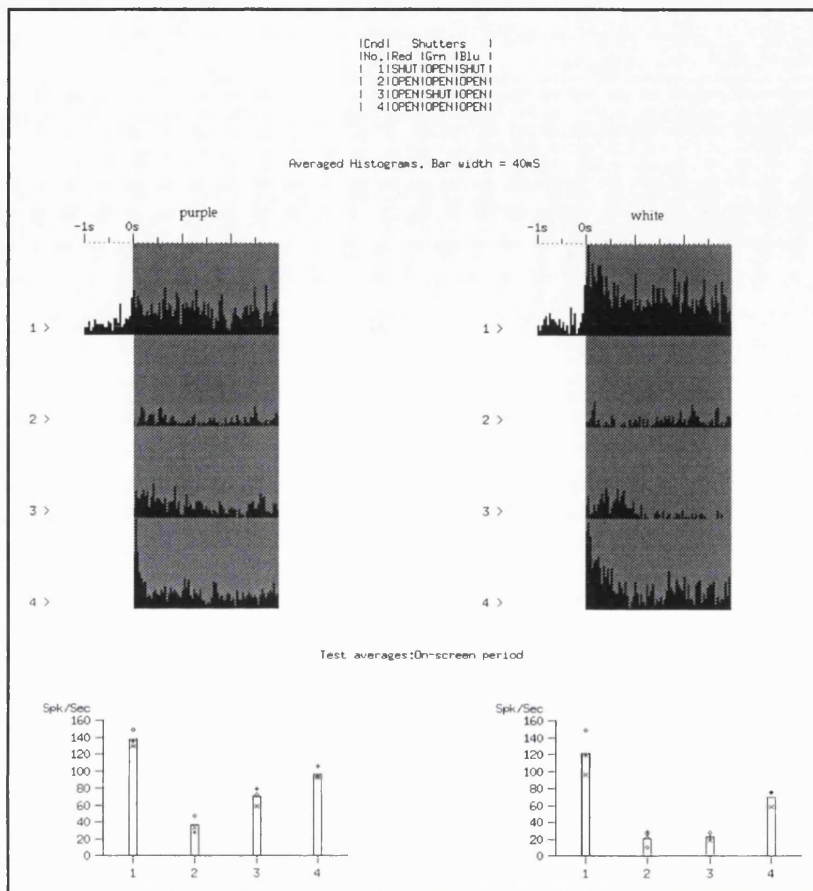
Figure 16c: As in Figure 16b, for a mid-wave selective cell. The response of the cell to the two different sequences of illuminating the Mondrian with the same wavelength combination (conditions 2 and 4) is recorded in the same test.

response of most of these cells also depends upon the sequence with which their preferred wavelength appears. The route taken is the determining factor, and depending on it these cells may or may not respond to a particular combination of wavelengths. These cell thus signal a change (increase or decrease) in the amount of their preferred wavelength relative to the amount of the other wavelengths, rather than just the relative amount of this wavelength with respect to the other two.

Mapping the visual space in V2

The evidence presented above confirms earlier results (DeYoe and Van Essen 1985; Hubel and Livingstone 1985; Shipp and Zeki 1985; Hubel and Livingstone 1987) and shows that cells registering different attributes of vision are grouped together and segregated from one another, and that each functional grouping is systematically related to the architecture of V2 and more specifically to the cytochrome oxidase stripes within it. This creates a problem in terms of mapping the visual space, because if a single continuous map is present in V2, without repeating the mapping of the same region for different attributes, not all the parts of the visual space will be mapped for each attribute. If, for example, there is a gap in visual field representation between two successive K stripes so that a part of the visual field is not mapped in either of them (i.e. is only mapped in the N and I stripes between them), area V2 would be "blind" at that part of the visual field with respect to the attribute processed by the K stripes. Therefore, in order to map all the visual attributes in the face of functional clustering, one would expect is at least three separate maps, intermixed with one another in the cortex so that the whole of the visual field is processed for each function separately. The CO stripes in V2 run parallel to lines of isoeccentricity, and perpendicular to lines of isopolarity (Rosa *et al.* 1988). Therefore when one records from V2 in a direction perpendicular to the CO stripes, going from the medial to the lateral part of the brain, the receptive fields of cells progress towards the centre of gaze; this is illustrated in **Figure 17**. By reconstructing the electrode track (see methods) it is possible to assign each of these cells to particular stripes and thus calculate the overlap in terms of visual field coverage between the different CO compartments.
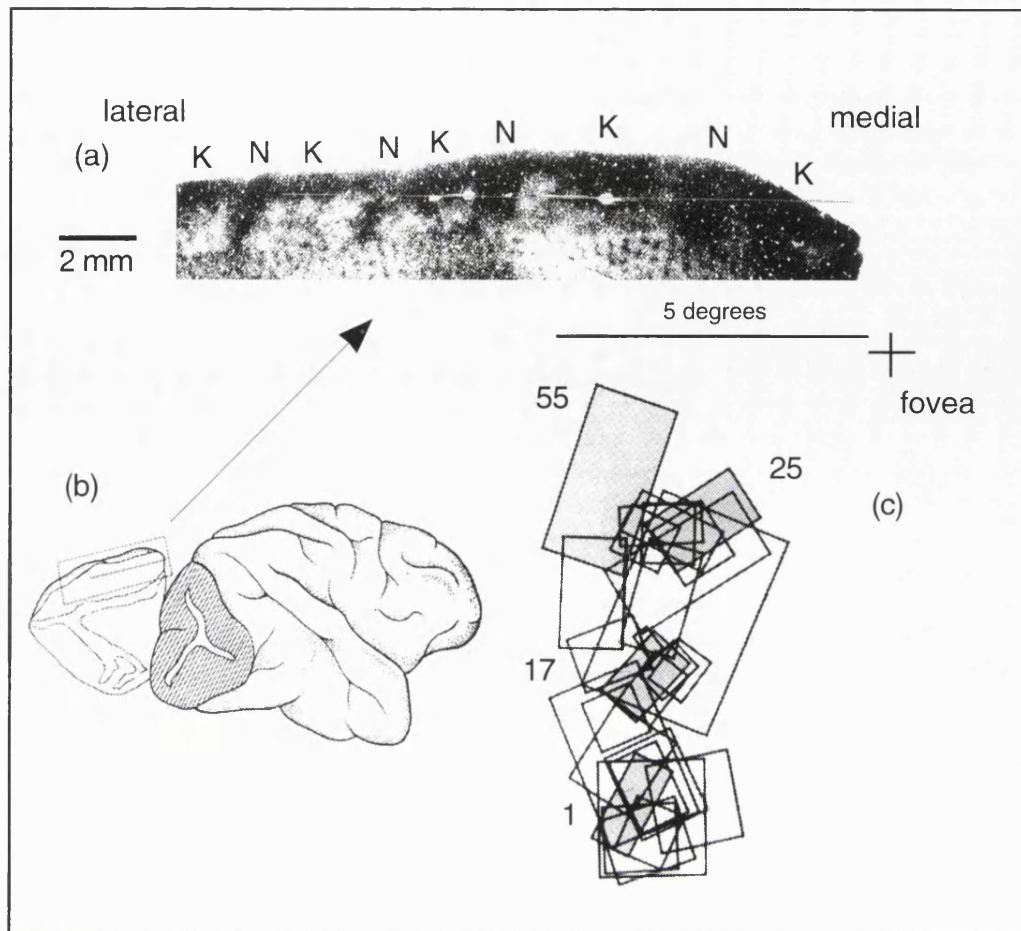
Figure 17: The change in receptive field positions of successive cells encountered during a long, tangential, penetration through area V2 of the monkey. The penetration in V2 advanced parallel to the V1/V2 border (V1 can be distinguished by its characteristic CO blobs) from medial to lateral and intersected several of the CO stripes, as shown in (a). The relation of the penetration's position to the rest of the brain is shown in (b). There was an orderly displacement of receptive fields towards the centre of gaze (cross), with the fields of cells at the lateral end of the penetration (cell 55) being close to the fixation point and those at the medial end (cell 1) further from it, as shown in (c).

The overall pattern of overlap between different stripe types

There is, overall, a systematic progression in receptive fields towards the centre of gaze as one moves from medial to lateral, perpendicular to the stripes. This progression, however, is much slower than that one gets when recording along stripes, and, when examined more carefully, is found to have a less orderly progression pattern than one might expect (see below). Quantitative results for the overlap between all combinations of successive stripes from all histologically identified

penetrations in which there were no detectable eye movements during the recording period (see **Table 3**) are presented in **Figure 18**.

penetration	stripe sequence	no. of cells per stripe	no. of mm per stripe	total no. of cells
sp7 pen15	KNIKNI	20,7,5,13,11,3	2,0.5,0.5,1.6,1.4,0.3	59
sp34 pen11	NIKINI	8,9,10,9,18,12	0.7,0.8,0.9,0.8,1.7,1.1	66
sp42 pen1	KINIK	27,18,16,10,11	2.7,1.8,1.6,0.9,1.2	82
sp3 pen12,13	IKINI	10,7,6,21,9	0.9,0.8,0.5,1.1,0.8	53
sp41 pen4	IK	12,9	1.1,1	21
sp43 pen3	KINIK ...NIK	11,6,7,8,7,9,8,4	1,0.5,0.6,0.7,0.6,0.8,1,0.3	60
lv85 pen1,2,3,6	KINK	14,11,13,12	1,0.8,1.1,0.9	50
lv88 pen1,2	NI	15,7	1.4,0.8	22
total no pen=13	K=12, N=10, I=16	total no of cells 413	total mm of cortex 38.2	413

Table 3: Summary of all penetrations used in the mapping study

Since the stripe pattern in V2 is periodically repeating itself, one could define a "cycle" as consisting of one K, one N, and two I stripes; depending on where one starts, there are four possible ways of dividing V2 into CO cycles (see discussion for more details). The lowest degree of overlap is found between K-K and N-N pairs, i.e. between stripes of the same type which definitely belong to different cytochrome oxidase cycles. Unfortunately we had only four values of N-N overlap, one of which was much higher than the others (perhaps due to an eye movement we could

not detect) and thus had a significant effect on increasing the average value (hence the big SE as well); thus, the value shown for the N-N overlap is probably larger than is the real case. The highest degree of overlap is seen between the I-K and I-N pairs. This is an expected result, since these stripes are very close to each other and can belong to the same stripe cycle in more than one way. On the other hand, K-N and I-I pairs might or might not belong to the same stripe cycle and thus the overlap between these pairs is intermediate.
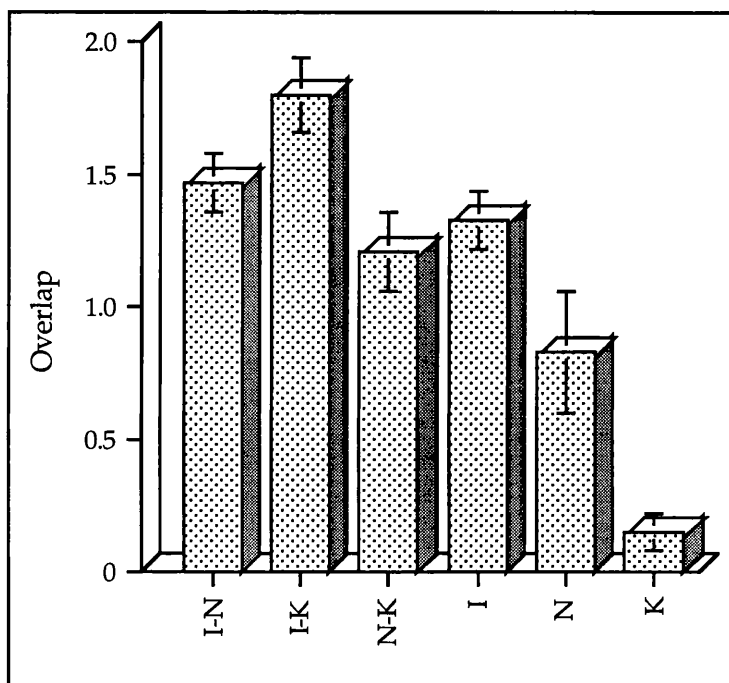
Figure 18: The average values of receptive field overlap between cells in the different cytochrome oxidase stripes in V2. All possible pairs of adjacent stripes are shown; only the overlap between stripes which were adjacent is included in these averages. Overlap is expressed as number of receptive fields (see Methods).

