The Weighting of Binocular Experience in Visual Cortical Development

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Summary

After birth the brain adapts to characteristics in the environment in order to optimise its resources with respect to the individual's circumstances. For instance, early monocular deprivation results in reduced cortical representation and visual acuity of the deprived eye. However, such a loss of visual function in one eye after only transient periods of compromised vision through injury or infection would seem to be maladaptive. I examined here whether cortical deprivation effects can be counteracted by daily periods of normal experience. Cats received variable daily regimens of monocular deprivation (by wearing a mask) and binocular exposure. Visual cortex function was subsequently assessed with optical imaging of intrinsic signals, visually evoked potentials, and extracellular electrophysiological recordings. Regardless of the overall length of visual experience, daily binocular vision for as little as 30 minutes, but no less, allowed normal ocular dominance and visual responses to be maintained despite several times longer periods of deprivation. Thus, the absolute amount of daily binocular vision rather than its relative share of the total daily exposure determined the outcome. When 30 minutes binocular exposure were broken up into two 15-minute blocks flanking the deprivation period, ocular dominance resembled that of animals with only 15 minutes binocular vision, suggesting that binocular experience must be continuous to be most effective. My results demonstrate that normal experience is clearly more efficacious in maintaining a binocular visual cortex than abnormal experience is in shifting the ocular dominance distribution. These findings contribute to the larger debate about how much nature and nurture, respectively, contribute to the development of the brain; they suggest that while experience plays a significant role, for some functions there may be an intrinsic bias towards a state that is optimally adapted to the most probable environment.

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Abbreviations

AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, 41
BCM	The Bienenstock-Cooper-Munro model of synaptic plasticity, 42
BD	Binocular deprivation, 33
BDNF	Brain-derived neurotrophic factor, 45
BE	Binocular exposure, 72
BOLD	Blood oxygen level dependent signal, 52
cAMP	Cyclic adenosine monophosphate, 41
CREB-1	cAMP response element binding protein-1, 41
DE	Deprived eye, 33
fMRI	Functional magnetic resonance imaging, 49
GABA	Gamma-aminobutyric acid, 45
GAD65	65kD isoform of glutamic acid decarboxylase, 45
hWhH	Half width at half height, 78
i.v.	intravenous, 74
IT	Inferotemporal cortex, 29
LGN	Lateral geniculate nuclei in thalamus, 17
LTD	Long-term depression, 41
LTP	Long-term potentiation, 41
MD	Monocular deprivation, 33
MT	Mediotemporal cortex (also known as area V5), 29

NE	Normal or nondeprived eye, 33
NGF	Nerve growth factor, 45
NMDA	N-methyl-D-aspartic acid, 41
NT-4/5	Neurotrophin-4/5, 45
OD	Ocular dominance, 19
PKA	Protein kinase A, 43
RF	Receptive field of a neuron, 15
RO	Reverse occlusion, 38
RoI	Region of interest, 58
STDP	Spike timing-dependent plasticity, 42
tPA	Tissue-type plasminogen activator, 46
V1	Primary visual cortex (Visual area 1 or Brodmann area 17), 19
V2	Visual area 2 (Brodmann area 18), 28
V3	Visual area 3 (Brodmann area 19), 29
V4	Visual area 4, 29
V5	Visual area 5 (also known as area MT), 29
VEP	Visual evoked potential, 76

Chapter I

Introduction

Nürnberg, Germany, 26th May 1828. A stray teenage boy is discovered in the city. He is unable to walk and, beyond stammering a few rehearsed words, can not communicate verbally. His only means of identification are two letters he carries on his person, which state his name to be Kaspar Hauser and explain that the child was brought up in solitary confinement...

The story of this foundling received much attention by the public at the time as it represented one of the first well-documented cases of feral children, stories that up until then had existed predominantly in the realm of legends. Eventually, Hauser learned to speak allowing him to elaborate on the alleged circumstances of his childhood, which he spent in a small cell deprived of any human interaction and without ever being taught any social skills (von Feuerbach, 1833). The reason for his relatively successful integration into civilisation is unknown, and this is the point where his biography contrasts starkly with that of most other feral children. Their inability to ever learn to talk or acquire basic civilised conduct has been taken to suggest that there is a critical phase in early life during which many skills and traits are established through exposure to the environment, and that lack of such early experiences leads to stunted and insufficient development of these skills (Leiber, 1997; Schneider, 2003).

Cases of feral children have thus contributed to the great nature-nurture debate that has been a focal point of not only scientific but also philosophical and sociological discourse throughout the ages. In how far are the traits of a person, from simple perceptual capabilities over general intelligence, sexual preference, criminal tendencies and any other behavioural characteristics, predetermined by their genetic make-up? On the other hand, to what extent does the organism adapt to its environment after birth? Ultimately, which is more influential in shaping a personality: heredity or experience?

Adopting a scientific view that skills and behaviour depend largely on brain function, we must surmise that most of these questions can be answered by studying the postnatal development of the brain and investigating the effects of early experience on its functionality. Evidently, many human traits are shaped extensively by our upbringing, such as our native language which depends on the language of our parents and the people surrounding us during infancy. Further, as the haunting stories of (non-mythological) feral children teach us, exposure to human speech in early life appears to be essential for the ability to ever achieve the proficiency to speak (Leiber, 1997).

Yet, it is the combination of the heritability of skills and behavioural traits as well as

the ability to adapt to individual circumstances which can grant the greatest evolutionary advantage. Genetic inheritance bestows skills on a species that can markedly bias natural selection in its favour. Conversely, an individual organism that cannot adapt would likely perish in an unusual environment. Therefore, the best guarantor for survival of the species is to possess both characteristics to an extent.

While exposure to speech may be required for the maturation of language skills, there may thus be an innate mechanism for language built into the human brain. Steven Pinker championed the concept of a universal grammar that is shared by all human languages and which young children use in order to learn the phonemes, syntax and grammatical peculiarities of their first language, usually even without being taught explicitly (Pinker, 1994). In the absence of any language exposure during the critical period for language acquisition this would prevent emergence of normal speech as seen in feral children. However, even limited experience with language may be sufficient for language proficiency to develop normally. For instance, infants start imitating the sounds they pick up from their parents independent of whether they are actively being spoken to or are merely overhearing conversations. Further evidence for the idea of a universal grammar comes from Creole languages of children whose parents themselves only speak Pidgin dialects: despite the lack of a full range of a verbal experience these people develop natural languages (Bickerton, 1981).

Even though the study of language acquisition is intriguing, it is at present very difficult, if not impossible, to investigate its underlying neurophysiology. On the one hand, current methods of analysing brain function lack the necessary resolution to dissect the neuronal processes underpinning the organisation and development of human language. More importantly, however, such research is largely limited to case studies as controlled experiments on this in human subjects would be highly unethical, while animal models are unsuitable as even those species most closely related to us do not possess language as complex and sophisticated as ours.

The visual system provides a much better model for investigating the experience-dependent reshaping of cognitive functions and their underlying neural mechanisms. It is evolutionarily much older than human language and preserved throughout many mammalian species. However, just like language acquisition during early life many properties of visual perception display experience-dependent plasticity within a critical period after birth, and the synaptic and molecular mechanisms on which this plasticity depends are relatively well-understood.

1

Plasticity of Human Vision

As the following section will describe in greater detail, the visual system of higher mammals, in particular primates and carnivores, exhibits a great degree of functional sophistication with specialised cell types encoding the great diversity of visual information. It is not difficult to imagine that such a system requires extensive fine-tuning in order to function optimally. Also, considering the large proportion of neuronal tissue that is allotted to the visual system, in the absence of any visual input it would be sensible, if the brain adapted to this unusual situation by reorganising itself to use its resources economically.

For instance, it is easily conceivable that there is plasticity for the many complex aspects of visual processing. The face recognition of children surrounded predominantly by members of the same race may be more sensitive to facial features common to that ethnic background and less to those of different races. There are adaptation effects for face stimuli based on gender, ethnicity and even facial expressions (Webster *et al.*, 2004), similar to those seen in basic stimulus attributes like orientation or colour. Comparable fine-tuning of the system towards faces typical in the subject's environment may very well be taking place during childhood. In the same way, the understanding of complex visual objects, based on perspective and other cues, probably depends on experiences of these relationships in early life. In fact, the phenomenon of ambiguous figures, which fluctuate between different perceptual interpretations, is only observed in children above a certain age (Rock, Gopnik, and Hall, 1994). There appears to be a crucial phase in development when these systems are established by instructive environmental input.

A great body of anecdotal evidence suggests that congenitally blind people have superior hearing and tactile perception than sighted subjects. Empiric studies have confirmed such changes of perceptual ability in the blind (e.g. Gougoux *et al.*, 2004;

Doucet *et al.*, 2005). Furthermore, with modern brain imaging techniques it has been possible to show that brain areas traditionally considered to be involved in visual processing are active in blind subjects when they engage in tactile or auditory tasks, Braille reading, or semantic processing, but such patterns of brain activity are not measured in normally sighted subjects (Sadato *et al.*, 1996; Amedi *et al.*, 2003; Pietrini *et al.*, 2004; Röder *et al.*, 2002). The extent of this reorganisation depends on the age of onset of blindness, lending support to the notion of a period of very high plasticity early in postnatal life and a decline of this adaptability as the organism matures (Burton, 2003; Burton *et al.*, 2002a; Burton *et al.*, 2002b).

William Molyneux, a friend of the English philosopher John Locke, raised the question whether a man born blind would even be able to visually distinguish simple objects like a cube and a sphere, if his sight was suddenly restored in adulthood (Locke, 1694). Only fairly recently medical advances permitted this to be tested effectively. In the most recent example, Fine and colleagues studied a man, Mike May (MM), who became blind[1] in both eyes through accident at the young age of three (Fine *et al.*, 2003). While his left eye was irreversibly destroyed, the right eye suffered extensive corneal damage but was otherwise unscathed. This allowed vision to be restored in this eye 40 years later, and subsequent psychophysical and physiological tests could assess his visual abilities.

Even though the optical properties of the eye and its retina were intact after surgery, MM's vision through it remained very poor, although his contrast sensitivity function was near to normal at very low spatial frequencies. The highest spatial frequency he could resolve was 1.3 $cycles/^\circ$ as opposed to at least 30-40 $cycles/^\circ$ in normal adults. Evidently, the restoration of a clear image on his retina did not suffice to restore a normal visual percept.

However, not all ability to distinguish visual stimuli was lost and there was gradual improvement in the time following surgery. His colour vision was normal as had been observed in many similar patients previously (von Senden, 1932). Further, MM was able to discriminate simple geometric shapes, analyse textures of a visual scene, albeit with somewhat lower accuracy than controls, and could perceive motion using it to conceptually

[1]Note that the ophthalmologic definition of blindness is complete insensitivity to light, whereas the colloquial (and legal) meaning, and the one most often used in scientific studies, is the absence of *useful* pattern vision. True blindness is usually the result of retinal or neural defects, which are far more difficult to treat and may cause different changes to visual brain areas than are observed in patients like MM, whose retinas and ocular media are intact and who thus retained some residual light sensitivity (Gregory and Wallace, 1963).

distinguish objects in stimuli like motion glass patterns.

Some monocular depth cues, like transparency and perspective, posed great difficulty for him, and he could not interpret a line drawing of a cube as a three-dimensional object. In the case of the Shepard Table illusion (Shepard, 1990), in which the perspective cues lead normal observers to misjudge the surface area of two tables, his lack of a perspective concept even permitted him to perform with high accuracy. Finally, his ability to distinguish faces and facial expressions was also impaired.

In summary, while the patient showed a severe loss of visual acuity, he was capable of some very basic form discrimination, but lacked the conceptual understanding of complex or more abstract images. His abilities were certainly well below those normal for children of the age at which he lost his eye sight. Therefore, a reduction of visual function had taken place during the subsequent decades, indicating drastic changes in the part of his central nervous system involved in visual processing.

Due to the fact that MM had had normal visual experience for the first three years of his life, however, some visual function was established during his childhood and appears to have been preserved throughout the long time when he was not able to see. While the visual cortex of a three year old is still highly susceptible to alterations of the visual input, many low-level functions may have developed by that time and are thus stable even in the subsequent absence of any visual experience.

Patients recovering after the removal of congenital (or early onset) ocular defects frequently show much more severe impairments of visual acuities than were described for MM. In many cases the ability to distinguish simple geometrical figures, such as triangles, squares, cubes, and spheres, appears to be severely disrupted. Motion sensitivity of patients with bilateral cataracts after restorative eye surgery is very poor (Ellemberg *et al.*, 2002), indicating that the development of receptors in the brain selective to motion depends on very early visual experience. There appears to be a critical period during which this development can be disrupted by the absence of eyesight, but this period concludes at a very young age, certainly well before the age of one year (Lewis and Maurer, 2005). This may explain why MM's performance in motion tasks was adequate. The lack of the concept of monocular depth cues in MM, which leads to his poor performance on a number of tasks, is mirrored by other patients (Gregory and Wallace, 1963) and lends support to the notion that the critical periods for more complex functions are longer than those for simple sensory abilities. For instance, colour vision appears to be well preserved in many individuals (von Senden, 1932; Carlson, Hyvärinen, and Raninen, 1986), although a case

with a possible deficiency in colour vision has been reported (Ackroyd, Humphrey, and Warrington, 1974) and often the true range of colours these patients can discriminate is unclear.