The overlap between K -K and N -N stripes

The length of a complete cycle of stripes, consisting of one K, one N, and two I stripes, is about 4-5 mm of cortex (Shipp and Zeki 1989b). Thus, when one records from two successive K stripes, or from two successive N stripes, the electrode moves for a considerable distance along the cortex. Figure 19 (left) shows the receptive fields of cells from two

successive K stripes, in a long penetration (shown in **Figure 17**) parallel to the long axis of V2, i.e. perpendicular to the stripes. The electrode first passed through a K stripe and then moved through one N and two I stripes, before entering the second K stripe. There is however no gap in visual field representation, the second K stripe taking over from where the first one ended. Nor was this unique to the K stripes: **Figure 19** (right) shows the same phenomenon between the two adjacent N stripes in a similar penetration.
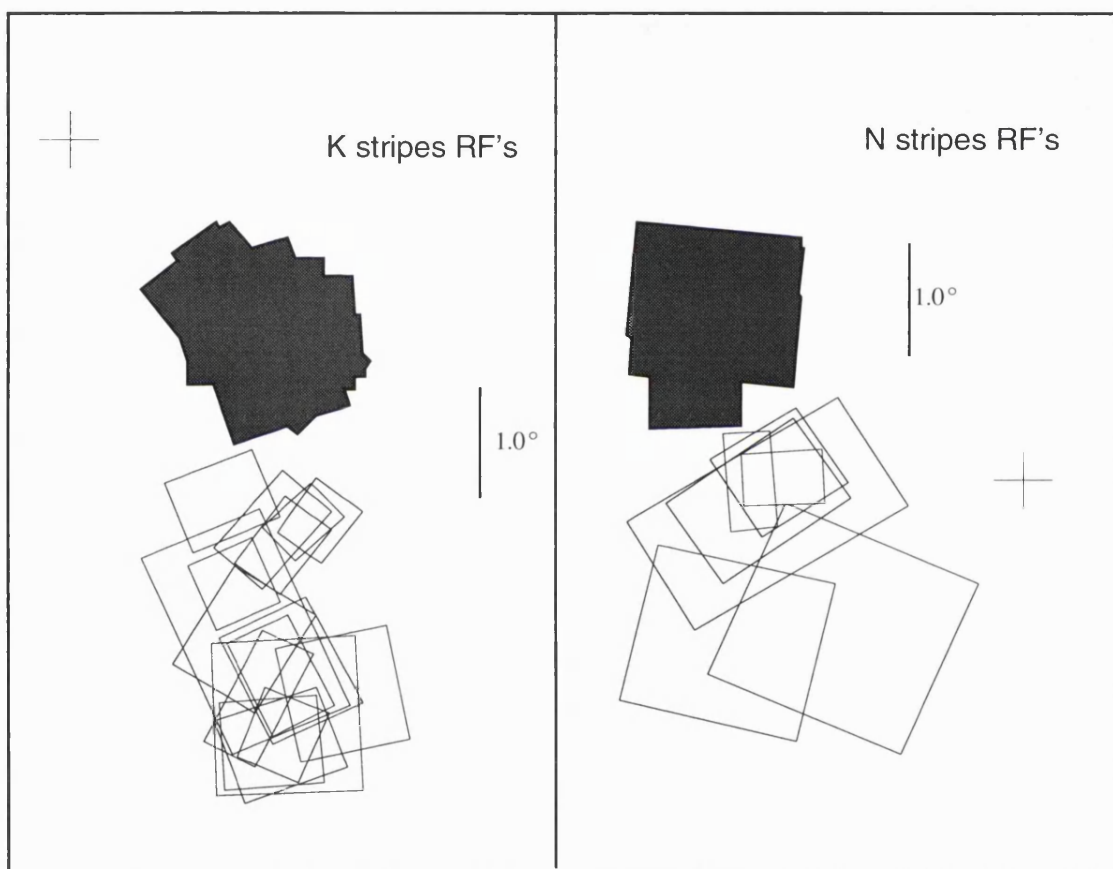
Figure 19: The receptive fields of cells belonging to two adjacent thick stripes (left) and two adjacent thin stripes (right) in two long penetration intersecting the cytochrome oxidase stripes at right angles. Fields belonging to cells in the more medial thick stripe (20 cells) are shown in white, and fields of cells belonging to the more lateral thick stripe (12 cells) are shown in black. Fields belonging to cells in the more medial thin stripe (7 cells) are shown in white, and fields of cells belonging to the more lateral thin stripe (10 cells) are shown in black. The fovea in the right part of the figure is probably plotted incorrectly, since dorsal V2 fields are always found below the horizontal meridian.

Fundamentally, the same picture was obtained when, instead of sampling from cells in a penetration made parallel to the cortical surface, several penetrations which were normal to the cortical surface and therefore parallel to the long axes of the stripes were made. This is illustrated in **Figure 20** which shows the receptive fields of the cells belonging to the first and last of the penetrations of **Figure 7**: both were in K stripes and the continuity in visual field representation between them is again evident.
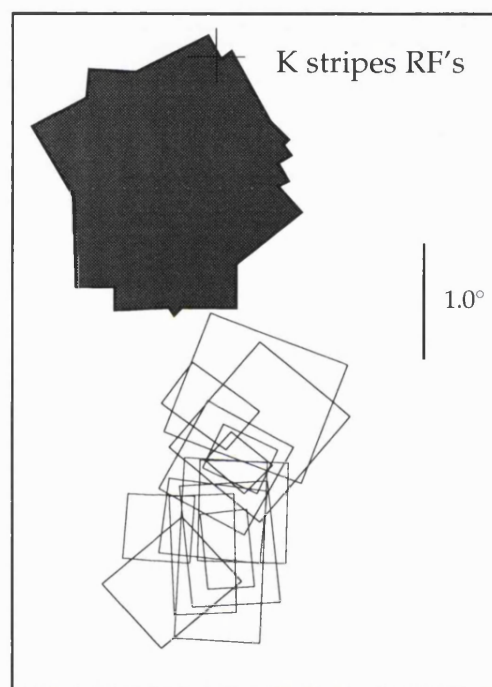
Figure 20: The receptive fields of cells belonging to the two thick CO stripes of the two vertical V2 penetrations shown in Figure 7. Fields belonging to cells in the more medial thick stripe (14 cells) are shown in white, and fields of cells belonging to the more lateral thick stripe (12 cells) are shown in black.

This pattern is to be contrasted with the substantial overlap that is evident when one records from three successive stripes, each belonging to a different type: a great deal of overlap is now apparent at a glance (see **Figure 21**). This result should be expected, since different stripes represent the processing of different attributes, and thus any part of the visual field

needs to be re-mapped in each different stripe separately. However, the amount of overlap between successive stripes of a different type is not always as high, since it also depends on the cycle pattern of the particular brain; this issue is addressed in more detail in the discussion.
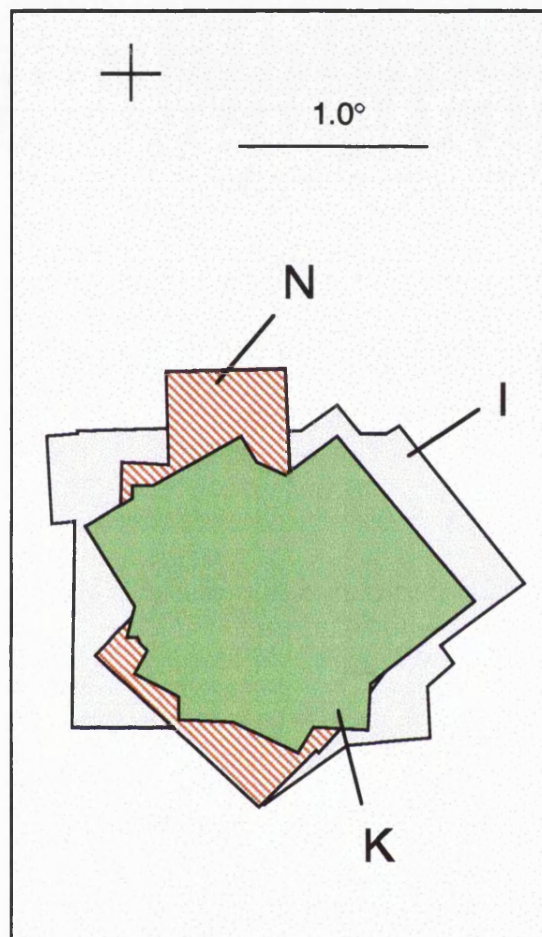
Figure 21: The receptive field position of cells belonging to three adjacent stripes of a different type (K, N, I). Receptive fields shown in red belong to a thin stripe, the ones shown in green to the following thick stripe, and those in blue to the interstripe between them. There is a high degree of overlap since the particular part of the visual field is re-mapped in each stripe separately.

The overlap between the I stripes

While there was a neat separation of overall receptive field position, and continuity in mapping, between two adjacent K stripes or N stripes, the results were less clear cut when the recordings were made from two adjacent I stripes, possibly because they are double in number

and much closer to each other than are adjacent K stripes and N stripes. **Figure 22** illustrates the results of another experiment and shows the receptive fields of cells belonging to two successive I stripes separated by a N stripe, in a long tangential penetration: this time, there is a considerable overlap in field positions. This and similar penetrations represent our general finding, namely that there is no neat separation in receptive field distribution between adjacent I stripes, in the way that we found between adjacent K or adjacent N stripes.
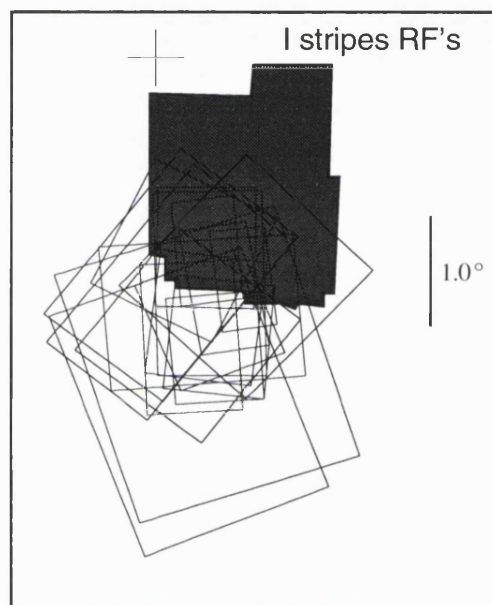
Figure 22: The receptive fields of cells belonging to two adjacent interstripes in a long tangential penetration perpendicular to the stripes, separated between them by a thin stripe. Fields belonging to cells in the more medial interstripe (17 cells) are shown in white, and fields of cells belonging to the more lateral interstripe (9 cells) are shown in black.

Receptive field centre elevation graphs

Another way of representing the re-mapping of the visual field in each different type of stripe is to plot the progression in receptive field centre position (towards the centre of gaze, see **Figure 17** above) from one cell to the next along a penetration made perpendicular to the CO stripes. **Figure 23** shows three such progressions of field centres along each stripe, the progression along the same part of the visual field being repeated in

adjacent stripes. The same part of the visual field is thus re-mapped separately in each different type of stripe.
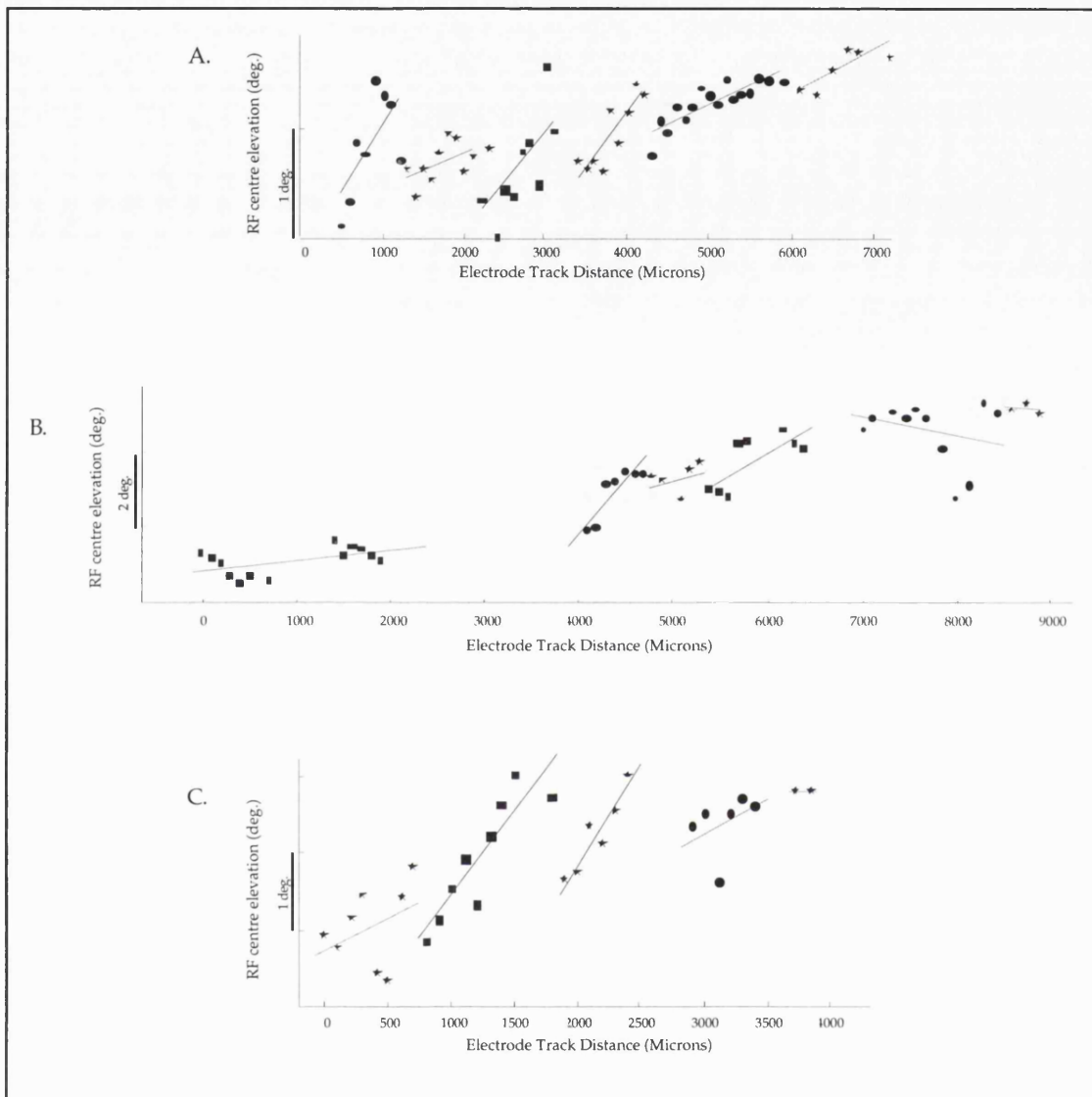
Figure 23: Reconstructions of the elevation of field centres against track distance in three different long penetrations through V2, transversing the cytochrome oxidase stripes. The receptive field centres of cells belonging to thin stripes are shown in circles, of thick stripes in squares, and of interstripes in stars. Regression lines (of elevation on distance) are drawn for each stripe; in most stripes, the rate of progression through the visual field is greater than the mean rate of the penetration as a whole, resulting in notable offsets in the representation of elevation when moving from one stripe to another.

Conclusion

In summary then, different populations of cells in V2 process signals related to different attributes of the visual field. Cells having the same selectivity and thus belonging to the same population are grouped together in clusters, and this clustering is directly related to the CO architecture of V2. Wavelength selectivity is represented in the thin stripes, directional selectivity in the thick stripes, and orientation selectivity in both the thick and interstripes. Furthermore, the entire visual space is represented in each type of CO stripe separately, so that each part of the visual field is processed for all the visual attributes. This clear separation of function in V2, and also in the rest of the visual brain, raises interesting questions concerning the binding of different attributes together in the final percept, some of which are addressed in the second part of this thesis.

Part II: Psychophysics

Summary

The results of the psychophysical experiments are presented in this chapter. The basic experiment was the one designed to test a possible time difference between the perception of colour and motion. This experiment was repeated with 9 subjects, and constitutes the basic study exploring perceptual time differences in the visual system, and the effect of this on the temporal binding of the different visual attributes belonging to the same object. A few modifications to the experiment were also tried, to verify the validity of the method and thus the results obtained. These variations were normally tested on 2-3 subjects, to make sure that similar results were obtained from different individuals.

The basic colour-motion experiment

For all 9 subjects tested on the basic colour-motion experiment described earlier (see Methods), the response curves were rotated anticlockwise, and for 8 of them the degree of rotation was proportional to the frequency of the colour and motion oscillations. The amount of rotation was not the same for each subject, suggesting a possible uniqueness to each individual. This is shown in **Figure 24 B and C** which presents the results of two extreme cases in the amount of rotation, and thus in the degree of mismatch between the perception of colour and motion. In both the response curves are rotated anticlockwise, suggesting that colour is perceived before motion (because of the stimulus configuration, see Methods). Furthermore, the degree of the rotation is in both cases greater for the higher frequency, a result which is in agreement with the time-difference hypothesis: the smaller the period of the colour and motion oscillations, the higher the fraction of it by which they are

displaced with respect to each other, and thus the greater the degree of mismatch.

Figure 24 D and E gives the average of the rotation for both frequencies in all nine subjects. What these curves show, in essence, is that subjects report one colour as moving upwards at phase differences where it is the other colour that predominates during upward motion, and vice versa for the downward motion. When drawing the best-fit "red/green reversal" line, the anti-clockwise rotations of the average curves were found to be 39.1° for the lower frequency and 56.5° for the higher frequency, giving an average value for the time difference Δt between the perception of colour and of motion of 78 ms and 84 ms respectively.

We wanted to see if we would get the same effect if, instead of all the squares having at any time the same colour and direction of motion, a mixture of both colours and directions of motion were present, while preserving the same relationship between them. Thus, in a modification of the stimulus we subdivided the screen into two halves, with each half having the reverse colour motion relationship than the other (phase differences differing by 180°). Moreover, in each half, the change in colour and direction of motion started at a different point in the sequence for each square, instead of being the same for all the squares. Subjects were asked to chose whether green was moving upwards and red downwards on the right or the left part of the screen. The results are shown in **Figure 24 F** and are identical to the ones obtained with the original method.
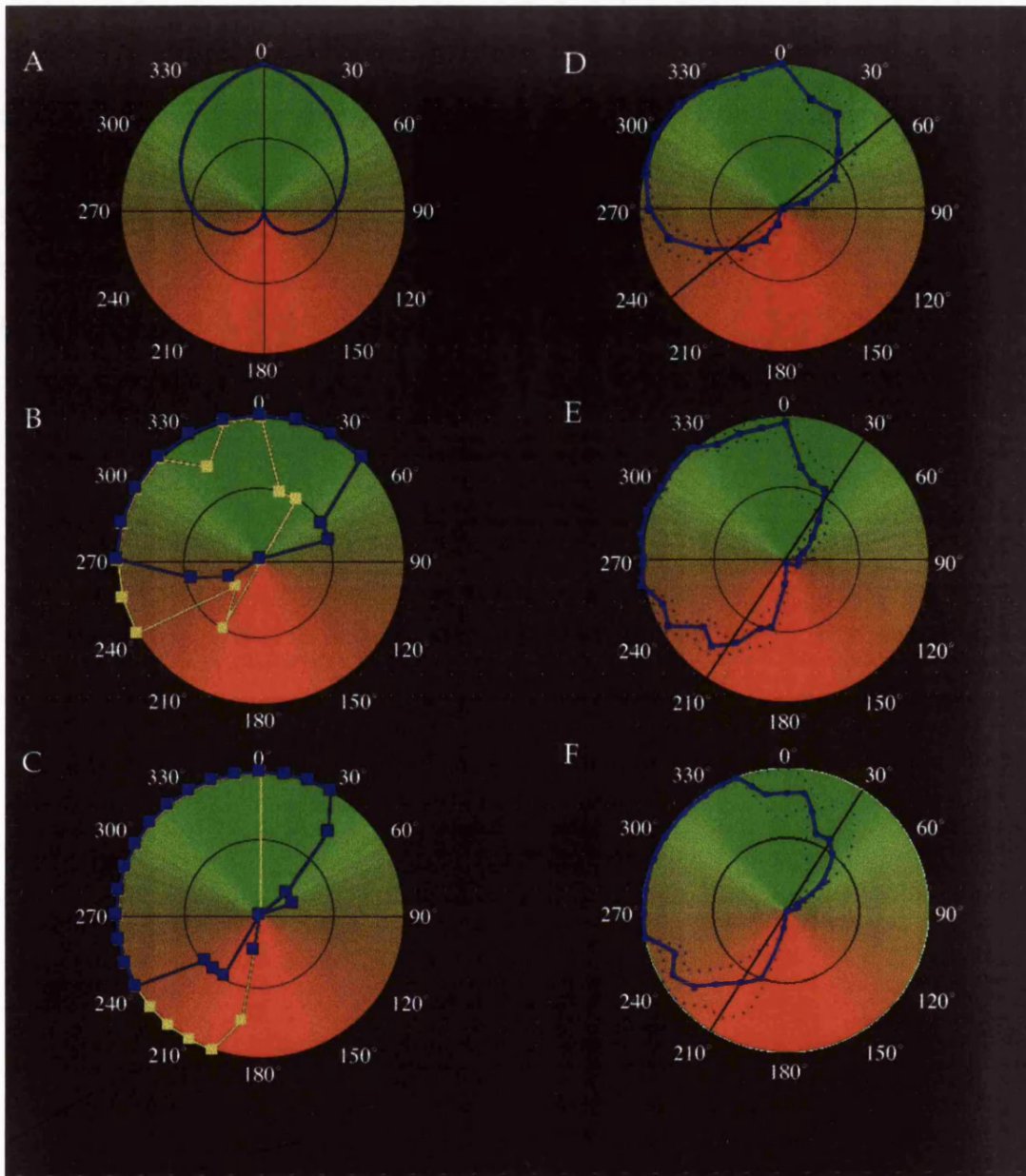
Figure 24: Response curves for the psychophysical experiments. A is a polar plot representing the relationship of the colour of the moving squares to their direction of motion, for all different phases, as produced by the computer. The percentage of time the green squares are moving upwards (and the red downwards) is plotted as a function of the phase difference between the colour and motion oscillations. The outer circle represents 100% and the inner circle 50%. A green-red gradient is also drawn to indicate the change in the upward movement as the phase difference varies. B and C represent the results for the perception of the colour of the moving squares in two subjects, at two frequencies, the lower shown in blue and the higher in yellow. D and E represent the average result obtained from all nine subjects, at the lower and higher frequencies respectively. The best-fit "red/green reversal" line is drawn and the curve describing the standard error of the means for each phase difference is also shown with dotted lines. F represents the average result of three subjects, for a slightly different configuration and presentation of the stimulus when the screen was divided into two halves, with each half having the reverse colour-motion relationship (see Methods).

Confidence levels against the possibility of developing strategies

If colour is perceived before motion, one should expect results like the ones we obtained in the experiments described above. The reverse does not necessarily hold true, however, and thus the same results could also be obtained because of some other reason. The possible use of various strategies in performing the task was one of our biggest worries. We tried to avoid this by instructing subjects to bind each direction of motion to a colour, and each colour to a direction of motion, pay no attention to position, and not follow any particular square but rather look at the screen as a whole. We furthermore specifically asked subjects not to use any method or strategy when doing the binding task.