Intriguingly, there are a number of differences between cases beyond the patients' colour vision. While MM was capable of distinguishing simple shapes (Fine *et al.*, 2003), other patients who lost their vision at a similar age were not (Ackroyd, Humphrey, and Warrington, 1974; Carlson, Hyvärinen, and Raninen, 1986). Conversely, Gregory and Wallace's subject SB was stricken by his ocular condition at the age of l0 months and was forced to wear bandages on his eyes - nevertheless his post-operative visual capabilities were akin to those of MM (Gregory and Wallace, 1963). This may be due to a great many factors, including the general residual visual quality prior to surgery or the time course of the pathology. For example, MM's accident led to an abrupt loss of vision, but naturally occurring conditions, in particular cataracts, may worsen gradually allowing for different (more or less) visual information to reach the retinas than is appreciated by a mere reading of the medical records, which in many older cases are very sketchy. Finally, the degree to which visual brain regions have been remodelled to accommodate other sensory or cognitive functions may be significant, and this is likely to depend on the very subjective experiences of the individual patients.

While the deficiencies of visual processing after the prolonged absence of adequate sight from an early age are striking, an equally startling loss of function is observed when the ocular occlusion is unilateral. Patients suffering for instance from cataracts, corneal abrasions, or *ptosis* (drooping eyelid) in only one eye for a prolonged time during infancy exhibit very poor acuity in that eye even after the eye defect has been repaired. This condition of poor vision in an eye despite full optical correction and in the absence of any persisting pathology is called *amblyopia* or colloquially "lazy eye."

The incidence of amblyopia is around 2-3% (von Noorden, 2001). It does not only occur after monocular occlusion, but is also caused by *anisometropia*, that is when the refractive states of the two eyes are significantly different (typically defined as a spherical equivalent difference of at least 2 diopters). Likewise, it can be found in patients suffering from *strabismus* (squint), in which the visual axes of the two eyes are misaligned. The image from one eye is suppressed in favour of the other dominant one in order to prevent double vision. However, the characteristics of amblyopia caused by these three conditions, especially between that caused by strabismus and occlusion, differ in various respects and also not all of the underlying biological mechanisms are likely to be the same.

Crucially, amblyopia only develops after abnormal visual experience in early life. In adulthood, monocular occlusion or anisometropia do not cause a loss of visual acuity in the affected eye. This lends further support to the notion that there is a sensitive period in childhood during which visual functionality does or does not develop. In humans this period likely lasts from very early in postnatal life until around 8-10 years of age (Mitchell, 1989).

Of course, studies of unilateral visual deficiencies are not complicated by the lack of a conceptual understanding of many visual phenomena, which can be very problematic when studying the recovery from long-term visual deprivation. Patients enjoyed a complete range of visual experience, albeit only monocular ones, allowing researchers to use the good eye as a reference against which to compare the effect of monocular visual deprivation in the other eye.

Much like the changes to the visual brain after blindness or severe visual impairment, the emergence of amblyopia during these conditions reflects the reorganisation of visual brain areas as a reaction to abnormal sensory input. Visual acuity of the amblyopic eye appears to be correlated with an imbalance between the neuronal representations for each eye in the primary visual cortex (Goodyear, Nicolle, and Menon, 2002). When there is a functional discrepancy between the two eyes, available neuronal resources appear to be allocated to the good eye to compensate.

In fact, a functional role for this plasticity of eye dominance in normally sighted individuals has been proposed (Horton, 2006). It appears to be necessary for the establishment of a seamless neural representation of the visual field and its effect can be extremely topical. For instance, in each hemisphere the region of the visual cortex corresponding to the optic disc (the "blind spot") is monocularly dominated by the ipsilateral eye, whose retina covers this part of the visual field. Even more striking, angioscotomata are very fine gaps within the brain map of an eye's visual field representing the pattern of large retinal blood vessels, whose shadows block the visual input to the underlying photoreceptors, causing a miniature form of monocular deprivation. There are large individual differences in this retinal vascularisation and a genetic mechanism for mapping the visual field around them is therefore very unlikely. Weighting of the inputs arriving from the two eyes appears to shape the visual brain thus that unstimulated parts of the retina in one eye are essentially removed from the representation of the visual field.

This raises the question about the exact balance between normal binocular vision and abnormal monocular experience that can counteract visual acuity loss and the associated

neuronal changes. To date, most research on the underlying mechanisms of amblyopia employed animal models with strict, continuous regimens of deprivation, usually by eyelid suture. This method allows for testing the effects of a complete loss of patterned visual input to the eye and typically results in rapid and extensive changes, if used at the height of the critical period. However, such rigorous manipulations of visual input cannot tell us about the efficacy with which binocular and monocular experience drive the plasticity of the visual cortex in early life.

Aims of this thesis: While an efficient adaptation to environmental abnormalities and individual peculiarities is clearly of benefit to an organism, a rapid and complete loss of vision in one eye after transient periods of occlusion would be catastrophic. Recent findings in animal models of amblyopia suggest that rearing regimens pairing binocular and monocular periods on a daily basis allow for a good outcome for visual acuity through both eyes even when the duration of eye patching outlasts the binocular period (Mitchell *et al.*, 2003; 2006; Wensveen *et al.*, 2006). This is independent of whether animals are reared with or without visual experience prior to the patching regimen, suggesting that it can not simply be due to normal vision in early life (Mitchell *et al.*, 2003). These observations imply that the plasticity of the visual cortex may be biased towards the normal, most typical, form of experience. Certainly, such a bias would be sensible from a teleological point of view. In light of the wider nature-nurture debate, this would mean that perhaps even traits known to be influenced heavily by experience nonetheless depend on an innate component that can only be overridden by a consistently abnormal environment.

The aim of this thesis is therefore to establish the balance of normal and abnormal experience that permits a normal development, using the neuronal representations of the two eyes in the primary visual cortex of cats as a model for experience-dependent brain plasticity in early postnatal life. My findings should have implications for the effective prevention of amblyopia in children, and also point towards further avenues of research into the physiological processes underpinning this alteration of brain function. For a better understanding of this investigation, it is necessary to review the sophisticated functional organisation of the mammalian visual system as well as the current body of evidence about cortical plasticity from animal models.

2

The Visual System

Before one can set out to investigate how experience influences the development of visual capacities, the complex mechanisms underlying visual processing must first be understood. Fortunately, as vision may well be the most extensively studied special sense, there is a great body of knowledge about the intricate functional organisation of the visual system within the brain.

2.1 Early visual processing

Light enters the eye through the pupil and passes the lens, which generates an upside-down projection of the visual environment on the back of the eye. Here, in the retina, visual information is transformed into neuronal signals by photoreceptors, which are adjacent to the sclera and underneath a rich network of blood vessels and neurons that lies in the light's path.

The retina contains a number of different neuronal cell types and represents the first stage of visual processing. Retinal ganglion cells, whose axons project onto neurons deeper within the central nervous system, form the innermost layer of cells of the retina. Each of these cells receives input from a number of photoreceptors spanning a small region within visual space known as the cell's receptive field (RF). The size of these fields varies between different ganglion cells depending on their position, with large RFs in the periphery but small ones in the central area of the retina known as the fovea, which is important for resolving fine visual detail. These fields tend to be circular in shape and are arranged with a characteristic centre-surround antagonism meaning that stimulation of the central part of the RF counteracts the effect of stimulation of its outer ring (Fig.

2.1). There are ON-centre cells, which become excited when light falls on their centre but not in the surrounding annulus, and also OFF-centre cells, whose response is suppressed when the centre is illuminated. This allows cells to respond preferentially to contours (or contrast edges) in the visual stimulus, which is of primary importance for extracting a representation of a natural scene from the retinal image. The presence of distinct on- and off-coding cell populations is repeated in hierarchically higher stages of the visual system.

Besides their different receptive field profiles retinal ganglion cells vary in their anatomical and functional characteristics. Originally, a different nomenclature was proposed defined by each of these investigations; however, the classification of different cell types has since become clear. In macaque monkeys (and humans), about a tenth of ganglion cells are parasol or M-cells, which have large cell bodies and dendritic trees and thus also have large receptive fields. They exhibit fast response latencies and high contrast sensitivity, which allows them to respond to moving stimuli. On the other hand, the much smaller P-cells (or midget cells) take longer to respond and have lower contrast sensitivity, but they are sensitive to colour contrasts and fine spatial detail because of their smaller receptive field sizes. This cell type makes up the vast majority of neurons in the human retina underlining the importance of high spatial resolution and colour perception in the primate visual system. Finally, the response latency, contrast sensitivity and spatial resolution of bistratified or K-cells, which were only identified recently, lie between that of the P- and M-cells. They also appear to play a role in colour perception as they are always excited by input from photoreceptors for wavelengths in the blue spectrum and inhibited by photoreceptors tuned to red or green wavelengths (Dacey and Lee, 1994).

In cats, the two principle types of retinal ganglions cells are the Y or α cells on the one hand, which correspond to the M-cells of primates as they are fast-responding, low spatial frequency filters, and the X or β cells on the other hand, which are the analogue (but probably not homologue) of primate P-cells concerned with the processing of fine spatial detail. In addition, there is a rather diverse group of retinal ganglion cells that have been collectively termed W cells (Boycott and Wässle, 1974). Some of these probably correspond to the K-cells of primates (Hendry and Reid, 2000) as they appear to have receptive fields integrating colour information from blue-sensitive cone photoreceptors. The excitatory input to these neurons is opposed by the input from cones coding for green wavelengths and that from rod photoreceptors, which are relatively unselective for wavelength but respond mainly to intensity during scotopic conditions (Hammond, 1978).

The projections from retinal ganglion cells leave the eye through the optic disc (the

"blind spot") together with the blood vessels sustaining the eyeball and form the optic nerve. The optic nerves from the two eyes meet at the optic chiasm (Fig. 2.1) where those axons serving the nasal halves of the retina from each eye cross over to the other side, but those arriving from the temporal retinas remain on the same side. The exact proportion of fibres crossing over depends on the species and is related to the anatomical arrangement of their eyes and thus to the size of the binocular area in their visual field. In humans and many non-human primates, whose eyes are aligned frontally rendering most of their visual field binocular, approximately half of the nerve fibres cross over. In felines, on the other hand, around 60% of fibres cross over (the exact proportion depends on the type of retinal ganglion cell; Rodieck, 1979), whereas in rodents, whose eyes are aligned laterally, the vast majority of axons do (Grafstein, 1971; Dräger and Olsen, 1980). Common to all these species, this means that the representation of the left visual field is in the right cerebral hemisphere and that of the right visual field in the left.

Beyond the chiasm the fibres run their separate ways within the two optic tracts until they reach their target cells in the lateral geniculate nuclei (LGN) of the thalamus (in cats, this nucleus is the dorsolateral geniculate nucleus, dLGN). This part of the brain is organised in a layered fashion with six such domains stacked on top of each other within the human LGN. Each layer contains a retinotopic map (ie. adjacent regions correspond to neighbouring areas of the retina). The inputs from the two eyes remain separate with layers 2, 3, and 5 of the LGN receiving input from the ipsilateral eye and the others from the contralateral eye. Thus, in humans three of the six layers are allotted to each eye, whereas cats only have four layers in the LGN. Layers A and C receive contralateral input, while layers A1 and C1 received input from the ipsilateral eye. There is an over-representation of the contralateral input, which corresponds well to the amount of cross-over in the optic chiasm, and it is also present at the next stages of the visual system (Rodieck, 1979).

Moreover, there is functional segregation of the input from different types of retinal ganglion cells arriving in the LGN. In primates, retinal M-cells project onto magnocellular cells in the ventral layers 1 and 2, which are concerned with fast processing of motion at the cost of fine detail, while P-cell input arrives in the slow-latency parvocellular cells in layers 3-6 that process fine spatial detail and colour information (Schiller, Logothetis, and Charles, 1990). Finally, information from retinal K-cells is processed by the koniocellular neurons of the LGN, which are found between the layers of this nucleus. They play a role in the processing of colour and may also be involved in the integration of other modalities

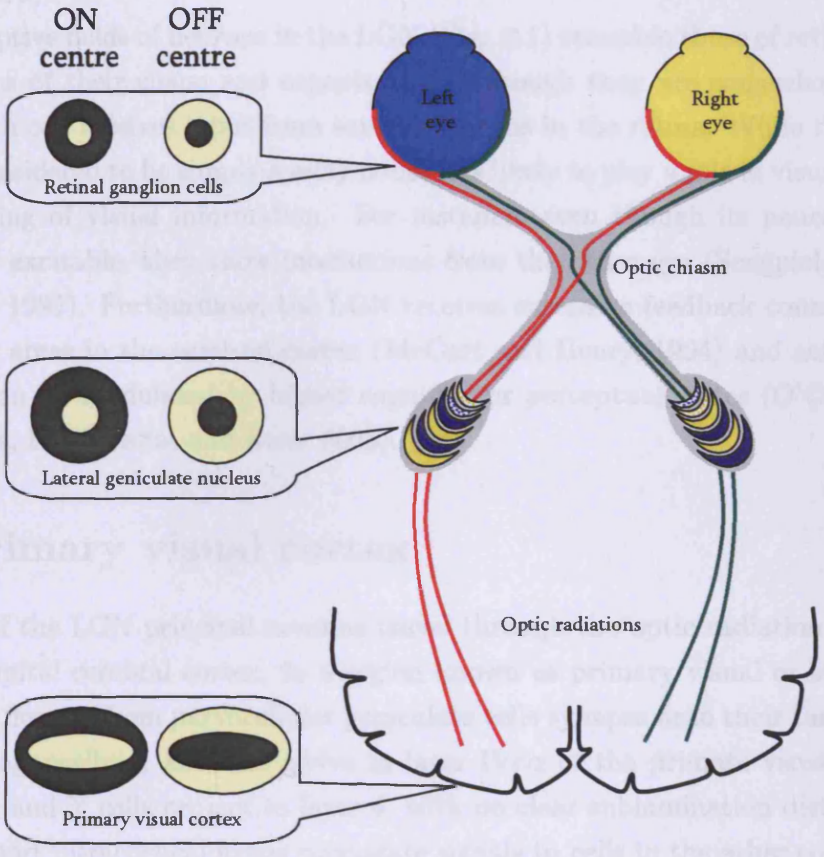


Figure 2.1: *Schematic of the optical path.* Axons from retinal ganglion cells project along the optic nerve out of the eye onto their target cells in the lateral geniculate nuclei. On the way there they pass the optic chiasm where the axons serving the nasal retinas cross over to the other hemisphere. Beyond the chiasm the axon bundles are called optic tract. Geniculate neurons in turn project along the optic radiation onto targets in the primary visual cortex. The insets on the left depict diagrammatic representations of the antagonistic surround receptive fields of neurons in each brain area. ON-centre neurons are shown in the left column, OFF-centre neurons on the right. Light presented to the yellow regions excites the cell; dark regions inhibit its response. For primary visual cortex only the RFs of simple cells are shown. (Inspired by Gray's *Anatomy of the Human Body*, 1918; not drawn to scale.)

with visual input (Hendry and Reid, 2000). In cats, both retinal X and Y cells project to the A and A1 laminae of the LGN, while Y and W cells project to the C and C1 laminae (Rodieck, 1979).