If some kind of strategy was used, it must have been the same for all subjects since very similar results were obtained by all. What is less easy to explain in terms of a strategy is why the rotation of the response curve with respect to the veridical is proportional to the frequency used, since this effect can only be explained in terms of the amount of displacement between the colour and motion oscillations (see Methods). Nevertheless, in order to determine more conclusively whether the response curves we obtained were the result of a genuine difference in the time required to perceive colour and motion, or of adopting some kind of strategy, we compared the confidence levels at each phase difference. **Figure 25** shows one subject whose response curve could be the result of him reporting the colour at the end of each upward excursion as the one which is moving upwards. However, he was most confident of having made the correct choices when there was an equal time span for red and green squares and least confident when the upward or downward motion was purely green or red. If the subject had developed the strategy described above to obtain a response curve rotated anti-clockwise by 90° but his perception had not

been tricked, one would have expected him to be more, or at least equally, confident at 0° (and 180°), than he was at 270° (and 90°). However, even when the nature of the experiment had been explained to him, the subject still reported that the green was much more obviously moving up (and the red down) at 270° than at 0°. Although the confidence rating varied from subject to subject, they were consistent within subjects and similar confidence level results were obtained from all the subjects. In other words, the confidence level curve always corresponded to, and could be predicted by, the response curve.
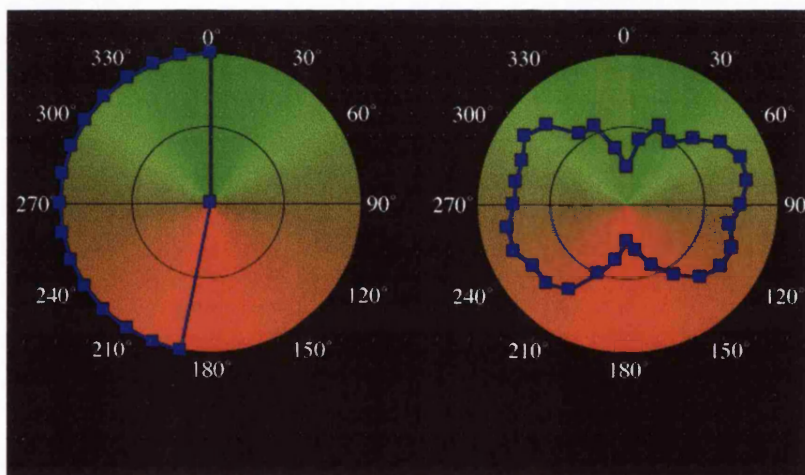
Figure 25: The response curve for one subject, plotted as before, is shown to the left: at the frequency used (T=0.478s), this curve was rotated anti-clockwise by about 90°. The confidence level curve, plotting average confidence levels at each phase difference, is shown to the right. The outer circle represents a confidence level of 4 (rarely chosen by this particular subject), and the inner circle a confidence level of 2. The subject was most confident of having assigned the correct colour to each motion when the colour was mixed, that is to say when during the excursion of the stimulus the colour changed midway from red to green, and least confident when it was pure, that is to say when it maintained its colour for a full upward (or downward) excursion.

Colour and motion "lesions"

As a further control, we manipulated our stimulus by deleting various segments of the colour or motion components, and tested to see whether we would obtain results which would be in agreement with our original hypothesis. These manipulations produce new veridical curves,

which should then be subject to the same amount of rotation with the original veridical curve, if motion and colour are not perceived at identical times. It can be deduced from **Figure 5** (left) that, if one "deletes" the first half of each motion from the motion oscillation, the veridical curve will be rotated by 45° anti-clockwise, and if one deletes the second half, the curve will be rotated by 45° clockwise, with respect to the curve obtained by the "normal" stimulus. The opposite is true for "deleting" the early and late halves of the colour changes. We thus selectively replaced either the early or the late motion component with stationary squares (having the same colour that they would have if moving), or the early or late colour component with grey squares (moving in the same direction that they would if coloured), and repeated the experiment. **Figure 26** compares the results of the 'normal' response curves with the ones obtained after performing the colour and motion deletions, one in each of two different subjects; for both subjects the new response curves are indeed rotated by 45° with respect to the original ones, in exactly the expected manner. These results are in agreement and can be explained by the model of the colour and motion oscillations of **Figure 5**.

Colour constancy experiment

In the original experiment, the wavelength composition of the light reflected from the (green or red) square pattern was kept constant. We were interested to see whether in this case the brain still undertakes all the comparison mechanisms necessary for assigning a colour to an object (see Introduction), irrespective of whether there is a variation in wavelength composition or not. We therefore tried a modification of the original method, in which the wavelength composition was continuously varied but, due to colour constancy, the colour of the red and green squares remained more or less unchanged. This was achieved by presenting the
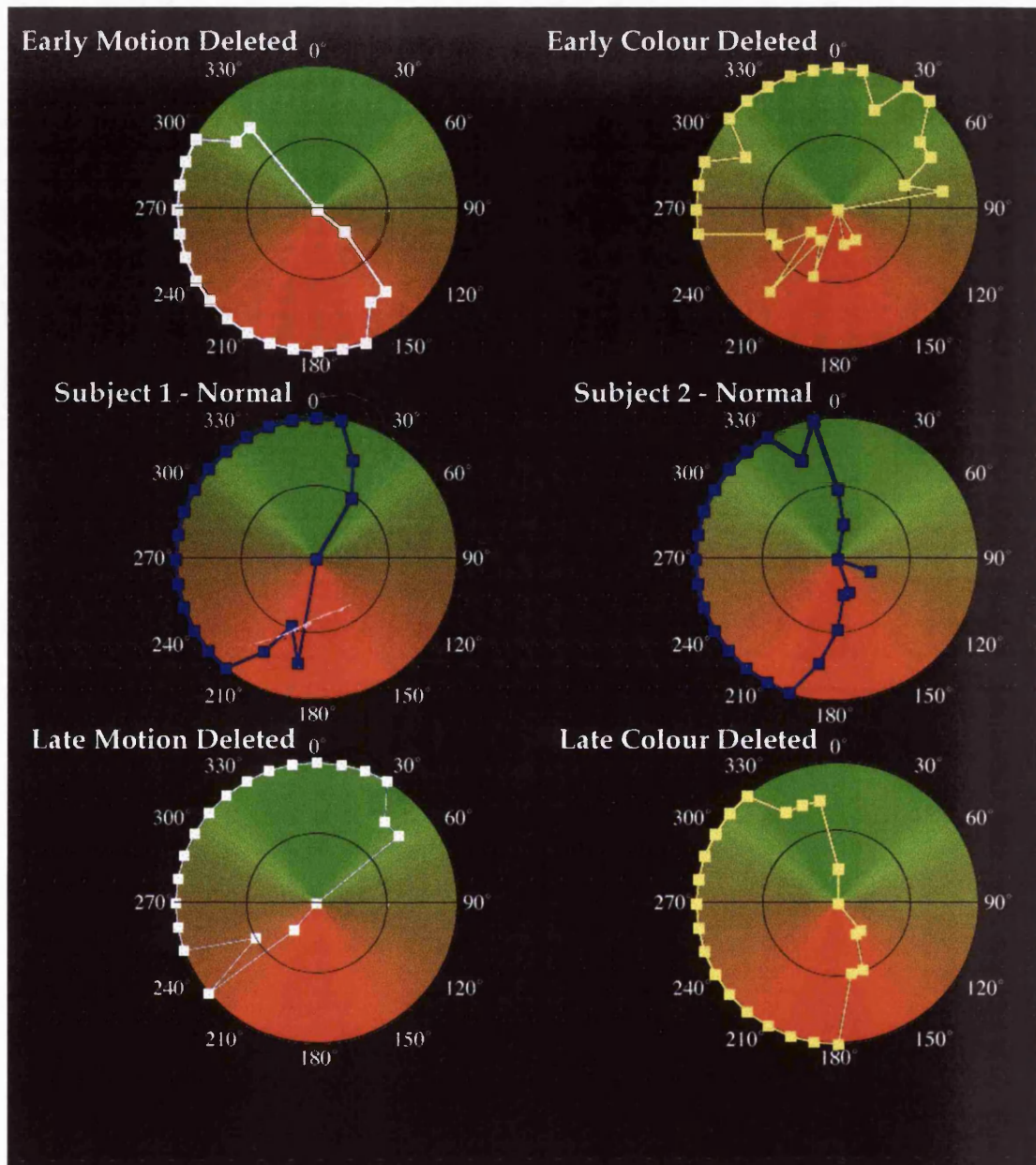
Figure 26: Comparisons between the response curves of two subjects, to the normal stimulus and to stimuli produced after deleting either the early or the late half of the motion or the colour. The deletions always produce a 45° (roughly) rotation towards the predicted direction (see text).

red and green squares against a Mondrian pattern with many other squares (instead of the black background used in the original experiment), the wavelength composition of the entire screen changing continuously. If colour constancy calculations were made by the brain in this case but not in the original experiment, one might expect colour perception to take
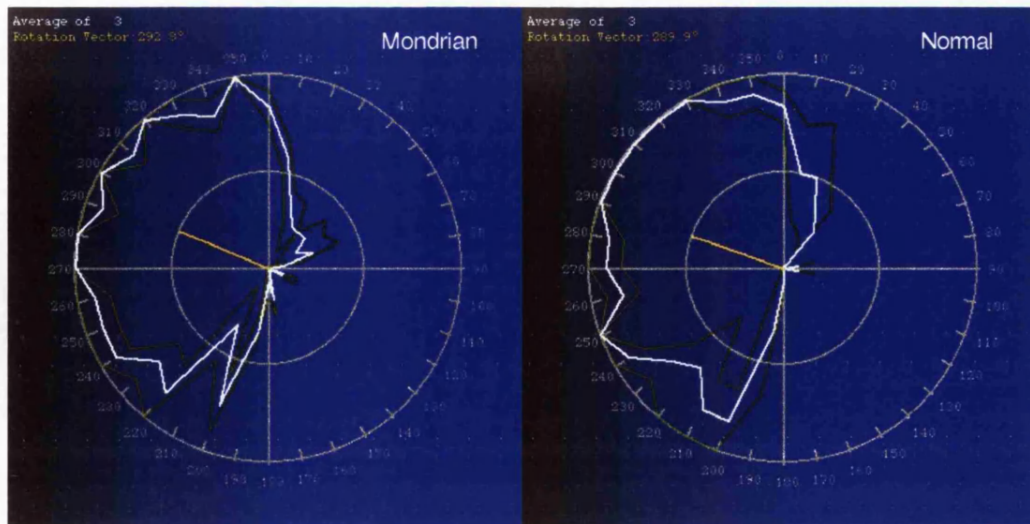
Figure 27: Average response curves for three subjects performing the colour/motion experiment in the standard way (right), and by continuously changing the wavelength composition of the red and green squares (left, see Methods). Standard error is shown in blue.

longer and therefore its time difference with respect to motion perception to decrease. **Figure 27** compares the average result of three subjects when the experiment was done in this way, with their result when the experiment was done keeping the wavelength composition constant. The response curves in these two cases are identical, implying that colour processing is not delayed in the Mondrian condition. One might have expected a different result if wavelength discrimination, which is probably faster than colour perception, was used by the brain in the original method; this, however, is not the case, since in both experiments the brain has to bind a colour percept to a motion percept. Therefore, colour perception is the end result of the processing of the colour system and should not be confused with wavelength discrimination, still achieved by areas V1 and V2 after lesions in area V4 in monkeys (Wild *et al.* 1985; Heywood *et al.* 1992; Walsh *et al.* 1992b; Walsh *et al.* 1993) and humans (Vaina 1994; Kennard *et al.* 1995).

Perceptual delays between other attributes

In addition to testing colour versus motion, we also tried other different combinations of visual attributes, in order to verify the validity of the method and also gain further insight into the temporal perceptual differences occurring in the brain. **Figure 28** shows the result of testing motion along the vertical direction versus motion along the horizontal direction (see Methods). This average response curve is indeed veridical, a result expected by the fact that the same (motion) system is involved in both perceptions. **Figure 28** also shows the average results from testing motion versus form, and colour versus form. As the figure shows, motion is perceived later than form, and form is perceived later than colour, a result compatible with the fact that colour is perceived earlier than motion. Furthermore, when one compares the exact values of the time differences between these three attributes, one can find the time difference in the perception of colour and motion by adding the difference between colour and form to that between form and motion.
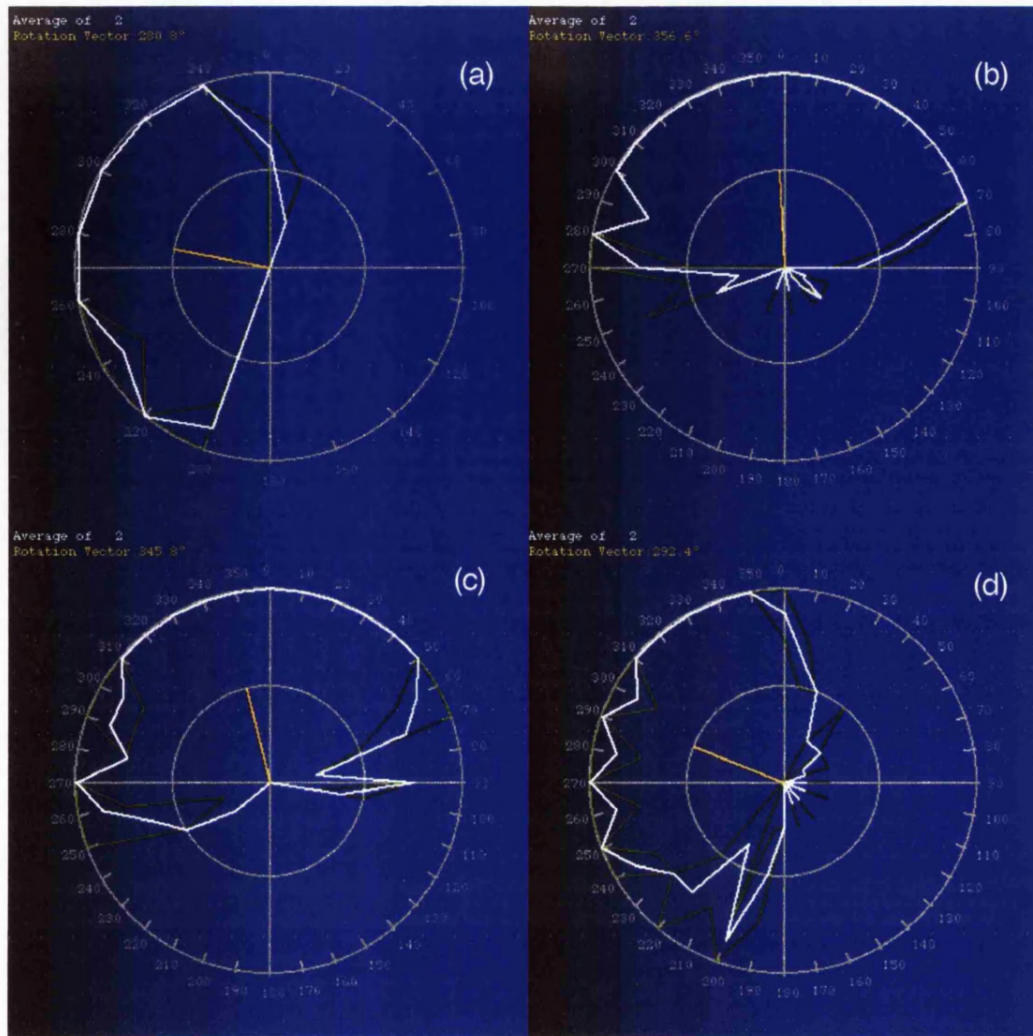
Figure 28: Average response curves for two subjects, comparing colour versus motion (a) with motion versus motion (b) with colour versus form (c) with form versus motion (d). If the angle of rotation of the colour/form experiment is added to the angle of rotation of the form/motion experiment (14.2+67.6=81.8), the result is very close to the angle of rotation of the colour/motion experiment (79.2).

DISCUSSION

Chapter 1: Functional segregation in V2

Separation of function

The most evident conclusion from the electrophysiological experiments is the fact that cells which have a selectivity for a particular attribute of the stimulus are not intermixed randomly in V2 but are instead grouped together with other cells having similar properties and thus kept separate from cells having different properties. This phenomenon is not special to V2: the striate cortex, area V1, is also divided into distinct compartments, each one dealing with the processing of a particular attribute of the visual scene (for a review see Livingstone and Hubel 1987b; Zeki and Shipp 1988). One can trace this compartmentalisation back to the lateral geniculate nucleus (LGN) of the thalamus, which is clearly divided into the magnocellular and parvocellular layers (Dreher *et al.* 1976), and even to the retina with its different types of retinal ganglion cells (Leventhal *et al.* 1981; Perry *et al.* 1984). At these early stages of the visual system, the separate classes of cells can be easily distinguished from one another through their morphology (e.g.. size, fibre diameter) as well as through their properties, which are related to basic functional characteristics of the cell: transient or sustained responses, high or low temporal or spatial sensitivity, slow or fast conduction velocities etc. At the cortical level, functional segregation remains a feature of the organisation but takes a more elaborate, sophisticated form. The appearance of cells with new selectivities leads to the formation of new functional groups, with cells having properties which did not exist in cells at previous stages. Cells in V1 already receive a mixed parvo and magno input, and therefore the idea of the M and P

systems as being totally segregated applies only to the LGN (Blasdel *et al.* 1985; Fitzpatrick *et al.* 1985; Lachica *et al.* 1992; Nealey and Maunsell 1994; Yoshioka *et al.* 1994). The appearance in the cortex of new cell selectivities such as orientation, direction, and disparity (wavelength is already present at the LGN), results in the formation of new systems, specialised for form, motion, and 3D vision. It is possible that the M system contributes more to one or the other of these new systems than the P system does, and vice versa (see Livingstone and Hubel 1987b for a review). However, it would not be correct to assume that each one of these new systems is nothing but a continuation of either the M or the P systems, and thus generalise in dividing the whole brain into two parts originating from a subdivision which is only truly present in the thalamus. What seems to be the general rule which applies to the whole of the visual brain is the fact that different functions are performed by different systems which are kept segregated and operate in parallel to each other, and each system may depend more on P or on M signals to execute its functions (Zeki and Shipp 1988).

The concept of separate segregated cell populations

As a direct result of functional segregation, different populations of cells are involved in the processing of different attributes of vision. This is indeed what we found in our experiments, and what has been reported by other laboratories both in V2 and also in the striate cortex (Livingstone and Hubel 1984a; DeYoe and Van Essen 1985; Hubel and Livingstone 1985; Shipp and Zeki 1985; Hubel and Livingstone 1987; Tootell *et al.* 1988b; Ts'o and Gilbert 1988; Tootell and Hamilton 1989; Ts'o *et al.* 1990). The basic principle is that if a neuron is selective for one attribute, it will not be selective for any other. This conclusion, which becomes obvious when one records from many single cells, also follows directly as a logical consequence of the segregation of cells with similar properties in clusters:

if cells had multiple selectivities, it would be illogical for the different functions to be processed in different parts of the brain, since more than one function would be processed at the same place by the same population of polyselective neurons. On the other hand, functional selectivity does not automatically imply functional segregation since, for example, cells selectively responding to colour could be widely distributed in the cortex, mingled with cells selective for other attributes. Two basic conclusions can thus be drawn regarding the way in which the visual system handles the different attributes of the visual scene. Firstly, it assigns the task of, say, the processing of colour and the processing of motion to different populations of cells. This is a logical implementation and maybe the most efficient way in solving the problem of dealing with different visual attributes, since, for example, the requirements for the perception of colour and the processing necessary for this achievement are very different from the requirements and processing necessary for the perception of motion. The second basic conclusion is that the brain does not leave these separate populations of neurons randomly distributed in the visual cortex, but rather groups cells dealing with different tasks to geographically separate locations. Bringing cells performing the same function next to each other probably makes it easier and faster for them to interact and perform the function with which they are assigned, since it simplifies the connectivity pattern between the cells. This also applies to the brain as a whole, since the part of the brain dealing with vision is kept separate from the part of the brain dealing with movement, and so on. Within the visual brain, the segregation is especially evident in the prestriate cortex, with the existence of separate visual specialised areas, among which the two best studied are the colour and the motion areas (Zeki 1973; Zeki 1974; Zeki 1977). Within areas like V1 and V2, where many different attributes of vision are represented (see Livingstone and Hubel 1987b for a review), the problem becomes more

complicated and the area itself has to be further subdivided. This is indeed the case, but a new method to achieve it is introduced: instead of splitting the area into, say, two parts, one concerned with colour and the other with motion, clusters of cells dealing with one or the other attribute alternate between them, creating a periodic pattern which constitutes the functional architecture of this area.

Different anatomy suggests different physiology

In addition to the creation of new types of selectivities when one moves to the cortical part of the visual system, it also becomes less easy to distinguish the different functional categories of cells on the basis of their morphology. In the LGN, magno and parvo neurons can be easily distinguished by their appearance, but the same cannot be said for the colour and orientation selective cells in V1 or V2. It takes more sophisticated methods of histological staining to show that the functional heterogeneity of these areas, which is evident when recording, has an anatomical counterpart. At present, the best indication of a functional segregation in these areas is staining for the activity of the enzyme cytochrome oxidase, which reveals the blob and stripe architectures of V1 and V2 respectively (see Introduction). The evidence for the fact that different morphology implies different function is overwhelming, not only from recording from these areas and relating cell properties to different CO compartments, as done in this study, but also from the anatomical connections between V1 and V2, and those between these two areas and areas V3, V4, and V5 (see Shipp and Zeki 1988 for a review). There is a great advantage in visualising the functional segregation of these areas, because one can then relate the connections and pathways of the different specialised systems throughout the brain. The basic conclusion from such studies is that the different attributes remain

segregated throughout the whole of the visual system, and that "like connects to like" is the basic principle of cortical connectivity (Livingstone and Hubel 1983; Livingstone and Hubel 1984b; Livingstone and Hubel 1984a; DeYoe and Van Essen 1985; Hubel and Livingstone 1985; Shipp and Zeki 1985; Hubel and Livingstone 1987; Livingstone and Hubel 1987a; Livingstone and Hubel 1988; Shipp and Zeki 1989a; Shipp and Zeki 1989b; DeYoe *et al.* 1994).

The oversimplification of functional segregation

On the other hand, interactions between the segregated systems can occur often, both by interconnections of different subdivisions within a single multi-functional area such as V2 (Levitt *et al.* 1994b) but also by way of connections between pathways, especially by means of "backward", less specific, connections (Zeki and Shipp 1988) or by convergence of projections of the different specialised areas (Shipp and Zeki 1995). Thus, functional specialisation and the processing of different attributes of vision by different systems does not imply that these systems are completely independent and cushioned from one another. The concept of functional specialisation is a very important one in trying to understand the way the visual system works, but it should not be oversimplified since the way in which it is used by the brain is far from known, particularly regarding the degree of independence and interaction between the specialised systems.

Although functional segregation as described above is the predominant impression we gained from our recording in V2, there are often some exceptions to the rule. For example, within a cluster of unoriented colour cells there are often one or two cells selective for orientation. Furthermore, not all cells selective for colour are found inside the colour clusters of V2; it is possible to find an occasional isolated colour

cell within a cluster of non-colour orientation or direction selective cells. This is the exception rather than the rule, and should not be taken to indicate that there is no functional segregation within V2 (for example see Gegenfurtner *et al.* 1996). All papers on V2 show functional segregation to a greater or lesser extent. The weakness of the techniques used, like for example the precision with which one can demarcate the exact type and boundaries of a CO stripe, can vary the extent to which the characteristic functional architecture of V2 is revealed. Segregation is not perfect; for example, not all cells in a thin stripe are colour selective, but colour clusters are placed in thin stripes. Similarly, orientation selective cells can be found in a thin stripe, but they are normally a few, and do not occur in clusters. The same is true for cells with more than one selectivities: most of the colour selective cells are unoriented, and most of the orientation and direction selective cells are indifferent to the colour of the stimulus, but one can occasionally find a cell which is both colour and orientation, or colour and direction selective. These cells, however, are the exception to the rule and are usually marginally selective for one of the two attributes. Furthermore, cells being the exception to the rule in one way usually follow the rule in another way: a directionally selective cell, for example, which is marginally selective for the colour of the stimulus as well, is more likely to be found within a cluster of non-colour, directionally selective cells of a thick stripe. There are thus exceptions to the rules, but the more one deviates from the rules the less frequently these exceptions are seen. To give another example, it is difficult to find a cell which is equally strongly selective for both colour and direction of motion, but if one does, it is very unlikely that it will be in the centre of an interstripe. Functional segregation, strongly related to the pattern of CO staining, is undoubtedly the basic principle of the organisation of V2.