The receptive fields of neurons in the LGN (Fig. 2.1) resemble those of retinal ganglion cells in terms of their shape and organisation, although they are somewhat larger and typically each cell receives input from several neurons in the retina. While the LGN had long been considered to be simply a relay node, it is likely to play a role in visual processing and the gating of visual information. For instance, even though its neurons are only monocularly excitable, they show interactions from the other eye (Sengpiel, Blakemore, and Harrad, 1995). Furthermore, the LGN receives extensive feedback connections from higher brain areas in the cerebral cortex (McCart and Henry, 1994) and activity in this brain area can be modulated by higher cognitive or perceptual states (O'Connor *et al.*, 2002; Haynes, Deichmann, and Rees, 2005).

2.2 Primary visual cortex

The axons of the LGN principal neurons travel through the optic radiation and project into the occipital cerebral cortex, to a region known as primary visual or striate cortex (area V1). Afferents from parvocellular geniculate cells synapse onto their targets in layer IVcβ and magnocellular afferents arrive in layer IVcα of the primate visual cortex. In cats, both X and Y cells project to layer 4, with no clear sublamination distinguishable. From here short intracortical axons propagate signals to cells in the other cortical layers.

Just like the LGN this brain area is organised in a retinotopic manner. It is the first stage in visual processing at which the input from the two eyes converges onto the same cells. In many higher mammals with relatively large binocular portions of the visual field, the geniculocortical afferents from the two eyes are segregated as they synapse onto their target cells in layer IV. Subsequent projections from these neurons, however, project onto cells in higher and lower layers, which receive input from both eyes and can be excited by left and right eye stimulation.

2.2.1 Ocular dominance

The efficacy of each eye in driving the response is known as the neuron's ocular dominance (OD). As David Hubel and Torsten Wiesel showed in their classic experiments in the early

1960s (Hubel and Wiesel, 1962), in the cortex of normal subjects the majority of cells is approximately equally excitable by the two eyes, while a smaller proportion of cells is dominated primarily by one or the other eye, and a relatively small number is monocular so that only one eye is able to elicit a response from them. By assigning each neuron to a category defined by the degree to which each eye can drive its response, ocular dominance histograms can be generated showing the degree of binocularity of the cortex as well as the number of cells receiving input from the left and right eyes (Fig. 2.2A).

Since then a great deal has been revealed about the functional organisation of V1. It is now known that in many mammals, in particular felines, ferrets, humans and Old World monkeys, neurons with similar ocular dominance are clustered into domains, so that there are patches of cortex responding more strongly to the left and right eye respectively (Fig. 2.2B-C). This is due to the spatial segregation of geniculocortical afferents in layer IV and because the cells in the other cortical layers at any particular location have a similar ocular dominance as those receiving the thalamic input. Thus ocular dominance is arranged in columns extending from the top to the bottom layers of the cortex (Fig. 2.2D). Presently, the question through which mechanism ocular dominance columns are formed remains unresolved. They develop regardless of visual experience prior to the time window when experience can influence cortical architecture (Crair, Gillespie, and Stryker, 1998; Crair *et al.*, 2001; Crowley and Katz, 1999; Crowley and Katz, 2000). So far no molecular cues guiding the segregation of contralateral and ipsilateral geniculocortical afferents have been identified. Possibly, OD columns are generated by intrinsic processes in cortical circuits, although a recent report proposed that blocking spontaneous retinal activity can disrupt their formation (Huberman, Speer, and Chapman, 2006). This is in contrast to the work of Crowley and Katz (1999; 2000) showing that early removal of the eyes does not disrupt ocular dominance segregation.

In Old World monkeys and humans, ocular dominance domains display a very regular arrangement perpendicular to the border between primary visual cortex and the adjacent visual area V2. In cats, on the other hand, the ocular dominance domains have a more patchy appearance, whereas in ferrets an even more irregular arrangement can be seen, with large rostral ocular dominance bands near the representation of the vertical meridian and smaller patchy OD columns in the remaining binocular part of V1 (White *et al.*, 1999). Because of the higher degree of organisation, for a long time it was assumed that there is a functional significance of ocular dominance architecture for sophisticated visual functions such as stereopsis. However, it was since found that many New World monkeys do not

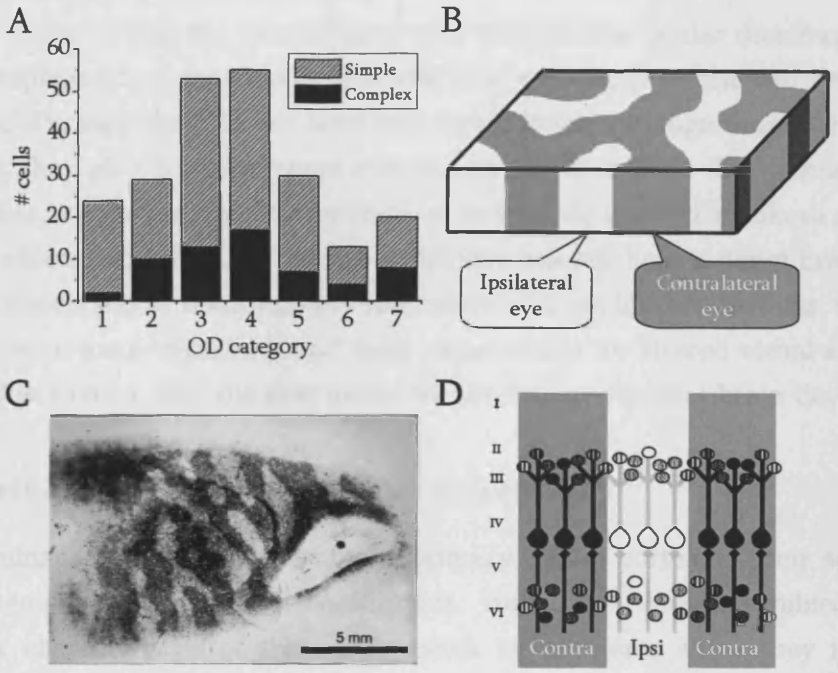


Figure 2.2: *Ocular dominance columns.* A proportion of the neurons in the primary visual cortex of many mammals can respond to stimulation of either eye to a varying degree. The efficacy with which the left or right eye can drive a cell's response is called ocular dominance. (**A**) Seven bin ocular dominance distribution histogram adapted from Hubel and Wiesel (1962), which shows monocular cells driven by the contralateral eye in category 1, monocular ipsilateral eye-driven cells in category 7, and cells with varying degrees of ocular dominance in the categories in between. Both simple and complex cells are shown. (**B**) Architecture of ocular dominance domains in primary visual cortex. Neurons with similar eye preference are clustered together in columns spanning all the layers of cortex and thus respond more strongly to stimulation of one eye compared to the fellow eye. (**C**) Ocular dominance montage of layer IVc sections of human primary visual cortex obtained post-mortem by staining for cytochrome oxidase activity. (From Horton and Hedley, 1984.) (**D**) Simplistic schematic of the circuitry in ocular dominance columns. In cats, cells of layer IV receiving geniculocortical afferents tend to be monocular, but subsequent projection targets and interneurons in upper and lower layers can often be excited by the other eye as well. In primate V1, fewer neurons are binocular.

have ocular dominance columns despite having exquisite stereoscopic vision. There are even species like the squirrel monkey, in which only some animals have OD columns but others do not (Adams and Horton, 2003).

It is now believed that the clustering of cells with similar ocular dominance is due to the spatial segregation of geniculocortical afferents arriving from the two eyes in layer 4 of the cortex. Perhaps they do not have any direct functional significance, but it is also possible that they play a role in some species but not in others. Furthermore, while it is unlikely that the presence of OD columns in individual squirrel monkeys correlates to stereoscopic vision (Horton, 2006), to this date there has not been a direct investigation of this issue. Independent of their putative role, as we will see in later sections, the presence of OD columns in many mammals and their vulnerability to altered visual experience in early life makes them a very suitable model for studying postnatal brain development.

2.2.2 Orientation and direction selectivity

Another dominant property of neurons in primary visual cortex is their selectivity to spatial and temporal attributes of the stimulus. Hubel and Wiesel stumbled across one of the major characteristics of these cells much by accident, when they noticed that a neuronal discharge coincided with the insertion of the projector slides they used for stimulation: unlike in the retina and LGN, the receptive fields of V1 neurons are elongated and thus respond best to lines or bars of light (Fig. 2.1). Different cells are tuned to lines of different orientations, together coding for the entire 180° range of orientation. Stimuli of non-optimal orientation evoke a lesser response from these cells and orientations perpendicular to the preferred one (the *null*-orientation) fail to produce a response as they do not line up with the excitatory region in the cell's receptive field. By plotting the response of a neuron elicited by a range of orientations (in Cartesian or polar coordinates), the sharpness of a cell's orientation tuning can be determined (Fig. 2.3A-B).

Similar to OD domains, in higher mammals with sophisticated visual systems orientation selectivity is clustered into cortical columns, with neighbouring columns preferring similar orientations. This leads to a mosaic of varying orientation columns across the cortex, whereby a whole set of orientation columns are arranged in pinwheels, in the centres of which one finds neurons selective to a wide range of stimulus orientation (Maldonado *et al.*, 1997; Ohki *et al.*, 2006). As Fig. 2.3C shows, these pinwheel centres tend to fall within the middle of ocular dominance domains, and the boundaries of orientation

columns tend to intersect the borders of ocular dominance columns at approximately right angles (Hübener *et al.*, 1997; Crair *et al.*, 1997a; Yacoub, Ugurbil, and Harel, 2006).

Hubel and Wiesel (1962) distinguished different types of V1 neurons based on their receptive field properties. *Simple cells* possess rectangular elongated RFs with clearly defined opponent on- and off-regions, which allow them to code for contours of particular orientations. In addition, there are also *complex cells* from which a response can be invoked when a bar stimulus falls anywhere in their receptive field, because on- and off-regions are interspersed or superimposed. Due to this, simple and complex cells can be easily distinguished even without mapping of the receptive field. Simple cells tend to show phasic bursts of firing in response to a grating, which is modulated by the spatial and temporal frequency of the stimulus, because it responds to each contour of the grating passing through its receptive field. Complex cells, on the other hand, exhibit a tonic, continuous response to a grating.

While the vast majority of neurons in primary visual cortex are orientation selective (apart from layer IVc cells in monkeys, which are non-oriented), about a third of them also display direction selectivity. Thus, while a neuron may respond well to a vertical bar moving rightwards, it will show only a weak or no response to the same stimulus moving to the left. An example of this is shown in Fig. 2.3B. Direction selectivity is also arranged in a columnar fashion and the mapping of orientation and direction domains is closely related (Shmuel and Grinvald, 1996), because the direction of motion of a line in an aperture like the receptive field is inherently linked to its orientation.

Furthermore, a small proportion of cells even distinguish between the motion of the stimulus itself and the motion caused by eye movement across the visual scene (Galletti *et al.*, 1984). This suggests that the striate cortex receives input from brain areas not involved with processing visual sensory information.

2.2.3 Spatial frequency, context, and colour

Neurons of the primary visual cortex are further tuned to many other attributes of the stimulus. Instead of using simple bar or line stimuli, modern vision science tends to employ grating stimuli, which consist of repeating parallel stripes. The spatial frequency of these gratings, that is the number of repeats within a degree of visual angle, is also encoded by neurons in visual cortex and is associated with the ability of the system to resolve spatial detail. Cells are tuned to spatial frequency in much the same way as to orientation or

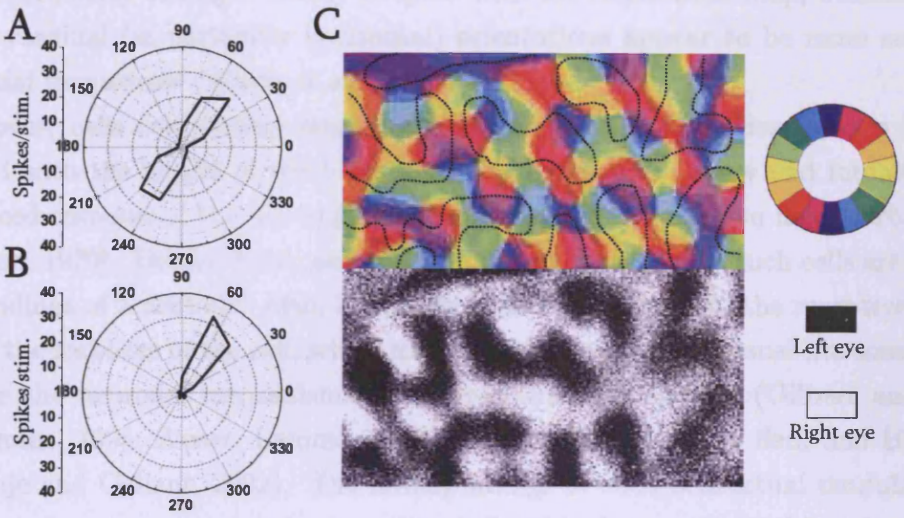


Figure 2.3: *Orientation selectivity.* Many V1 neurons exhibit selectivity to stimulus orientation. While the preferred orientation evokes the strongest discharges, the further the stimulus is tilted with respect to this orientation, the smaller the response. (**A**) Polar orientation-response plot of a typical neuron that responds best to gratings rotated approx. 45° clockwise from vertical. (**B**) A direction-selective neuron that prefers gratings rotated approx. 45° clockwise sweeping in one direction. (**C**) Orientation preference map, which arises from the clustering of cells with similar preferred orientation into columnar domains, and below, the ocular dominance map for the same cortical area. Different orientation domains are colour-coded (cf. colour key). Boundaries of ocular dominance domains are indicated by the dashed black lines. Orientation patches and ocular dominance domains intersect at approximately right angles. Pinwheel centres of the orientation map tend to fall near the centres of ocular dominance domains. (Adapted from Hübener *et al.*, 1997.)

direction, such that there is a maximally efficacious spatial frequency and the response falls off gradually as this property is increased or decreased. There is also evidence that spatial frequency is organised in columns (Shoham *et al.*, 1997; Hübener *et al.*, 1997; Issa, Trepel, and Stryker, 2000) much like ocular dominance, orientation and direction. According to a recent report on ferret visual cortex, however, the columnar architecture of spatial frequency tuning is closely coupled with the orientation map, because neurons tuned to cardinal (in particular horizontal) orientations appear to be more sensitive to high spatial frequencies (White *et al.*, 2006).

Moreover, cells can display length summation, that is their response is positively correlated with the length of the bar stimuli. Other neurons show end inhibition, that is a reduced response if the bar extends past their classic receptive field (Orban, Kato, and Bishop, 1979b; Orban, Kato, and Bishop, 1979a). Essentially, such cells are detectors for the endings of a contour. Also, the presence of stimuli outside the receptive field can influence the response of the cell, which may explain a number of visual illusions and may constitute the neuronal mechanism for figure-ground segregation (Gilbert and Wiesel, 1990; Lamme, 1995; Zipser, Lamme, and Schiller, 1996; Sengpiel, Sen, and Blakemore, 1997; Vinje and Gallant, 2002). The underpinnings of such contextual modulations are probably an interneuron network connecting cells with those with neighbouring receptive fields (Das and Gilbert, 1999). One of the great challenges of vision research is to dissect the computational mechanisms by which these specialised receptors encode the complex, detailed scenes encountered by the visual system in everyday life without the need for an infinite number of different cell types (Gross, 2002; Revonsuo and Newman, 1999).

It has been suggested that the columnar arrangement of separate feature maps cannot explain the complexity of visual processing. While conventional theories state that the stimulus at a particular location in the visual field can be accurately described by the intersection of the various feature maps at this location, assuming that cortical coverage of all parameters is uniform (Swindale *et al.*, 2000), Basole and colleagues (Basole, White, and Fitzpatrick, 2003) proposed a different model by which striate cortex contains only one map of spatiotemporal energy. Orientation maps as well as single-cell orientation tuning curves are strongly affected by the direction of motion of a stimulus when textures consisting of individual bars, as opposed to the more commonly employed gratings, are used.

Another important aspect of vision for many higher mammals, in particular primates, is of course colour perception, which depends on the presence of cone photoreceptors in

the retina selective to specific wavelengths of light, and on separate processing of inputs from different cone types within the visual pathways. Old World primates mostly have trichromatic vision as they possess three types of retinal cones, whereas many other mammals only have dichromatic vision with lower density of retinal cones and less integration of colour opponency by ganglion cells, suggesting poorer colour sensitivity. For instance, cats were for a long time believed to lack colour vision. It was since found that they only seem capable of some colour discrimination (eg. blue vs. green and blue vs. grey) when the stimuli subtend a large visual angle (Loop, Bruce, and Petuchowski, 1979), which implies a lower spatial resolution of their colour filters. Due to the prevalence of rod photoreceptors in the cat retina their colour perception is likely also much less saturated than in primates.

Small patchy anomalies are scattered throughout the cortical map containing neurons with poor orientation preference but which in primates are selective to colour. These anomalies are known as blobs due to their typical appearance in cytochrome oxidase staining. Individual blobs contain cells with similar colour opponency, with red-green opponency being more common than blue-yellow opponency, again underlining the separate processing streams of these two systems in the early visual system (Ts'o and Gilbert, 1988).

Cytochrome oxidase blobs are also present in cat V1 (Murphy, Jones, and Van Sluyters, 1995), which contain neurons coding for low spatial and high temporal frequencies (Shoham *et al.*, 1997). A role for them in colour processing has not been demonstrated.

2.2.4 Retinal disparity and binocular interactions

Finally, an important property of many cells in primary visual cortex is related to their binocularity. The conventional view defines a binocular cell as being equally excitable by stimulation of either eye. Many neurons, even completely monocular ones on standard monocular tests, display interactions when the other eye is stimulated alongside the dominant one (Blakemore, 1970). For instance, neurons can be tuned to the retinal disparity, ie. the separation between the location of a stimulus on the left and right retinas, between the images arriving from the two eyes (Ohzawa and Freeman, 1986b). This is thought to be a fundamental prerequisite for stereoscopic vision. When fixating on a plane within visual space, stimuli that are nearer or farther to the observer will appear disparate to the two eyes. The brain is capable of lining up the contours seen by the two eyes and can

thus encode depth information about an object in space.

Meticulous electrophysiological recordings by Freeman and his colleagues showed it is predominantly simple cells that show response modulation by the spatial phase disparity (Ohzawa and Freeman, 1986a). It appears that the spatial receptive field profiles (in other words the phase of the excitatory and inhibitory regions of the RF) are often different for the two eyes, which explains how phase-selectivity is achieved (DeAngelis, Ohzawa, and Freeman, 1991; Anzai, Ohzawa, and Freeman, 1999).

A recent report proposed that disparity tuning in V1 is organised in a columnar or clustered structure (Kara, 2006) similar to the aforementioned stimulus characteristics, whereas earlier electrophysiological investigations failed to reveal such an organisation and at best reported that there is a correlation between a cell's ocular dominance category and whether it is disparity-selective at all or not (LeVay and Voigt, 1988; DeAngelis *et al.*, 1999; Freeman, 2003). It is certainly possible that the principle of cortical clusters, which contain neurones sharing similar functions, is present throughout the brain and holds true for any stimulus attribute for which there are receptors. However, the brains of rodents do not display orientation maps (Hübener, 2003), although their neurons are tuned to stimulus orientation suggesting that columnar architecture is not necessary to achieve selectivity for certain response properties.

Beyond coding for binocular disparity there are other forms of interaction between the inputs arriving from the two eyes. Presenting vastly different stimuli to the two eyes also drastically suppresses the firing rates of many V1 neurons, while a similar stimulus can cause an enhancement of the neuronal response (Sengpiel, Blakemore, and Harrad, 1995). For example, when the dominant eye views the cell's preferred orientation simultaneous presentation of most orientations to the other eye results in suppression. However, in many cells showing an orientation near the preferred one in the second eye leads to a facilitation of the cell's firing. This may underpin the phenomena of binocular rivalry and binocular fusion. While in the former a subject's percept fluctuates between what is seen by the two eyes when each one views different stimuli, binocular fusion occurs when both eyes view similar stimuli resulting in a merged percept.

The circuitry underlying such interactions may involve short horizontal connections via interneurons between cells in left and right eye ocular dominance columns. It may prove interesting to test whether there is a correlation between the presence of segregated ocular dominance architecture and the nature of binocular interactions. For instance, during binocular rivalry an intermediate perceptual stage (piecemeal rivalry) can occur

during which the visual field appears fragmented into the different stimuli seen by the two eyes[1]. The pattern of this fragmentation in a particular retinotopic location might depend on the degree of ocular dominance segmentation in its cortical vicinity. Recent advances in human neuroimaging, which allow the mapping of cortical columns (Goodyear and Menon, 2001; Yacoub, Ugurbil, and Harel, 2006), may help address this issue in the near future. However, regardless of whether they have any relationship with the OD architecture, perceptual phenomena such as these show that a neuron may be functionally connected to both eyes even when on simple analysis it appears to be completely monocular.

2.3 Extrastriate visual cortex

The neurons of the primary visual cortex project on to the higher brain areas of the visual system. Taken together these areas are frequently referred to as *extrastriate visual cortex*. This nomenclature is in reference to *striate cortex*, a relatively old-fashioned name for V1, which stems from the typical myelin stripes, the Stria of Gennari, found in that area.

The next stage after V1 is area V2, which lies directly adjacent and anterior to it. V2 also contains a retinotopic map, which shares the vertical meridian with that in V1. Its cells also code for orientation, spatial frequency, colour and disparity. However, many V2 neurons additionally display responses to illusory contours evoked by surrounding objects (von der Heydt, Peterhans, and Baumgartner, 1984), evidence of the higher level of processing taking place in this area. The receptive fields of its cells are larger than those of V1, and thus their spatial frequency tuning peaks at lower (wider) frequencies. Further, unlike primary visual cortex, in humans and primates V2 is not segregated into ocular dominance domains, which allows for a delineation of the area boundaries (in cats, whose area 18/V2 receives direct thalamocortical afferents, ocular dominance patches are found in V2 also; Stone, 1983).

Primate V2 displays another notable functional architecture. There are stripes running perpendicular to the V1-V2 border, which are defined by their appearance after staining with cytochrome oxidase. The thick stripes receive projections from the magnocellular

[1]Recent neurophysiological, psychophysical and neuroimaging data indicate that binocular rivalry can be dramatically influenced by inputs from higher stages in visual processing. Piecemeal rivalry does rarely occur when two rivalling images of coherent, complex objects are viewed by the two eyes, suggesting that parts of the brain associated with object recognition override rivalry of local stimulus attributes. However, when only one eye sees a complex object, but the other views a simple grating, piecemeal rivalry is observed (Alais and Melcher, 2007).

pathway, while the pale stripes receive the input of the parvocellular pathway and the koniocellular projections arrive in the thin stripes. Because of this, neurons in the thick stripes are primarily tuned to direction of motion and retinal disparity. Further, there have been observations of a regular stripy arrangement of near- zero-, and far-disparity domains (Ts'o, Roe, and Gilbert, 2001). Thin stripes contain colour-selective neurons, whereas the pale stripes consist of orientation-selective cells that are involved with the analysis of contours and form. As we can see, the different stripes in V2 thus continue the functional segregation present in earlier stages of the visual system.

This segregation continues onwards into higher visual areas beyond V2. There are two parallel streams of visual processing consisting of separate, albeit highly interconnected, brain areas. The *dorsal stream* runs on through V3 and V5 (mediotemporal area, MT), areas organised in a retinotopic fashion whose neurons code for the spatial location of a stimulus. Cells in V5 are further tuned to the direction of motion of a stimulus and, due to their larger receptive fields combining the input from numerous direction selective neurons in V1, process global motion as opposed to the local processing in lower areas.

Area V5 also exhibits neurons tuned to the retinal disparity of stimuli and is thus heavily involved in stereopsis. Both direction and disparity tuning appear to be organised into cortical columns underlining the notion that columnar architecture is a functional property common to many parts of the brain (Albright, Desimone, and Gross, 1984; DeAngelis and Newsome, 1999).

On the other hand, the areas of the *ventral stream* are concerned less with the spatial and temporal properties of a stimulus but rather with its identity and form. After V2 the subsequent stages in this pathway are area V4 and inferotemporal cortex (IT), the latter showing remarkable selectivity to complex objects and scenes. Neurons in V4 are selective to various shapes and orientations as well as the colour of stimuli, even though the complexity of such stimuli is not as large as that observed in studying IT neurons. Importantly, there has been evidence that also in these visual areas cells with similar response characteristics are grouped into cortical columns (Zeki, 1973; Wang, Tanaka, and Tanifuji, 1996; Wang, Tanifuji, and Tanaka, 1998; Tsunoda *et al.*, 2001). The rules governing the exact arrangement of these feature maps are presently unknown, because any systematic relationship between their different stimulus properties (like orientation or direction in V1) remains elusive.

One of the most remarkable properties of the brain is its adaptability and flexibility. It is likely that at all stages of visual processing there is potential for experience-dependent

changes, not only during infancy but throughout life. Some of the more complex processes may require instructive visual input in order to function adequately as is suggested by the visual deficiencies of many sight-recovery patients I reviewed earlier. However, the developmental plasticity observed in the primary visual cortex remains the best studied form of visual cortical plasticity, in particular in the light of research on the mechanisms underlying amblyopia, because of the ease with which its functional organisation can be studied, and because the comparably simple, low-level information that it processes can be manipulated in a systematic fashion.

3

Experience-dependent plasticity

As the previous paragraph already implied, experience-dependent plasticity is observed in the brain and the behaviour of an organism throughout its lifespan: it constitutes memory. Irrespective of the precise nature of these changes and whether they are readily visible in neuronal populations, it is of course obvious that learning occurs on a permanent basis and the establishment of new experiences must therefore be represented in the central nervous system in some way. Such memory traces remain elusive; however, a likely candidate mechanism has been identified.