The processing of colour in V2

One of the visual attributes processed in V2 is colour. This area is interposed in the cortical colour pathway, between the wavelength selective cells in V1 and the "real colour" cells in area V4 (Zeki 1980; Zeki 1983a); colour information is also found in the inferotemporal cortex, but the role of this area in colour vision is less extensively studied (Desimone *et al.* 1984; Tanaka *et al.* 1991; Kobatake and Tanaka 1994). There is a very ordered organisation in the way in which colour information is transferred along this pathway. Wavelength selective cells are first found in the parvocellular layers of the LGN, which project to the CO rich blobs of layers 2&3 in the striate cortex. The blobs of V1 in turn project to the thin CO stripes of V2, where the vast majority of wavelength selective cells in this area is found (Livingstone and Hubel 1984a). A direct projection from the blobs to V4 has also been shown (Yukie and Iwai 1985), the role of which is however not clear. Finally, thin stripes project to area V4, probably to regions different from the ones to which interstripes project (Zeki and Shipp 1989; DeYoe *et al.* 1994).

The properties of wavelength selective cells in V2 do not differ much from the ones in V1 (see Results). These cells are usually not selective for the orientation of the stimulus, and respond well to both stationary or moving stimuli of the appropriate wavelength. For most, the stimulus size is crucial and the cells will stop responding if the stimulus is made larger than the excitatory region of their receptive field. Such a behaviour reveals a suppressive surround, which is selectively inhibited by the same wavelength which excites the centre. Furthermore, with rare exceptions, most cells have an inhibitory opponent mechanism at the excitatory region of the receptive field. This mechanism is usually revealed by a weak response of the cell to white stimuli, although in a few

cases an inhibition below the spontaneous firing rate can also be seen with stimuli of the opponent colour.

In trying to find cells whose responses correspond to the colour as perceived by a normal human observer, Zeki recorded from cells in both V1 and V4 (Zeki 1983a). All of the cells in V1, and also many of the cells in V4, responded in a way which correlated with the wavelength composition of the light reflected from their receptive field. These cells were thus selective for the wavelength of the stimulus and would respond to an area of any colour in a Mondrian display, as long as it was made to reflect a certain amount of their preferred wavelength, and less amounts of the other two wavelengths. These cells were contrasted by Zeki with the real colour selective cells in V4, which responded to the an area of their preferred colour irrespective of the wavelength composition of the light coming from it, and would not respond to areas of a different colour no matter how much energy of one or the other wavelength was reflected by them. Colour cells can be further distinguished by the fact that they require the screen to be illuminated by all three projectors and full colour to be perceived by the experimenter before they respond. On the other hand, wavelength selective cells respond immediately when the projector of their preferred wavelength is switched on and the Mondrian illuminated by light of that wavelength only.

Since V2 is between V1 and V4 in the colour "hierarchy", we were interested to see whether cells in this area are able to signal real colour, i.e. whether colour constancy could be achieved before reaching V4. Our results showed that this was not the case, since the cells of area V2, when tested using a Mondrian, responded in exactly the same way as cells in V1 did. This is in agreement with lesion studies, which show that colour

constancy is lost after removal of V4, whereas wavelength discrimination is not, probably due to the integrity of areas V1 and V2, in both man (Vaina 1994; Kennard *et al.* 1995) and monkey (Wild *et al.* 1985; Heywood *et al.* 1992; Walsh *et al.* 1992b; Walsh *et al.* 1993). A further similarity we found between wavelength selective cells in V1 and V2 is the fact that the route one takes to reach a particular triplet of energies is a determinant of the response of the cell (Zeki 1983b). If the projector of the preferred wavelength is the first one to be switched on, cells respond strongly but then stop responding when the other two wavelengths are added. On the other hand, if the other two projectors are the first ones to be switched on, the cell remains silent but responds strongly the minute its preferred wavelength is added to the other two. The final energy triplet illuminating the Mondrian is in the end the same in both cases, but the cell only responds in the second case. These cell thus signal a change in the amount of their preferred wavelength with respect to the other two, being excited when there is an increase and inhibited when there is a decrease.

What can the role of cells in V2 with respect to colour be? Although it is possible that we have not sampled enough cells, our results suggest that wavelength discrimination but not colour constancy can be performed at this stage of the colour pathway. However, colour constancy in V4 must be the emerging property resulting from the characteristics of cells in the areas providing an input to it. Taking Land's Retinex Theory as a guide to the processes involving colour vision, a number of roles can be proposed for the corresponding cells in V2 (and V1 as well). Cells having an inhibitory surround of the same wavelength preference could be useful in comparing the relative amount of a particular wavelength between two different regions of the display, and thus assist in the construction of lightness records for that particular wavelength. Cells which signal an

increase or decrease of a particular wavelength reflected from an area are also useful, since the wavelength composition coming from objects in the real world is continuously changing, and the brain could use this type of information to compensate for these changes in order to keep the colour of each object constant. A role for cells with an inhibitory centre response to the opponent colour is more difficult to find, since a direct comparison of different wavelengths of the same area is not proposed by Land's theory. However, although this theory works very well, one cannot be sure that the brain follows a strategy which is identical to the one suggested by Land, when assigning colours to different objects. For example, it could be that the brain first uses colour opponent cells to compare the relative amounts of wavelengths within a single area of the Mondrian display, and then compares these comparisons between them to reach the same result Land's method would have reached as well.

Concluding, the similarities between V2 and V1 in terms of colour are more striking than the differences. Cells in V2 are binocular and have larger receptive field than the ones in V1, and the functional architecture between the two areas is also different. What is common, however, is a regional analysis of the visual field (not only for colour but for other attributes as well), rather than a global processing of a particular attribute. This property is especially significant in terms of colour, since the requirement for integration over space cannot be satisfied at this stage of cortical processing.

Chapter 2: Mapping the visual space in V2

Summary

Given that V2 is an area in which multiple attributes of vision are processed in a segregated manner (see above), it is interesting to investigate the strategy which the brain uses to map the visual space in this area. Given the functional architecture of V2, if it contained a continuous retinotopic map in which adjacent parts of the cortex represent adjacent parts of the visual field continuously and without repetition, each part of the visual field would be mapped for a single attribute only. A more realistic possibility, suggested by this thesis and also by previous findings (Zeki and Shipp 1987; Zeki 1990c; Roe and Ts'o 1995) is the existence of several separate maps of the visual space in this area. These maps are interleaved with one another, in a pattern identical to the CO functional architecture of V2. There is thus a distortion of the continuity of the overall mapping pattern in V2, which is characterised by a repetitive representation of the visual field for each attribute in turn. It was therefore interesting to record continuously from thin, thick, and inter CO stripes in long penetrations, and verify that each set of stripes belonging to one type contains collectively a complete representation of the visual field; any different result would raise strong arguments against the idea of a functional segregation in V2.

Previous mapping results not directly related to CO histology

If there is functional specialisation between the different cytochrome oxidase compartments of V2, it is reasonable to suppose that the visual field is mapped for a single function only along a particular stripe, whereas along the different types of stripes it is re-mapped separately for each of the different functions. Therefore, a dorso-ventral

displacement of recording sites, if coincident with a given stripe type, would result in a continuous and relatively quicker (in mm of cortex/degree of RF displacement, a measure known as "cortical magnification factor") receptive field displacement from the vertical to the horizontal meridian, compared to a medio-lateral displacement of recording sites; the latter would result in an irregular, repetitive, and thus relatively slower receptive field displacement towards the centre of gaze (see **Figure 17**). Previous detailed studies of V2 topography (Rosa *et al.* 1988), not related to cytochrome oxidase architecture, have suggested that this is actually the case: the cortical magnification factor was found to be smaller when recording along the isoeccentricity lines of V2 (parallel to the stripes) than when recording along the isopolar lines (perpendicular to the stripes); in the second case, receptive fields corresponding to recording sites separated by a cortical distance of up to 4 mm could represent the same portion of the visual field. Our present results are consistent with the above notion: we find that there are no gaps in visual field representation when moving from one stripe to the next stripe of the same type, and thus each attribute is continuously represented in one set of stripes and contained within the overall map of V2. It follows that one should find little or no overlap in field representation between adjacent stripes of the same type and maximum overlap between adjacent stripes of different types. The latter, however, can vary quite a lot depending on the phase relationship of the different maps in each particular brain (see below).

The results of previous CO-related mapping studies

The repetitive mapping of the visual space in V2 was first suggested by Zeki and Shipp (Zeki and Shipp 1987; Zeki 1990c). They made long parallel penetrations, intersecting the CO stripe pattern at right angles, and

assigned receptive fields to different stripe types. They found that (a) there is a continuity in the representation of visual space between two adjacent stripes of the same type, with a small overlap (but no gap) between the receptive fields belonging to cells of the two, (b) there is a much greater overlap between the receptive fields of cells belonging to adjacent stripes of different types, i.e. the same part of the visual field is re-represented when moving from one stripe to the other, and (c) the progression of receptive field centres towards the centre of gaze, as a function of electrode distance when recording from medial to lateral, follows a smooth pattern within each stripe but "jumps back" when crossing the borders between stripes, thus demonstrating a re-representation of the particular part of the visual field in different types of CO stripes.

While the present work was in progress, a very similar study was published by Roe & Ts'o (Roe and Ts'o 1995). The latter authors have used a somewhat different approach, classifying stripes according to function rather than according to architecture, and concentrating on disparity rather than motion. An advantage of dividing V2 in terms of physiology instead of anatomy is that the results are not influenced by any artefacts in the CO staining, since the grouping of receptive fields is done using the cell properties as a guide. On the other hand, because the segregation of function is not perfect in V2 (see above), the presence of a cell with the "wrong" selectivity or the absence of the expected selectivity by some cells in a stripe, might lead to an incorrect division of V2 into functionally separate regions. The deviation of Roe & Ts'o from the CO architecture is most pronounced with respect to the interstripes: they claim that orientation selective cell clusters (their equivalent of interstripes) are half the size of the colour (thin stripes) and disparity (thick stripes) clusters; the CO architecture of V2, however, suggests that interstripes are larger than

thin stripes and smaller than thick stripes, with an approximate ratio of K:N:I equal to 2:1:1.5 (Shipp and Zeki 1989b). Nevertheless, the basic pattern of separate mapping that emerges in this study remains the same. The finding that there is no gap in visual field representation between subsequent stripes of the same type is common between the Roe & Ts'o paper and the present thesis, and confirms the earlier results of Zeki and Shipp. This is probably the most significant result, since it shows that each part of the visual field is separately mapped in each of the three sets of stripes. A basic difference, however, between the present study and the study of Roe and Ts'o is the overall pattern of overlap between the receptive fields of cells in the different CO stripes. They report, after recording from large numbers of stripes in several penetrations, that there is maximal overlap between adjacent stripes of a different type, and minimal overlap between adjacent stripes of the same type. Since they have as well used a quantitative method to calculate overlap, they give numerical values and standard errors, showing that this statement is generally true and consistent. We agree that this can be the case sometimes, but in our view does not constitute a general phenomenon. Our basic finding is that there is minimal overlap between adjacent stripes of the same type, with the exception of the interstripes, between which the overlap is significant. However, the overlap between adjacent stripes of a different type might be large, medium, or small, depending on the overall pattern of overlap between the different maps (see below). This difference leads us to propose a model of visual field representation in V2 that is different from the one they propose.