Synaptic plasticity, that is the modulation of the efficacy of a synapse between two neurons, satisfies most of the characteristics required for a putative memory trace and of environment-driven changes to the brain (Martin, Grimwood, and Morris, 2000). The efficacy of many synapses in the central nervous system can be increased as well as decreased depending on the input it receives as well as on the post-synaptic activity. This way the experiences of an organism can tune the system to its environment. Many lines of research have investigated these mechanisms, which may play a role not only in long-term learning but even be involved in the short-term adaptation effects to prolonged stimulation found in the visual system (Yao and Dan, 2001).

Crucially, postnatal plasticity allows the brain to adapt to its environment, such as the acquisition of our native language, the extensive reorganisation of the visual system during early blindness, or the development of amblyopia; many of these processes share mechanisms that also underpin learning and memory. While the long-term changes associated with postnatal visual cortical plasticity are more structural and considerable changes in the connectivity are observed (Horton and Hocking, 1997), at least as far as the immediate reactions to altered experience are concerned, the regulation of synaptic

weights seems to play a major role. In this view, the critical periods for various brain functions are merely a phase in early life during which neurons are plastic well above the lifespan average, and wide-spread remodelling of neuronal circuits takes place.

3.1 Clinical studies

Modern technology can give insight into the neuronal events associated with experience-dependent plasticity. Brain imaging using functional magnetic resonance imaging (fMRI), for example, can visualise the activity in cortical areas making it possible to compare the visual cortices of sighted and blind subjects performing various tasks as described above.

For example, as already discussed earlier, it was shown that the visual cortices of congenitally blind subjects are active during non-visual tasks, while the functional connections from subcortical visual brain regions are atrophied (Amedi *et al.*, 2003; Pietrini *et al.*, 2004; Röder *et al.*, 2002). This brain activity is likely through corticocortical connections between visual and non-visual brain areas, which may have become upregulated in blind subjects. There have been proposals that such connections are however also present in normally sighted adults and can be "unmasked" by visual deprivation and training with a non-visual modality (Bass-Pitskel *et al.*, 2006). The tactile training in the blind may very well cause considerable new connections and structural changes, but the less pronounced changes in normal control subjects show that plasticity persists throughout life.

Long-term blind patients, whose eyesight was restored in adulthood, do not regain good visual quality and often fall into depressions when the disappointment of this fact hits (Gregory, 2003). Individuals like MM (Fine *et al.*, 2003), who shows remarkable improvements in acuity and visual function and who enjoys the help of a loving partner during his ongoing recovery period, are notable and encouraging exceptions. The case of an Indian woman, whose early onset cataract was removed at the age of 12 and who enjoyed 20 subsequent years of sight-recovery resulting in close to normal vision (Ostrovsky, Andalman, and Sinha, 2006), suggests that even if treatment is started relatively late in childhood substantial recovery of visual function can be achieved. A greater understanding of the differences between plasticity in the critical period for visual development and the plasticity observed in adults is necessary in order to improve the treatment of such conditions in the future.

It remains very questionable, whether it will ever be possible to completely restore

normal vision in the congenitally blind, especially as long as the impact of such treatment on their entire quality of life due to the reorganised brain systems is unknown. On the other hand, the outlook is far more hopeful for the plasticity associated with amblyopia, in which the neuronal representation of one eye (and thus vision through it) is diminished, while the visual system per se shows a nonetheless relatively normal organisation.

However, while recent technical and methodological improvements have allowed the imaging of the ocular dominance pattern in living human subjects (Goodyear and Menon, 2001; Goodyear, Nicolle, and Menon, 2002), current methods are not suited for characterising the intricate function of cortical networks. Also, comparisons of visual function with brain organisation are difficult, because conditions like amblyopia are only studied alongside the ophthalmologic treatment, and the medical history of each afflicted individual is not known with the same degree of reliability as is typical of an animal experiment. Further, such studies would be very costly and unlikely to be of much benefit to the individual.

Animal models of these disorders have therefore proven to be invaluable for understanding the cortical effects of various forms of visual deprivation or abnormal experience. They have opened up a wealth of knowledge about the cellular, synaptic, and molecular processes involved in brain plasticity. This research led to the refinement of medical treatment (Mitchell and MacKinnon, 2002) and also has great potential for the eventual development of interventions to prevent amblyopia in the first place.

3.2 Animal models

The pioneers of single-cell electrophysiological investigations of visual cortex, Wiesel and Hubel (1963; 1965), were the first to quantify the neuronal changes caused by visual deprivation. Testing the effects of bilateral and unilateral eye occlusion on the properties of individual V1 neurons, they discovered that with time binocular deprivation (BD) by eyelid suture causes a reduction of visually responsive cells and abnormal receptive fields in many of the remaining units. In contrast, monocular deprivation (MD) leads to much more drastic and faster functional changes causing the ocular dominance of V1 neurons to be shifted considerably or even totally towards representation of the nondeprived eye (NE). An example of this can be seen in Fig. 3.1A. The overwhelming majority of cortical cells is driven exclusively by the normal eye, a mere fraction of binocular cells remains, and only very few neurons can still be excited by stimulation of the deprived eye (DE). In

contrast, binocular deprivation leaves a fairly typical ocular dominance distribution, albeit in a reduced overall number of responsive cells (Fig. 3.1B). It is important to note that dark-rearing is not equivalent to binocular deprivation through diffusing lenses or eyelid suture. These methods of deprivation eliminate patterned visual input to both eyes, but partial light sensitivity is usually retained. Dark-rearing on the other hand is complete visual deprivation. As was shown in experiments conducting monocular deprivation on kittens with prior dark-rearing (Mower, Christen, and Caplan, 1983), the critical period appears to be prolonged in these animals as cortical neurons remain susceptible to MD at an age when little plasticity would be observed in normally reared animals, which suggests that the cortex is retained in an immature state. Visual input seems to cause changes in the cortex triggering the onset and the eventual closure of the plastic period. Conversely, at least in rats ten days of dark-rearing in adulthood appears to reset the cortex into a more immature state and restores juvenile levels of ocular dominance plasticity (He, Hodos, and Quinlan, 2006).

Strabismus, that is misalignment of the axes of the two eyes such that the two retinal images are discordant, also causes a severe change in the response characteristics of V1 neurons. After a prolonged period of strabismic binocular experience, the majority of binocular cells is lost and interactions between the inputs from the two eyes become predominantly suppressive. Thus only two large monocular populations driven exclusively by one or the other eye remain (Hubel and Wiesel, 1965; Van Sluyters and Levitt, 1980; Crawford *et al.*, 1996).

The shift in the ocular dominance distribution after monocular exposure is associated with a dramatic reorganisation of the functional architecture of the cortex. Early studies injected a tracer agent like radioactive proline or 2-deoxyglucose into one eye of animals in order to map topographic segregation of geniculocortical afferents in layer IV of primary visual cortex into ocular dominance bands. In the brains of amblyopic subjects, the bands corresponding to the closed eye appear markedly shrunken, whereas the area representing the good eye is enlarged (Hubel, Wiesel, and LeVay, 1977; Shatz and Stryker, 1978; Horton and Hocking, 1997).

Improvements in the scientific methodology have since allowed functional mapping of the primary visual cortex *in vivo*. Thus modern imaging studies confirmed the changes to cortical ocular dominance observed by electrophysiological recordings and postmortem anatomical experiments. Not only are there fewer afferents from the deprived eye arriving in V1 (Antonini and Stryker, 1993; Antonini and Stryker, 1996), but the responsiveness of

neurons in other layers above and below the recipient layer IV is also biased towards the open eye (Kim and Bonhoeffer, 1994; Gödecke and Bonhoeffer, 1996; Crair *et al.*, 1997b).

In a similar vein, strabismus often causes a more sharply delineated ocular dominance map (Shatz, Lindström, and Wiesel, 1977; Löwel *et al.*, 1998), because the architecture of columns is retained but the binocularity of neurons (in particular near the border of OD columns) is lost. In many cases, subjects alternate the eye they use for fixation; however, some fixate predominantly with the same eye and as a consequence, the input from the other eye is suppressed. This is accompanied by shrinkage of this eye's ocular dominance bands similar to that seen after monocular deprivation (Horton, Hocking, and Adams, 1999).

The effects of various other forms of abnormal experience on visual cortical development have been studied. For example, rearing animals wearing goggles with stripes painted on the lenses (Hirsch and Spinelli, 1970) or in a cylindrical environment with striped wall patterns (Blakemore and Cooper, 1970) causes an over-representation of the orientation of the exposed stripes in visual cortex. More recent studies, which combined these two approaches by rearing kittens wearing cylindrical lenses (essentially a model for severe bilateral astigmatism) in an environment that contains only this orientation, found that orientation columns for the exposed orientation are enlarged with respect to those of other orientations (Sengpiel, Stawinski, and Bonhoeffer, 1999; Tanaka *et al.*, 2006).

The development of the direction selective system is also susceptible to abnormal experience. Binocular deprivation leads to a loss of motion sensitivity in humans (Ellemberg *et al.*, 2002). Further, the direction map of ferret primary visual cortex only emerges after eye opening, indicating that the experience of moving stimuli is essential for it (Li, Fitzpatrick, and White, 2006). This is further supported by experiments in which kittens were reared under stroboscopic illumination, which allows normal spatial detail to be seen but interrupts the percept of motion. Such animals lacked direction-selective neurons both in V1 as well as the lateral suprasylvian cortex (Cynader and Chernenko, 1976; Pasternak *et al.*, 1985; Spear *et al.*, 1985), which corresponds to the motion areas V5 in monkeys and MT+ in humans. Rearing kittens in a rotating drum causes the majority of direction selective neurons to be tuned to the experienced direction of motion (Daw and Wyatt, 1976). Finally, exposing anaesthetised ferret kittens shortly after eye opening to a moving stimulus for about 8-10 hours results in the emergence of direction selective domains coding for only this axis of motion, while other directions still fail to produce a

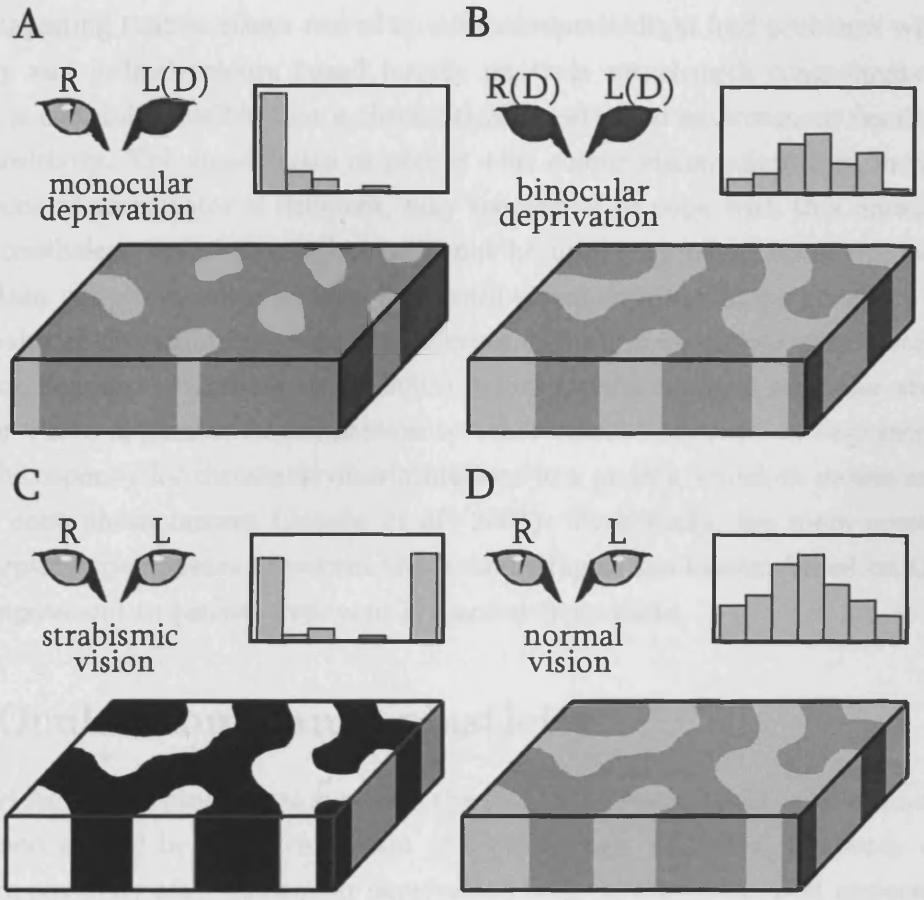


Figure 3.1: *Deprivation-induced ocular dominance changes.* Schematic depiction of differences in ocular dominance architecture and, in insets, OD histograms after rearing animals with monocular eyelid suture (**A**), binocular eyelid suture (**B**), surgically induced strabismus (**C**), and under normal control conditions (**D**). While monocular deprivation leads to a considerable shift in ocular dominance towards cells driven exclusively by the open eye, binocular deprivation only causes a loss of visually responsive neurons, but leaves the overall OD distribution unchanged. Strabismus leads to a pronounced loss of binocular neurons and thus a more sharply delineated ocular dominance map.

response (Li, White, and Fitzpatrick, 2006), suggesting an instructive role for experience in the development of motion perception.