A general model of mapping in V2

Since the CO stripe pattern in V2 is periodic, one can define a "cycle" as consisting of one K, one N, and two I stripes. According to Roe & Ts'o, the visual space can be divided into segments, each one of which is mapped three times (one for each one of the three types of stripes) by a single CO cycle. Different cycles map different parts of the visual space, and thus a single module is proposed for V2, which could be called the "functional cycle". Our results show that this model is an oversimplification. Returning to the histology, area V2 can be divided in CO cycles in four different ways, depending on which one of the four different type of stripes (K, N, I medial to K, I medial to N) the sequence begins. Therefore, the CO pattern cannot determine which stripes belong to the same cycle and which ones do not. A convenient way of dividing stripes into cycles would be to include in the same cycle stripes which map exactly the same part of the visual field. For example, a N stripe would clearly belong to the same cycle with the K stripe medial to it if they map the identical part of the visual space; the K stripe lateral to it (which histologically could also be grouped together with the N stripe in the same cycle) would then map a completely different part of the visual space, and thus belong to a different cycle. What happens, however, if the receptive fields of the cells in a N stripe partly overlap with the receptive fields of cells in both the K stripes before and after the N stripe? The attribution of cycles in this case is not straightforward any more: the two K stripes by definition belong to two different cycles, but to which one of the two would the N stripe between them belong to? Our results show that all possibilities of overlap patterns can be found in V2: a N stripe can overlap substantially with the K stripe medial to it and very little with the K stripe lateral to it, or vice versa, or overlap more or less to the same extent with both. The term "cycle" thus should only be used to describe the periodic

stripe pattern in V2, and not to imply a functional module constituting the basic building element of V2 (see Roe and Ts'o 1995). What we have found in V2 in terms of mapping is three complete maps of the visual space, based on the three different types of stripes, superimposed on each other with various phase differences between them. The "in phase" model proposed by Roe & Ts'o, where all three maps are in precise register, is only a (rare) single possibility out of many, not the basic rule of the retinotopic organisation of V2. It would therefore be better, instead of thinking in terms of well defined functional cycles mapping adjacent parts of the visual field, to think in terms of several independent maps having various phase differences (along the cortex) with one another, with these differences not being large enough to distort the overall topographic map within V2.

A schematic representation of this argument is shown in **Figure 29,** using a painting by Matisse in which we have tried to re-create as faithfully as possible what could happen in a segment of cortex such as the one illustrated in **Figure 17,** when a subject fixates the cross on the figure and views objects (in this case human figures), of a particular colour (red), moving in a particular direction (counter clockwise). We explore how a segment of the figure will be represented within the multiple maps in the three systems of stripes in V2. Assuming that each stripe represents 1° of visual field, a given portion of the lower field of view subtending 4° can be mapped within the three cytochrome oxidase systems of V2, making the assumption that there are independent maps of the visual field centred around the K, N and I stripes and that each alternating set of I stripes contains an independent map as well. In model (a), the different maps are in perfect phase register; in such an instance, there would be no overlap between adjacent K and K stripes, total overlap between adjacent
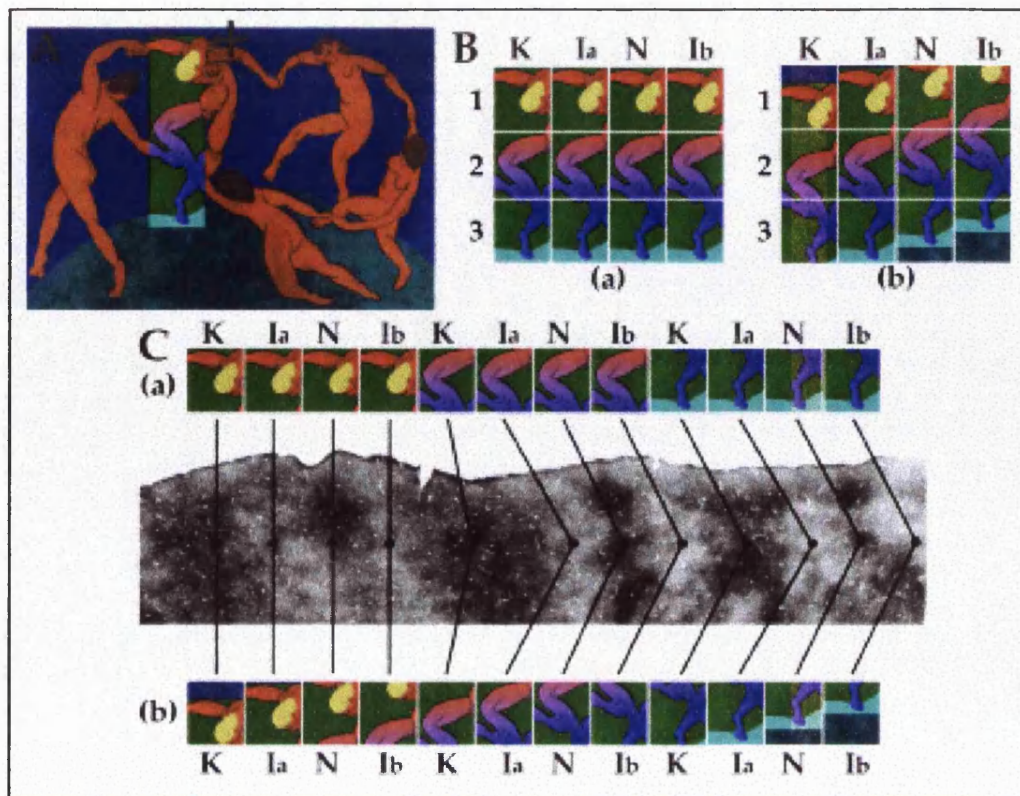
Figure 29: An example of the way in which the visual space is mapped in area V2. A subject is looking at the painting of Matisse shown in A, fixating on the cross; we will consider how the highlighted part of this painting is represented in 3 stripe "cycles" of area V2. Two models of dividing the image between them are shown in B: in both models, each one of the four different types of stripes contains a complete copy of the image. In model (a), however, all adjacent stripes of a different type contain exactly the same copy of part of the image, whereas in model (b) the copies are slightly out of phase with one another. These two possible mapping strategies are also shown in C, in the way they would occur across the cortex of V2.

K and N stripes belonging to the same "mapping cycle" and no overlap between K and N stripes belonging to different cycles; there would however still be substantial overlap between adjacent I and I stripes. In model (b), the maps in the three sets of stripes are out of phase with each other, and it is not as meaningful to assign stripes to particular cycles, since the overlap between different stripes is partial rather than complete. In such a model, the relationship between adjacent K and K and adjacent N and N stripes would be the same as in model (a), while the relationship between adjacent K and N stripes would vary according to the phase

difference of the K and N maps, and also the relative medio-lateral position of the two particular stripes.

Our results tend to favour the second model, since, for example, we have never encountered a case in which the degree of overlap of a K stripe was 100% with the N stripe on one side and 0% with the N stripe on the other. More usually, the degree of overlap was variable, the only noticeable difference being that it was more on one side than on the other, and on some occasions there was an almost 50% overlap on either side. We have found a high degree of overlap between successive I stripes, making the existence of two different types of I stripes possible (although we have not found any physiological evidence for this). In this, our results differ from those of Roe and Ts'o who find a lack of overlap between successive I stripes to be the rule. The fact that we find a high degree of overlap between adjacent I stripes is in agreement with the fact that there is double the number of I stripes available than K and N stripes, mapping the same extent of visual space. A possible explanation for this discrepancy in the results Roe and Ts'o is that their stripes are based upon functional properties alone and are therefore not necessarily co-extensive with our anatomical CO interstripes. Their (physiological) I stripes are reported to be half the size of the N stripes, whereas in real histological terms (see Shipp and Zeki 1989b) I stripes are actually bigger. Hence many cells which would be included in an I stripe on the basis of (our) anatomical definition might have been excluded from theirs, giving the impression of an absence of overlap in their results.

What is the reason for having multi-modal areas?

The existence of multi-modal areas, such as V1 and V2, could lead one to ask why, instead of having a single area with alternating zones of,

say, colour, orientation, and direction, one should not have three separate areas instead. A possibility is that areas V1 and V2 are undertaking a uniform function, which is the same and necessary for each one of the different visual attributes. What this function might be remains unknown, but it could very possibly be related to topography, that is, assigning visual information to the appropriate parts of the visual space. Areas V1 and V2 are characterised by a very systematic and detailed retinotopy, a property which is lost as one moves to the "higher" areas of the visual system (Zeki 1978a). If the function of V2 is strongly related to topography, it is not surprising that colour and orientation clusters alternate in this area: in this way, parts of the cortex dealing with different functions in the same part of the visual field are placed next to each other, so that they can interact or be subjected to the same operation more effectively. In V2 such interactions are indeed possible, via horizontal interconnections between CO stripes of a different type (Rockland 1985; Levitt *et al.* 1994b). In our opinion, a key question for understanding the function of V2 is the following: is the processing that takes place in V2 (whatever it might be) the same or different for each one of the different visual attributes? It is possible that V2 is a single area with a single function (in our opinion related to the determination of the spatial position of visual events) which is common and necessary for the processing of each one of the different visual attributes. Colour and motion signals, for example, could be processed in the same way (although separately) within a single area with a single function, area V2, before being sent to areas V4 and V5, two different areas with different functions, in order to be processed differently according to the specific different requirements necessary for the perception of each attribute. If the above hypothesis is true, the role of V2 (as well as V1) in vision is a very fundamental (and unknown) processing, necessary and common to all the

visual attributes, before specialised processing takes place in the higher visual areas.

Chapter 3: Psychophysics

Summary

Although functional specialisation and the existence of different (in their temporal properties as well) systems has been widely studied in vision (see Introduction), a unitary visual perception has always been assumed to be the end result, due to "binding" mechanisms in the brain bringing different visual attributes together in the correct spatio-temporal order. Given that colour and motion are processed by separate systems in the brain, we were interested to see whether there is any difference in the time necessary for the perception of these two attributes. We have used a moving abstract square pattern which was continuously changing both in its colour and direction of motion, so that any difference in perception time between colour and motion would lead subjects to pair colour and motion "incorrectly". The results of the experiment suggested that the colour of the pattern at time t is perceived together with the motion of the pattern at time t-Δt, where Δt=70 to 80 ms. Therefore, a particular colour and a direction of motion that occur simultaneously are perceived separately, i.e. functional segregation is maintained at the perceptual level. Furthermore, the brain does not compensate for the differences in perception time between its specialised systems, and thus binds together attributes which are perceived simultaneously, rather than attributes which occur simultaneously. We propose that the perception of each attribute is separately achieved by the specialised system involved with its processing, irrespective of the other specialised systems.

Perceptual time differences in the visual system

Since colour and motion are processed separately in the brain, it is possible that the colour and motion systems do not achieve perception at

exactly the same time. For example, consider the appearance of a red object moving to the right: given functional segregation, the possibility arises that its colour will be perceived before its motion or vice versa, instead of the colour and motion information being available to the brain at exactly the same instant. We tested the hypothesis that the perception time, defined as the time elapsed between the appearance of the stimulus and the perception of its colour (or motion) by the brain, is different for different attributes. The basic idea of the experiment was the following: if one attribute, say colour, is perceived before another, say motion, then the colour of an object at time t is perceived together with the motion that the object had at an earlier time t-Δt, where Δt is the perception time difference between colour and motion. Therefore, if the direction of the object's motion at time t-Δt is different from that at time t, then the colour of the object at time t is perceived together with a motion which is different to the motion of the object at the time it had this colour. Similarly, if the colour of the object at time t+Δt is different from that at time t, the motion of the object at time t will be perceived together with a colour different to the one the object had when moving in this way. Thus, by continuously varying both the colour and the motion of an object at a rate comparable to Δt, one can introduce a perceptual pairing of colour and motion which is different from the one happening in reality.

Our results show that there is indeed a difference in perception time between colour and motion, and that colour is actually perceived, on average, 70-80 ms before motion. We have tested the experiment on many people and the result is identical. What differs between individuals is the exact value of the time difference, which can range from 50 to 100 ms. This variation should be expected, firstly because the performance of individuals varies in most psychophysical tests, and also because the

limited accuracy of the method could allow for some variation in the results. However, the most important phenomenon regarding the reliability of the method, is the fact that the rotation of the response curves with respect to the veridical curves is proportional to the frequency of the oscillations used. Unfortunately, due to the limited capabilities of our computer, we could not test a bigger range of frequencies. It would be nice, for example, to make the period equal to 2Δt, in which case (according to our theory) an impressive 180° rotation would occur, i.e. the colour and motion relationship would completely reverse in terms of perception.

The outcome of the control experiments reinforced our confidence to the original result. The possibility that subjects might be using some kind of unknown "strategy", which resulted in the deviation from the veridical curves, was eliminated by the fact that the confidence level curves were always rotated (with respect to a hypothetical veridical one) by an amount equal to the amount of rotation of the response curves, i.e. they always followed the pattern of the subject's answers, suggesting that the "wrong" answers given by subjects were indeed the result of a "wrong" perception. Furthermore, concerning the generality of the rotational effect and its independence from the shape of the veridical curve, the results on the "lesion" experiments showed that the perceptual curves will rotate by a certain angle with respect to the veridical curves, irrespective of the position of the latter and therefore of the exact stimulus configuration.