One recent investigation further addressed experience-dependent plasticity of colour vision, suggesting that monkeys reared in monochromatic light had problems with colour constancy and judged colours based largely on their wavelength components (Sugita, 2004). It is certainly possible that a chromatically restricted environment results in poor colour sensitivity. The visual brain of people with colour vision anomalies, in which one type of cone photoreceptor is deficient, may reorganise to cope with this unusual visual input. Nonetheless, visual input alone may not be necessary to set up colour perception per se. Many subjects recovering from long-term visual deprivation are generally observed to be capable of discriminating between different colours immediately after restoration of vision (von Senden, 1932; Fine *et al.*, 2003), which points towards a greater stability in the colour vision apparatus in comparison to other visual functions. A very recent study showed the capacity for chromatic discriminations in a genetic knock-in mouse expressing a human cone photopigment (Jacobs *et al.*, 2007). Essentially, the mere presence of a novel receptor type appears to permit the remodelling of the system based on these new sensory inputs and to generate relevant behaviour from them.

3.3 Ocular dominance plasticity

All the evidence presented above supports the idea of a plastic brain, whose functionality is tuned and shaped by the environment at a young age. However, the study of ocular dominance plasticity after monocular deprivation remains one of the best understood and most effective manipulations for vision research. One reason for this is that disturbances of the visual input through one eye are far more likely to occur naturally than a striped environment, stroboscopic or monochromatic illumination; the study of its effects is therefore likely to be more relevant to our understanding of human vision and visual disorders. Moreover, many other visual functions appear to be relatively stable. For example, orientation selectivity emerges very early in life, likely well before birth, and the map of orientation columns in the cortex is remarkably preserved after selective exposure to a restricted orientation environment (Sengpiel, Stawinski, and Bonhoeffer, 1999). While a clear change in the number of cells and size of orientation columns can be observed, the cortex does not become selective exclusively to the experienced orientation. Rather, the fundamental architecture of the map is relatively normal.

Conversely, the ocular dominance and binocularity of cortical neurons as well as the response modulations they display to dichoptic stimulation are highly susceptible to altered visual experience: strabismic misalignment of the eyes can lead to a complete loss of binocular interaction and an associated loss of binocular depth perception. At the level of cortical circuits there appears to be a loss of the interocular facilitatory mechanisms present in normal subjects (Sengpiel and Blakemore, 1994) leaving only the suppression afforded by inhibitory circuits in primary visual cortex (Sengpiel *et al.*, 2006). Furthermore, after monocular occlusion or anisometropia the excitability of cortical neurons through the deprived eye, and accordingly its visual acuity, can be drastically reduced to the point of blindness. Ocular dominance plasticity appears to underlie the seamless mapping of visual space in primary visual cortex (Horton, 2006). In this context, it is important that individual peculiarities like angioscotomata (i.e. small areas of blindness in one eye caused by the shadow cast by blood vessels onto the retina) or topical occlusions of the retina are addressed effectively by the visual system. Further, in species with binocular depth perception it is of utmost importance that the images conveyed by the two eyes are in register during early development such that stereovision can develop. Therefore, it is not surprising that the effects of abnormal experience on ocular dominance are much more dramatic than those on other aspects of vision.

3.3.1 Cellular mechanisms of ocular dominance changes

Early investigators proposed a competitive mechanism as an explanation why the outcome of monocular deprivation is so much more drastic than when both eyes are occluded (Wiesel and Hubel, 1965). For a long time optometrists therefore prescribed patching of the formerly good eye, after vision in the afflicted eye was restored in childhood, as treatment for amblyopia. The rationale was that blocking the input from the formerly good eye influences the competition in favour of the now restored deprived eye, allowing it to develop the best possible acuity.

Extensive studies with the aim of finding the optimal patching regimen were conducted by Donald Mitchell, who assessed the outcome of visual acuity in kittens after a variety of different reverse occlusion (RO) paradigms. Patching of the formerly nondeprived eye generally leads to a better recovery of the acuity through the formerly deprived eye (i.e. a higher visual acuity is achieved in the end of the RO period) than does binocular recovery during which both eyes were open (Mitchell, 1988). However, many combinations of initial

monocular deprivation and subsequent reverse occlusion eventually result in worse acuity in one or both eyes (Mitchell, 1989). Although some treatment regimens are initially effective in restoring acuity of the DE, this gain is subsequently lost after treatment has been terminated, often resulting in bilateral amblyopia (Murphy and Mitchell, 1986). In contrast, the recovery from MD afforded by concordant binocular exposure (i.e. simple reopening of the sutured eye) is initially not quite as complete but more permanent (Kind *et al.*, 2002).

On the other hand, when a short binocular period is intercalated between the end of MD and the beginning of reverse occlusion a better outcome can be achieved (Mitchell, 1991) and this is mirrored by the changes ocular dominance maps in V1 undergo under similar conditions (Faulkner, Vorobyov, and Sengpiel, 2006). In the initial phase immediately subsequent to reopening of the deprived eye, binocular experience appears to be more effective at permitting recovery than reverse occlusion (Mitchell and Gingras, 1998; Mitchell, Gingras, and Kind, 2001), which casts doubt on the notion of a purely competitive mechanisms underpinning this recovery.

By pairing binocular periods with periods of reverse occlusion on a daily basis, good visual acuity can be obtained for both eyes even when 70% of daily visual experience is monocular (Mitchell, 1989). Similar outcomes are seen in human patients, who wear an eye patch over the formerly good eye after surgical correction of a unilateral ocular defect (Lewis, Maurer, and Brent, 1986). These findings suggest the question about the efficacy with which the two different kinds of experience, monocular and binocular vision, can drive ocular dominance plasticity. A regimen of daily eye patching of the good eye appears to be important for restoring functional connections from the formerly deprived one, but relatively short daily periods of binocular vision seem to be effective at maintaining the good eye's gain and finally allowing a beneficial result for both eyes. Accordingly, such mixed-experience regimens are nowadays routinely used to treat amblyopia (Mitchell and MacKinnon, 2002).

Quite in contrast to these encouraging results, it has been reported for monkeys that restoration of binocular vision to both eyes is insufficient to permit recovery from MD effects (Blakemore, Vital-Durand, and Garey, 1981). The reason for this apparent discrepancy is unknown, but it may be related to a misalignment of the visual axes of the two eyes. Recovery from monocular deprivation with binocular but discordant (strabismic) vision does not result in an improvement of deprived eye acuity (Kind *et al.*, 2002). Monocular occlusion (or other abnormal optical conditions) can often cause the develop-

ment of a minor strabismus (Quick *et al.*, 1989). A deviation of the visual axes measuring only a few degrees is unlikely to be problematic for allowing binocular recovery in cats, whose normal visual acuity is an order of magnitude lower than that of humans. In primates on the other hand, such micro-strabismus may be detrimental due to the smaller size of their receptive fields.

In spite of the undeniable differences between the feline and primate visual systems, studies on cats have shed light on mechanisms of MD-induced cortical plasticity and have highlighted the importance of binocular exposure during recovery from MD. The fundamental cellular mechanisms involved are likely to be similar in both species.

3.4 The underpinnings of plasticity

Long before the processes of synaptic transmission were known Donald Hebb postulated a theory of how memory and experience may manifest in a biological system. This concept is now frequently described as *Hebbian* learning mechanism meaning the notion that "cells that fire together, wire together," a simplistic generalisation of the far reaching hypothesis Hebb (1949) put forth in his book *The Organization of Behavior*:

> Let us assume then that the persistence or repetition of a reverberatory activity (or "trace") tends to induce lasting cellular changes that add to its stability. The assumption can be precisely stated as follows: When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.

While Hebb is now widely known for this ground-breaking theory of learning and memory, much of his book in fact focused on visual perception and on the question as to how far nature or nurture determine the perceptual capacities of an organism. In his book he referred to many of the case studies of patients recovering from blindness, which Marius von Senden (1932) had accumulated. Further, Hebb realised the strong link between neuronal plasticity, memory, and perception, and that only the study of the entire system, with all its details put into the bigger picture, will lead to a better understanding of the brain and the mind. Finally, over a decade before Hubel and Wiesel published their seminal work on the receptive field properties of V1 neurons, Hebb noted the necessity of specialised receptor neurons in the cerebral cortex.

3.4.1 Synaptic plasticity

It would take nearly another 20 years until an experience-dependent process similar to that envisioned by Hebb was discovered by Timothy Bliss and Terje Lømo (Bliss and Lomo, 1973). They showed that delivering a high-frequency train of electrical stimulation (*tetanus*) to a presynaptic neuron has a lasting effect on the efficacy of the synapse, such that a normal stimulus arriving in the terminal after the conditioning tetanus will elicit a stronger response in the postsynaptic cell than before.

This long-term potentiation (LTP) of the synapse can possibly persist for a lifetime, therefore fulfilling a requirement of memory to be stable. Subsequently, it was found that there also exists a process of long-term depression (LTD) by which the weight of a synapse can be decreased. The conditioning stimulus to achieve this is a prolonged period of low-frequency stimulation that activates the postsynaptic cell at a low frequency (Dudek and Bear, 1992).

The molecular processes underpinning these changes are subject to on-going investigation, however, a number of key parameters have been identified: the substrate for a subset of the processes reported as LTP and LTD are N-methyl-D-aspartic acid (NMDA) receptors and their associated complex of molecular signalling pathways within the synapse (Morris, 1989; Bliss and Collingridge, 1993; Bear and Malenka, 1994). Other mechanisms through which LTP and LTD can be induced have also been identified (Malenka and Bear, 2004).

The initial stimulus inducing NMDA-mediated LTP is conveyed through glutamate receptors of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type, which are the substrate for normal glutamatergic transmission. Opening of AMPA receptors causes an influx of positively charged ions (including calcium, Ca^{2+}) into the postsynaptic cell, whose membrane potential is in turn depolarised and NMDA receptors are activated. This causes a further influx of Ca^{2+} into the neuron setting off a cascade of intracellular signals that alter the response of this neuron to subsequently arriving presynaptic signals. This occurs initially through an increase in the excitability of AMPA receptors and voltage-gated ion channels by Ca^{2+}-dependent protein kinases (Malinow, Schulman, and Tsien, 1989; Sweatt, 1999). Further, intracellular AMPA receptor molecules are mobilised and inserted into the postsynaptic membrane enhancing the response to glutamate (Esteban, 2003).

Late processes in LTP are mediated through gene transcription and the synthesis and degradation of proteins. The main transcription factor involved in this mechanism appears

to be cAMP response element binding protein-1 (CREB-1; Dash, Hochner, and Kandel, 1990; Bartsch *et al.*, 1998). Ultimately, this results in the formation of new synaptic connections (Murphy and Segal, 1997).

While the initial subcellular response to a tetanus is only relatively short-lived, this late phase of LTP-induction fulfils the criteria for a molecular mechanism of long-term memory. However, it is only in combination with LTD that such a process can function, as LTP on its own would likely lead to an eventual saturation of synaptic strengthening throughout the neuronal network. LTD is also dependent on the presence of NMDA receptors. Low frequency stimulation (Dudek and Bear, 1992), or anticorrelated pre- and postsynaptic activity (Stanton and Sejnowski, 1989), triggers molecular pathways resulting in a desensitisation of ion channels, a reduction in the number of AMPA receptors by internalisation (Malinow and Malenka, 2002; Holman, Feligioni, and Henley, 2007), and the eventual loss of synaptic connections (Zhou, Homma, and Poo, 2004; Nägerl *et al.*, 2004).

One mechanism by which an imbalance in the inputs from the two eyes could lead to changes in synaptic weights in visual cortex is spike timing-dependent plasticity (STDP), a model for describing when and how the efficacy of a synapse changes in response to stimulation (Dan and Poo, 2004). Instead of a simple Hebbian rule, synaptic plasticity is thought to be dependent on the precise timing of activity in pre- and postsynaptic inputs: if a weak input is concurrent with or followed shortly after by a strong input, potentiation of the weak input occurs, whereas the reverse order will result in depression. By this logic, the input from a deprived eye (or a non-fixating one in strabismus) no longer correlates with the input from the fellow eye and thus undergoes synaptic depression.

On the other hand, the Bienenstock-Cooper-Munro (BCM) model of synaptic plasticity (Bienenstock, Cooper, and Munro, 1982) proposes a sliding modification threshold for synaptic remodelling based on the average activity of the postsynaptic neuron. According to this theory, postsynaptic activity below the threshold will result in a depression of the synapse, whereas activity above it will lead to the induction of LTP.

It has been suggested that this modification threshold is determined by the subunit composition of NMDA receptors (Philpot *et al.*, 2001; Philpot, Cho, and Bear, 2007). The trans-membrane receptor molecules are heterodimers and always contain a pair of NR1 subunits. In addition, they also contain a pair of NR2 subunits of which there are various types, named NR2A-D, which seem to control the molecular response to receptor activation - and thus the efficacy and possibly the direction of plasticity. The different

types of NR2 subunits show different patterns of distribution across the brain and different developmental time courses of expression (Monyer *et al.*, 1994).

In immature visual cortical neurons there exists a preponderance of receptors containing the NR2B subunit, which appears to predestine these receptors for rapid changes of synaptic weight as they permit longer charge transfer than the NR2A subunit found in mature cortical neurons. The decline of the critical period for ocular dominance plasticity is accompanied by a gradual replacement of NR2B containing receptors with those containing NR2A, which means that the efficacy with which synaptic changes can be induced is diminished (Nase *et al.*, 1999).

The same also occurs when a dark-reared subject is transferred to a normally lighted environment. As discussed earlier, dark-rearing has been proposed to maintain the visual cortex in an immature state as it prolongs the critical period (Mower, Christen, and Caplan, 1983). As soon as a few hours after being exposed to visual input the neurons in the dark-reared animal begin to exchange NR2B subunits for NR2A (Quinlan *et al.*, 1999).

The described mechanisms of synaptic plasticity are implicated in the shaping of visual cortical function by early experience, because elimination of various components within the NMDA receptor complex can severely disrupt the ocular dominance shift induced by monocular deprivation: blocking the action of NMDA receptors reduces the ocular dominance shift (Roberts, Meredith, and Ramoa, 1998). Moreover, elimination of signals downstream of NMDA receptors, such as protein kinase A (PKA) and CREB-1, result in diminished ocular dominance shifts (Beaver *et al.*, 2001; Mower *et al.*, 2002).

The process by which monocular experience reorganises geniculocortical afferents to the primary visual cortex during the critical period appears to be a two stage modulation of synaptic weights: the initially response is a depression of the deprived eye input, which occurs over the first three days, whereas in the mouse the efficacy of nondeprived eye neurons has been shown to be potentiated with a delay of a few days (Frenkel and Bear, 2004). In adult rodents beyond the critical period this potentiation of nondeprived eye responses persists but MD no longer has a suppressive effect on deprived eye responses (Sawtell *et al.*, 2003). The delay between changes to the drive from nondeprived eye in comparison to the faster loss of the deprived eye input, as well as the differential expression of these processes between juveniles and adults, are in contrast with purely competitive mechanisms underlying ocular dominance shifts.

Finally, there have also been recent suggestions for an involvement of homeostatic

mechanisms in synaptic plasticity, which is thought to be important for keeping input-dependent potentiation and depression from running rampant. In this theory, a neuron may exert modulation on the weights of its synapses opposed to those caused by Hebbian plasticity (Turrigiano and Nelson, 2000). Synaptic scaling, that is the calibration of all the synaptic inputs, thus ensures a stable target level of postsynaptic activity. At the level of neuronal circuitry, similar mechanisms may also cause regulation of feedback loops that ensure stable activity in neurons (Turrigiano, 1999).

In regards to visual cortical plasticity, homeostatic mechanisms could be an explanation for the delayed potentiation of nondeprived eye responses after monocular deprivation (Frenkel and Bear, 2004). Another indication for this may be found in very recent reports of enhanced responsiveness of monocular deprived eye-dominated neurons remaining after monocular deprivation (Mrsic-Flögel *et al.*, 2006).

Certainly, at present direct evidence for a straightforward involvement of synaptic plasticity mechanisms such as LTP and LTD remains elusive. While the experiments by Frenkel and Bear (2004) showed that blocking spontaneous retinal activity prevents the rapid depression of deprived eye inputs (thus suggesting a role for LTD), there is no clear indication for LTP in the enhancement of nondeprived eye responses, as it is only inferred from the NMDA-dependence of this process (Sawtell *et al.*, 2003).

A largely unexplored possibility is that the enhancement of nondeprived eye responses reflects a form of perceptual learning that would allow the animal to compensate for the loss of vision in one eye. Recently, it was shown that selective overexposure of a visual stimulus can cause enhanced responses to this stimulus in the visual cortex of adult mice (Frenkel *et al.*, 2006). This mirrors the enhanced responsiveness to nondeprived eye stimulation after monocular deprivation in adult rodents (Sawtell *et al.*, 2003). Since the reduction of deprived eye responses is restricted to the critical period, only the delayed enhancement of nondeprived eye responses can be observed in adulthood. While this form of adult plasticity appears to be specific to murine animal models, it may merely be more subtle in other species. The fact that an ipsilateral eye can show such extensive increases in responsiveness in rodents is perhaps due to the extensive contralateral bias in these species.

For example, a recent neuroimaging study on perceptual learning in humans (Furmanski, Schluppeck, and Engel, 2004) showed that training on the detection of an oblique oriented grating can enhance the response to this stimulus to the level of a vertical grating, thus seemingly abolishing the cardinal bias (the representation of cardinal orientations

is stronger than that of oblique ones; Furmanski and Engel, 2000; Coppola *et al.*, 1998) present in primary visual cortex. This finding bears a haunting resemblance to the enhancement of ipsilateral eye responses after monocular deprivation, as it allows for an equalisation of previously imbalanced inputs. The much smaller contralateral bias in the visual cortex of primates and higher mammals compared to rodents could indicate that there is simply less "room for enhancement" of the weaker responses.

3.4.2 Other factors

In light of the fact that evidence for a purely synaptic mechanism underpinning ocular dominance changes has not been established, there have been studies on putative structural, cellular, and molecular processes that may be involved. There could be competition between the afferents from the two eyes for endogenous agents for the generation or maintenance of synapses.

For instance, a role for neurotrophic growth factors has been suggested. In one study, exogenous infusion of brain-derived neurotrophic factor (BDNF) or neurotrophin-4/5 (NT-4/5) in young kittens prevented the formation of ocular dominance columns (Cabelli, Hohn, and Shatz, 1995), which suggests a role for the receptor molecule TrkB in the development of visual cortex. The expression pattern of this receptor molecule also changes over the course of cortical development (Cabelli *et al.*, 1996). Furthermore, it has been shown that administration of BDNF eliminates the contralateral bias observed in the visual cortex of normal or dark-reared kittens, while another factor, nerve growth factor (NGF), had no effect on monocular deprivation-induced changes, but was effective at restoring ocular dominance plasticity in adults (Galuske *et al.*, 2000).

This connection to growth factors has led some researchers to presume a role for environmental enrichment, because a more stimulating environment in terms of sensory, motor and social input increases BDNF levels in mice (Pham *et al.*, 2002). It has subsequently been shown that environmental enrichment appears to accelerate the critical period (Cancedda *et al.*, 2004) thus representing the converse of dark-rearing.

These neurotrophic factors may be linked with the maturation of cortical circuits and the balance of excitatory and inhibitory systems in the cortex. In this view, inhibitory circuits could gate sensory plasticity, either at the level of individual spikes (which would suggest a role for STDP), or within large neuronal assemblies firing in synchrony, which would be sensitive even to slight offsets such as uncorrelated inputs from the two eyes

(Hensch, 2005). Over-expression of BDNF leads to an accelerated maturation of OD plasticity and of intracortical levels of inhibition (Huang *et al.*, 1999). The maturation of the visual cortex is accompanied by the maturation of the major inhibitory neurons. The main inhibitory neurotransmitter in the brain is gamma-aminobutyric acid (GABA). Intracortical inhibition appears to be a key factor in ocular dominance plasticity, since a genetic knock-out of glutamic acid decarboxylase (GAD65), which reduces GABA release by inhibitory neurons, eliminates the shift in cortical responses to the nondeprived eye following monocular deprivation (Hensch *et al.*, 1998). Pharmacological inhibition restores plasticity in these knock-outs (Iwai *et al.*, 2003), and it appears that the start of the critical period for OD plasticity is triggered by a threshold level of inhibition (Fagiolini and Hensch, 2000). At least in rodents, dark-rearing in adulthood even returns the balance of excitatory and inhibitory receptor subunits to juvenile levels, thus reopening susceptibility to monocular deprivation (He, Hodos, and Quinlan, 2006).

Also associated with the emergence of inhibitory neuronal activity in the visual cortex from infancy to adulthood are structural changes in the cortical tissue. Perineuronal nets consisting of chondroitin sulphate proteoglycans in the extracellular matrix surround these inhibitory interneurons (mostly parvalbumin-positive basket cells) from around the same time as the closure of the critical period. These glycoproteins may constitute a physical barrier for the formation of new synaptic connections and may therefore impede changes (Berardi *et al.*, 2003; Berardi, Pizzorusso, and Maffei, 2004). By digestion of these macromolecules with chondroitinase ABC, ocular dominance plasticity can be restored in adult rats (Pizzorusso *et al.*, 2002) and reverse occlusion therapy, which is normally ineffective in the mature visual system, can be used to restore vision to the formerly deprived eye (Pizzorusso *et al.*, 2006).

In this context, the motility of dendritic spines (i.e. the protrusions of the cellular membrane which constitute points of synaptic contact on the dendrites of a postsynaptic cell) has also been reported to be involved in ocular dominance plasticity. Spine density and dynamics are closely linked to the critical period (Berardi, Pizzorusso, and Maffei, 2004; Mataga, Mizuguchi, and Hensch, 2004) and visual deprivation causes enhanced motility during its course (Majewska and Sur, 2003). Motility increases with degradation of the extracellular matrix through the tissue-type plasminogen activator (tPA)/plasmin cascade (Oray, Majewska, and Sur, 2004).

Finally, it has been suggested that intracortical myelin, in particular in the geniculocortical recipient layer IV, blocks the outgrowth of neurites in visual cortex, and that it

is the maturation of myelination of cortical neurons which puts a halt on plastic changes in adult cells. Myelination is essential for fast neuronal transmission, but it may constitute a hard-wired state, which impedes the reorganisation of neuronal circuits. Insertion of Schwann cells into the ventricles of rats at the age of eye opening inhibits plasticity (Pizzorusso *et al.*, 1994). On the other hand, the closure of the critical period can be prevented by disrupting the interaction between myelin and neurons: the neuronal Nogo-66 receptor, whose substrate is a membrane protein in the myelin sheath around axons, normally inhibits the outgrowth of neurites. By targeting this receptor or its associated proteins, ocular dominance plasticity can be made to persist throughout life (McGee *et al.*, 2005).

From the account in this section, it should become clear that there is a large number of proposed mechanisms that could underlie sensory plasticity. It is important to keep in mind that the cerebral cortex is not a homogenous tissue, but a layered structure containing numerous types of different cell types and a complex arrangement of neuronal circuits. Due to this it is highly unlikely that any one process can explain all of the changes caused by abnormal sensory input. It will be critical to establish the exact interplay between all of these mechanisms and their pattern of expression over the course of development.

Moreover, in light of the evidence from cellular and molecular biology, it is of great importance to understand the precise prerequisites for postnatal experience to permit normal sight through both eyes and the associated establishment of a normal functional organisation of the visual brain. As I discussed on page 14, there are clear indications that binocular and monocular experience may not be weighted equally in shaping the primary visual cortex and for the development of good visual acuity. In spite of this, as of today no other study has addressed this issue systematically.

Chapter II

General Methods

4

Optical Imaging of Intrinsic Signals

An ideal method for illuminating the intricate function of neuronal circuits in the living and behaving organism, while causing it minimal discomfort, has not yet been discovered. Yet, over a century since the era of Ramon y Cahal and Camillo Golgi, when neuroscience was limited to anatomic and histological assessments of nervous tissues, a wide range of powerful techniques for visualising brain function *in vivo* have been developed.

From the actual electrical activity associated with neuronal discharges, over the weak magnetic fields they generate, the metabolic demand of neurons, to the molecular processes within them a variety of physiological changes can be used as a measure of brain activity. Each of these methods has advantages and disadvantages with regard to their accuracy and reliability. Excellent spatial or temporal resolution often comes at the cost of greater invasiveness generally limiting such methods to animal models. In rare cases when they are employed in human subjects, they are used primarily as a tool for medical diagnostics in preparation of neurosurgery. Therefore, the observations from any potential scientific investigations, which could be conducted on these patients, are often difficult to interpret, since these studies are confined to pathological brain regions and subject to time constraints, because the health of the patient is of course a greater priority than scientific investigation.

When interested in mapping the functional organisation of primary visual cortex *in vivo*, optical imaging of intrinsic signals (Bonhoeffer and Grinvald, 1996; see experimental setup, Fig. 4.1) is one of the most suitable methods available. It permits a time efficient visualisation of the architecture of cortical columns within a relatively wide region of the cerebral cortex with high spatial resolution ($\sim$ 50 μm). It samples a large number of neurons overcoming the sampling bias inherent even to multi-array electrode recordings.

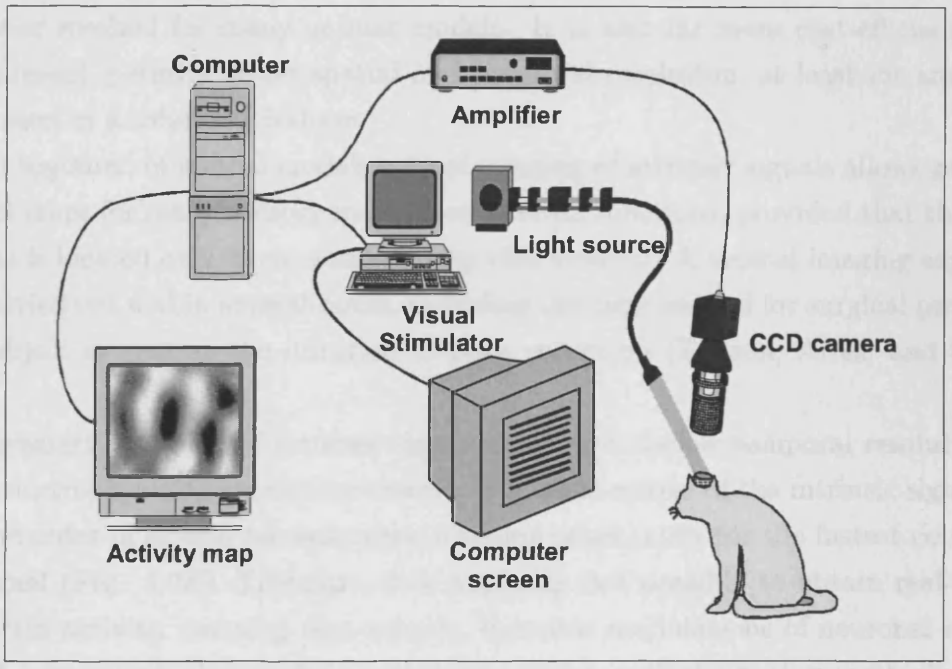


Figure 4.1: *Optical imaging experimental setup.* While the animal views stimuli presented on a computer screen, a slow scan CCD camera captures image frames of the exposed brain, which is illuminated by near-infrared light (shown here in grey). The images are amplified and digitised and then an activity map can be calculated. Usually, images are range-fitted and filtered offline. (Adapted from Bonhoeffer and Grinvald, 1996.)