What would the prediction of the literature be?

In addition to the physiological evidence described in the Introduction, a separate processing (but not perception) of colour and motion has been also suggested by some psychophysical experiments. The perceived velocity of equiluminous gratings is slower than that of

luminance gratings moving in the same way, suggesting that colour information is not available to the motion system (Cavanagh *et al.* 1984). This is also confirmed by the loss of apparent motion at equiluminance (Ramachandran and Gregory 1978; Cavanagh *et al.* 1985). A further indication for an independent processing between colour and motion is the fact that in some cases, under the same stimulus condition, the motion system can be integrating information coming from the two eyes whereas binocular rivalry is, at the same time, taking place with respect to the colour system (Carney *et al.* 1987).

Given the existence of the two different systems, what evidence is there to suggest that one is faster than the other? A direct comparison between the temporal properties of the colour and motion systems, using maximum alteration frequencies, was done by Livingstone and Hubel, who concluded that the motion system is faster (Livingstone and Hubel 1987b). Another way of measuring the temporal properties of different systems is by means of their temporal integration periods. These can be measured by relating stimulus presentation time to the threshold of detection. The "integration time", or "critical duration" value of the minimal necessary presentation at the lowest threshold, gives an idea of the amount of information necessary to be integrated by the system before perception of the stimulus can be achieved. Nevertheless, temporal integration periods give a very imperfect guide to perception times, since they are principally determined at a very early stage in the visual system, and thus do not reflect the properties of the system as a whole (Krauskopf and Mollon 1971). Perception time is the end result of the processing of the system as a whole; different psychophysical measures of sensory latency (such as temporal integration period) are probably related to different elements of the neural response, not to the whole perceptual process (see

Sternberg and Knoll 1972). Furthermore, integration times vary depending on the conditions and the method used: for example, it can vary depending on the wavelength used (Pokorny *et al.* 1979) or the adaptation level (Kawabata 1994); with motion, humans are more sensitive in the temporal detection of direction changes than of speed changes (Nakayama 1985; De Bruyn and Orban 1988). Therefore, no thing such as a "standard temporal integration period" for motion or colour exists, and one cannot generalise and come to a conclusion on whether colour or motion is seen faster by comparing results between different studies.

Psychophysical evidence is not the only one available: the temporal properties of the colour and motion systems have also been studied physiologically. Here the unanimous view seems to be that the M system, which largely feeds the motion pathway, is faster than the P system, which feeds the colour pathway (Dreher *et al.* 1976; Schiller and Malpeli 1978), that the colour cells of V2 are activated after the non-colour cells (by 10- 20 ms -- see Munk *et al.* 1995; Nowak *et al.* 1995), that the thick stripes and interstripes of V2, which are relatively poor in colour cells, are activated before the thin stripes which are rich in wavelength selective cells, and finally that direction selective cells are activated earlier than cells lacking such a selectivity (Munk *et al.* 1995). Moreover, area V5, specialised for motion, receives signals (from fast moving stimuli) before V1- see (ffytche *et al.* 1995) and its cells are among those which have the shortest latencies (Raiguel *et al.* 1989). Furthermore, signals reach V5 before they reach V4 (see Buchner *et al.* 1994 and ffytche *et al.* 1995).

Motion as a temporal derivative

An observation that might favour colour as being faster than motion is that the latter is defined as a change in position with respect to

time, i.e. it is a temporal derivative. However, it is now generally accepted that motion is a fundamental biological sense, rather than an elementary cognitive process reconstructed very late in the visual system in terms of the memory of a position over time (Nakayama 1985). Examples of the dissociation between motion and position are the "waterfall" illusion (Nakayama 1985), the Leviant illusion (Leviant 1996), and also the stimuli used by Newsome in V5 monkey studies (Newsome and Pare 1988). On the other hand, colour vision requires integration over space (Land 1974), a rather complicated task which may involve a respectable amount of processing time. The above argument concerned us because since a motion change requires 3 frames on the screen whereas a colour change only 2, colour could be given a 1 frame advantage in time. We wondered whether we should subtract 15 ms (1 frame) from the 75 ms average value given to the colour and motion perception time difference, still leaving colour with a 60 ms advantage. For the reasons described above, however, we do not believe this is the case. For example, consider the last frame of a 100% green excursion, which is a green square at the uppermost position of its trajectory: the instant the following frame appears, consisting of a red square placed one position below, information about both a change in colour *and* a change in direction of movement is available to the brain. We believe, therefore, that the motion information becomes available in the same frame as the colour information does.

Separate perceptions and temporal binding

Whether colour is perceived first, or motion first, although very interesting, does not in fact affect the basic finding of this experiment. We were not interested in the processing but rather in the final perception times of colour and motion: subjects were asked to pair two percepts which are processed separately with one another. Our results on

performing the experiment by varying the wavelength composition of the squares also show that our task was indeed a perceptual one, as opposed to, for example, simple wavelength discrimination. Due to a perception time difference between the colour and the motion systems, a particular colour and a particular type of motion which occur at the same time are perceived separately. Therefore, our findings extend the concept of functional segregation into the perceptual level and the time domain: colour and motion are not only processed separately, but also perceived separately, and at different times. Thus, the motion system is on its own able to perceive motion, and will do that in its own time; the same is true for the colour system. What is the effect of this on the binding of different attributes together? The colour, motion, form, and stereoscopic depth that an object has at a certain place and at a certain time have to be combined together in the correct spatio-temporal way, in order to perceive (after a small delay) the object as it really appeared. With our method we have been able to disturb the temporal dimension of this binding, and thus lead to the perception of objects which do not exist in reality: the subjects report seeing green squares moving upwards and red squares moving downwards, when the reality on the screen is the opposite. What does this tell us about the way in which the brain brings together different attributes belonging to an object? The possibility of a "synchroniser", either in terms of lateral interconnections within a multi-functional area like V2 (Levitt *et al.* 1994b) or in terms of "feedback" and convergence connections between different areas (Zeki and Shipp 1988; Shipp and Zeki 1989b) is not consistent with the present results. It is clear from our psychophysical experiment that the brain does not bind together attributes which occur together, but rather attributes which are perceived together. The "real-time" synchrony between two attributes is thus not the same with the "brain-time" synchrony, and it is the latter rather than the former which

the brain uses as a guide to binding. This feature of the brain is not a problem in the real world, since it is very unlikely that an object will ever keep changing colour and motion at such high rates. It can only be revealed under extreme experimental conditions, but nevertheless gives valuable information concerning the binding problem.

As soon as functional specialisation in vision was discovered, the question of binding arose. If different visual attributes are processed separately in the brain, how are they in the end combined together to give a final, unified percept? Our psychophysical results raised a somehow similar but also quite different question: given that there are differences in the perception times between the different attributes, is the brain able to take these differences into account when binding these attributes together? In other words, does the visual brain have a mechanism to compensate for the differences in perception times between its specialised systems, and thus achieve correct temporal binding? Such a mechanism could, for example, be the existence of a higher "perception area" to which all the systems would eventually report, and which would be able to arrange and combine information of different attributes in the correct manner, taking into account the temporal differences between the different specialised systems. Such an area could also partly help to solve the original binding problem as well, since a unified percept would result after integration of colour, motion, form, and stereoscopic depth information in it. However, simple anatomy speaks against such a solution since there is no single area to which all other areas project. Instead of taking place in the "higher" visual areas, a possibility for integration could arise in a "lower" area such as V2, where the different attributes of vision are all present and interactions between them are possible via horizontal interconnections between different CO stripes (Rockland 1985; Levitt *et al.* 1994b). A

difference in the arrival of motion and colour signals, however, has already been demonstrated in V2 (see Introduction), and motion related signals are the ones to arrive before signal related to colour. In terms of perception, however, we have shown that colour is perceived before motion. Therefore, the electrophysiological events taking place in V2 are not a good indication of the final perceptual result of the visual processing. Since area V2 is involved in the processing of both motion and colour, we believe that it influences the way in which each one of these attributes is finally perceived, and thus also the way in which the colour and the motion of an object are paired together. However, we find the idea that the function of this area is to integrate the different visual attributes between them (Shipp and Zeki 1989b; Roe and Ts'o 1995) an oversimplification.

In trying to solve the binding problem, it is first worth asking whether this problem really exists. We believe that there is an independence of the specialised systems involved in the processing of the different visual attributes all the way to the perceptual level. The perception of an attribute is therefore the sole result of the processing taking place in the specialised system involved with it; both this processing and its end result, perception, are separate and independent from the processing and perception in other specialised systems. The simplest way to account for the "misbinding" of our psychophysical experiment is to suppose that attributes are bound together if, and only if, they are perceived at the same time (although separately) in corresponding spatial locations of the visual space. No areas of convergence or integration are necessary in this way, since binding occurs automatically as a result of the spatio-temporal synchronisation between the percepts of the different specialised systems.

The generality of the phenomenon

One could question the generality of our psychophysical results, i.e. whether colour is perceived before motion in general or just under the specific conditions that we used. We have repeated the experiment by randomly varying the hue, saturation and brightness, as well as the background colour (black, white, and grey), speed of motion, number of squares, size of squares, pattern of squares on the screen, viewing distances, fixation versus free gaze, monocular versus binocular viewing etc. We have failed to see any deviation from the original phenomenon due to these factors. However, it is possible that if, for example, colour is presented at threshold its perception time will increase and therefore our Δt value will decrease. Such experiments would be indeed very interesting but not very easy to perform, since the replies of subjects are very inconsistent at threshold levels, and a very large number of repetitions would be necessary to get a significant result. However, even if under certain conditions the performance of one or the other system is impaired, the demonstration that there is such a thing as a difference in perception time between visual attributes at suprathreshold levels, and the fact that this can result in incorrect binding, is a general discovery about the organisation and functioning of the brain.

The generality of the finding is also evident from the fact that it applies not only between colour and motion, but also colour and orientation, and orientation and motion as well. Results from these experiments were used as a further control on the psychophysical method introduced, since, for example, if colour was found to be faster than motion, and motion faster than orientation, but orientation was found to be faster than colour, our results would be questionable. As it happens, our

results were consistent between them, showing that colour is the fastest and motion the slowest in terms of perception times. In fact, the numerical precision of the comparative results surprised us, given the expected low accuracy of any measurements obtained with our method. Nevertheless, the power of this method lies in its ability to be applied between any combination of attributes, or any sub-modalities within an attribute, and determine the time difference with which the brain achieves their perception.

EPILOGUE

This study really started as an enquiry into the problem of integration at the level of area V2. The integration that was envisaged was of two kinds, spatial and temporal The spatial integration is implicit in the generation of colour, since colour itself is the result of a comparison of signals coming from relatively large parts of the field of view. V2 promised to give some insights into how this is achieved, because the receptive fields of its cells are larger than those of V1 and smaller than those of V4 and also because V2 stands midway between V1 and V4 in terms of topography - not as precise as V1 but much more so than V4. The very topographic arrangement of V2, with cells signalling different attributes of the visual scene being clustered together into identifiable groupings that are connected, also carried with it the hope of giving us some insights into how the representation of the separate attributes of vision is brought together to give us our unitary experience of the visual world, where all attributes are seen in precise spatio-temporal registration.

In a sense, we failed at both these aims, for we saw no evidence of a spatial integration that is extensive enough to generate colour-coded cells and no hint of the kind of integration that would lead to precise spatio-temporal registration. But that very failure brought its own rewards, in showing for the first time that the search for such integration at *any* level of the visual brain is probably a superfluous exercise. This is because of our finding that the perceptual systems are distinct, just as are the processing systems, and that there is, therefore, no precise temporal integration, as we had supposed. Rather, such integration as occurs is that of *asynchronously* perceived signals. The route to such a conclusion nevertheless yielded many interesting results -- about the nature of the map in area V2, about

what is perceived first, about the selectivities of cells, and much else besides. But the conclusion itself has perhaps, in its own small way, opened a different and I hope insightful way of studying the visual brain.

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