Furthermore, unlike multiple electrode penetrations or the imaging of voltage-sensitive dyes, this technique does not require the insertion of foreign substances into the nervous tissue, leaving the brain mostly intact for subsequent electrophysiological or histological studies, which can be targeted to specific locations of interest based on the brain maps obtained with imaging (e.g. orientation pinwheel centres or ocular dominance domains). Finally, while it is not as non-invasive as modern functional magnetic resonance imaging (fMRI), which may have the future potential for acquiring data of similar quality, it is the superior method for many animal models. It is also far more cost-efficient and, at least at present, permits better spatial and temporal resolution, at least for areas which are organised in a columnar fashion.

Taken together, in animal models optical imaging of intrinsic signals allows generation of cortical maps for many sensory modalities or brain functions, provided that the imaged brain area is located on a gyrus accessible by this method. A typical imaging experiment can be carried out within several hours, including the time needed for surgical preparation of the subject as well as the duration of data collection (Zepeda, Arias, and Sengpiel, 2004).

The greatest downside of intrinsic signal imaging is its low temporal resolution compared to electrophysiological measurements. The time course of the intrinsic signal develops on the order of several seconds after stimulus onset, even for the fastest components of the signal (Fig. 4.2E). Therefore, it is normally not possible to obtain real-time images of brain activity, meaning that minute, dynamic modulations of neuronal responses can not be measured. For such investigations faster optical imaging methods, such as voltage-sensitive dyes (Shoham *et al.*, 1999) or flavoprotein imaging (Tohmi *et al.*, 2006), are more suitable. The limited temporal resolution of intrinsic signal imaging, however, does not pose a problem for the mapping of relatively stable functional architecture, like orientation domains or ocular dominance bands, which can only change over the course of days.

4.1 Sources of intrinsic signals

Intrinsic signals are changes of light reflectance that occur in active neuronal tissue. These changes depend on the wavelength of the illuminating light and are due to the combination of different physiological phenomena associated with neuronal and metabolic activity (Fig. 4.2): first, light scattering by cortical tissue, second, changes in blood oxygenation in local

capillaries, and third, increases in blood volume due to vascular recruitment (Bonhoeffer and Grinvald, 1996).

The fastest component is related to the light scattering by biological tissues (Fig. 4.2B), which originates in the ionic/molecular movements associated with synaptic transmission and neurotransmitter release as well as the cellular metabolism. The most rapid subcomponents of the light scattering signal are very closely coupled to electrical activity resulting from the reconfiguration of the cell membrane (Foust and Rector, 2007). More delayed events are the swelling and contraction of neuronal somata and extracellular spaces due to water movement and glutamate uptake by astrocytes after its release at the synapse (Gurden, Uchida, and Mainen, 2006; Buchheim *et al.*, 2005). In general, during neuronal activity the light scattering is reduced, which results in a more transparent tissue and thus more light absorption (Frostig *et al.*, 1990; Rector *et al.*, 1997). The light scattering signal is most effective at near-infrared wavelengths from 700 nm and above[1].

Further, the oxygenated and deoxygenated forms of haemoglobin have different light absorption spectra (Fig. 4.2F) and thus the amount of reflected light can be correlated to the concentration of oxygen in the tissue. When illuminating the cortex at a wavelength of $\sim$ 600 nm these differences dominate the reflected signal. At this wavelength deoxy-haemoglobin absorbs more light than oxy-haemoglobin, and thus more metabolically active domains (in which neurons take up more oxygen from the blood) appear darker (Fig. 4.2C). This aspect of the signal has been suggested to correspond to an "initial dip" in the blood-oxygen level dependent (BOLD) signal, which is measured in fMRI. In fact, functional maps of primary visual cortex have been obtained in anaesthetised cats using only this negative BOLD[2] signal (Kim, Duong, and Kim, 2000).

Changes in blood volume dominate the optical signal at an illuminating wavelength of 570 nm, an isobestic point at which the absorption spectra of oxy-haemoglobin and deoxy-haemoglobin are identical (Fig. 4.2F). Essentially, the increase in blood volume causes an increase in the tissue haemoglobin content and thus more red light is absorbed

[1]It must be pointed out that all of these components play a role in the intrinsic signal. While light scattering strongly dominates the signal at near-infrared wavelengths, the different components are all involved.

[2]Note that other types of a negative BOLD response, which should not be confused with the initial dip, are also used in the literature. For instance, it has been shown that increases in oxygenation in active brain areas (the positive BOLD signal) can be accompanied by a down-regulated haemodynamic response in unused areas (Smith, Williams, and Singh, 2004).

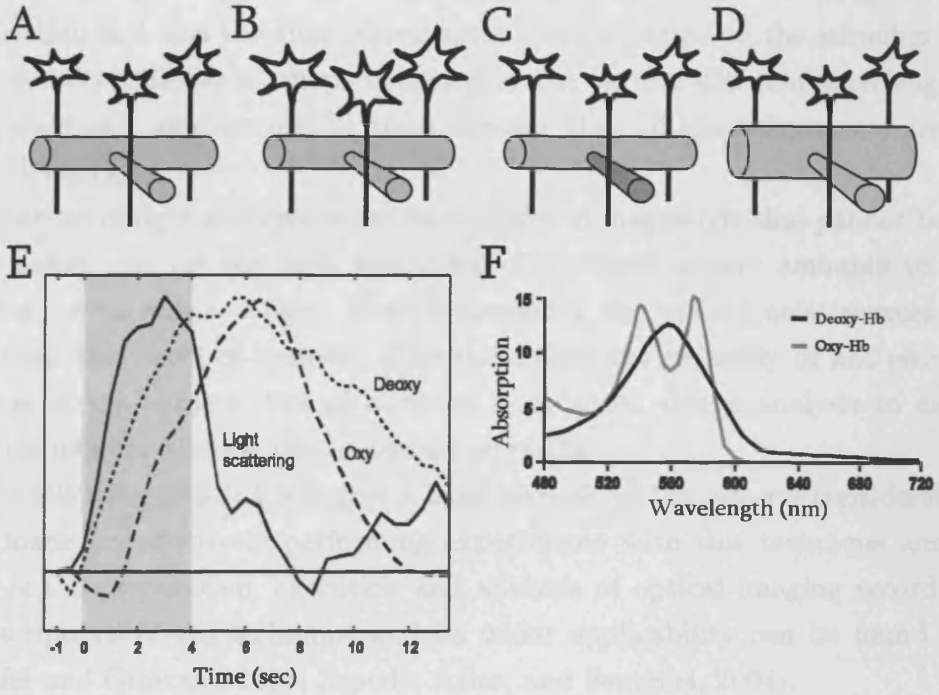


Figure 4.2: *Components of the intrinsic signal.* (**A-D**) Schematics representing the changes in cortical tissue during neuronal activity. (**A**) Unstimulated cortex at baseline activity. Shown are an arteriole, a capillary branching off it, and three neurons in the vicinity. (**B**) The first reaction to increased neuronal activity is a decrease in light scattering. The cortex becomes more transparent and thus more light is absorbed. (**C**) Associated with the increased metabolic activity of active neurons, blood oxygenation in nearby capillaries is decreased. More light is absorbed by deoxygenated haemoglobin than by the oxygenated state. (**D**) Finally, a delayed effect of metabolic activity is an increase in blood volume transporting fresh oxygenated blood into the active area. (**E**) The various components of the signal have distinct time courses. Signal amplitude is plotted as a function of time relative to stimulus onset. The light scattering signal is most closely correlated to the sensory stimulation (shaded area) as it rises most rapidly after stimulus onset and decays steeply back to baseline once the stimulus is turned off. (**F**) The two oxygenation states of haemoglobin have distinct absorption spectra. Light absorption of oxy- (grey) and deoxy-haemoglobin (black) are plotted against the wavelength of the illuminating light. (**E-F** Adapted from Bonhoeffer and Grinvald, 1996).

(Fig. 4.2D).

All these signal components have slightly different spatial resolution and signal time courses (Fig. 4.2E), with the light scattering signal being the one with the best spatial localisation and also the time course most closely related to the stimulus (and thus neuronal activity). However, maps obtained at the various different wavelength of illumination yield very similar cortical maps showing that all the components are similarly suited for imaging.

The changes in light absorption are on an order of magnitude that cannot be detected with the naked eye, for the light scattering component usually amounts to less than 0.1% of the overall reflected light. Most importantly, the various noise sources are much stronger than the signal of interest. This underlines the necessity of not only cautious preparation of the subject, but of complex and careful image analysis to extract the neuronal component filtering out unwanted artefacts.

On the following pages, I will give a brief account of the general considerations that must be made for effectively performing experiments with this technique and offer an overview of the preparation, execution and analysis of optical imaging recordings. Detailed descriptions of the technique and its wider applicability can be found elsewhere (Bonhoeffer and Grinvald, 1996; Zepeda, Arias, and Sengpiel, 2004).

5

Preparation

5.1 Surgery

The surgical preparation for the recording is performed under deep anaesthesia, which can be induced through an intramuscularly injected cocktail of ketamine and xylazine. A tracheotomy is conducted in terminal experiments, because it permits prolonged ventilation of the subject for days. In chronic experiments, when the animal is allowed to recover after the initial session and several subsequent recordings are performed, it is of course preferable to use endotracheal intubation for ventilation. Tubes for pediatric applications are suitable for this purpose.

Ventilation is achieved by means of a surgical respirator with a 60:40 mixture of nitrous oxide and oxygen, and 1.5-2.5% of gaseous anaesthetic, preferably isoflurane. The vital signs of the animal, like the electrocardiogram and heart rate, end-tidal expired carbon dioxide[1], respiratory pressure, and rectal temperature are monitored continuously, ideally by means of a patient monitor system (instruments used in my experiments are described in the Experimental Methods section, p. 74).

While near-infrared light can penetrate the tissue of the skull and thus enter the brain, it is of crucial importance to clear the region of interest of any obstructing material in order to achieve the best signal and highest possible spatial resolution. The more non-brain tissue there is in the path of the light, the less accurate the localisation of signals

[1]In very small animals the expired CO_2 can only be sampled intermittently with some gas analyser models, because the samples taken remove a certain amount of gas from the ventilated volume and in very small animals this volume could be large enough to disrupt sufficient respiration.

caused by actual neuronal sources.

This means that usually a craniotomy must be performed to expose the brain. Sometimes, the dura must also be resected to reveal the surface of the brain. In smaller mammals like mice or tree shrews, however, it is often possible to image through the thinned skull bone, because it is transparent enough to allow good illumination of the underlying brain. In felines and primates, on the other hand, this approach can not be used.

For studying the primary visual cortex of cats the trepanation is made over and anterior to lambda such that the posterior parts of the two lateral gyri are exposed. Care must be taken not to open the bone posterior to lambda, as this will rupture the venous sinus and can cause terminal bleeding. In young animals, a heart shaped opening with the dip of the heart located just anterior to lambda is used. In older subjects, it is advisable to make two openings on either side of the midline and leaving a bone bridge in the middle to prevent bleeding.

The bone is opened with a dental drill. It is imperative that the drilling is done with great caution, in order to prevent damage of the underlying brain tissue. Also, one should not perform prolonged drilling in a single location as the friction of the drill generates heat that can also damage the cortex. Often, blood vessels in the bone are damaged and the bleeding must be stopped with bone wax. It is imperative that the area for drilling is frequently cleared of bone dust, either with pulses of pressured air or by flushing it with saline solution. This also cools the drilled bone, which minimises any problems caused by friction.

Once the boundaries of the window have been drilled, the bone is carefully detached from the dura by scraping off the connective tissue with a metal spatula. This step is often the single greatest cause of minor bleeding spots on the surface, since this process can rupture small dural blood vessels. The surface of the brain is then cleaned with cotton swabs and small cotton triangles, which have the potential to absorb great amounts of fluid (Sugi, Kettenbach, Germany).

The next important stage is the creation of an environment that minimises mechanical disturbances in the organism. Due to vascular motion, the brain pulsates regularly with every heart beat, but more importantly, it also contracts and expands with every respiratory cycle, because of waves propagating through the cerebrospinal fluid system.

This is less problematic for non-invasive procedures like fMRI in which the skull is a closed system containing these reverberations. A craniotomy opens a point of exit for the

pressure changes in the brain, which amplifies these pulsations. This sometimes becomes even more significant when the dura has been sectioned, especially when the opening is only very small as used for electrode penetrations. The internal pressure can cause a swelling of brain tissue through the dural gap (essentially, a miniature brain hernia).

Therefore, it is essential that the preparation is stabilised by a pressured environment. A normal titanium head chamber used for chronic electrode recordings can be used for this. The chamber must be attached firmly to the cranium by means of dental cement around its outside. On the inside, the gap between bone and chamber is sealed with a layer of dental wax.

Chambers with two inlets are very suitable as they allow the controlled filling of the interior with silicon oil, which is used to create the pressure inside. An oil-filled syringe is attached to one inlet, whereas a tube (for example the other end of a "butterfly" intravenous catheter) is connected to the other one. When the chamber has been filled, the inlet is blocked by means of a three-way cock. The top of the chamber is closed with a glass cover slip that is placed on a rubber ring and screwed tight with a threaded metal ring. This makes the second inlet the only opening through which pressure can leave the chamber (provided the chamber was attached and sealed properly). By clamping its tube the pressure can be regulated with great precision.

It is of great importance to remove any particles from the oil before closing the chamber. Bone dust, debris from the dura or bone wax, tiny air bubbles, and blood can all cause problems. In particular, floating objects moving in the chamber, like an air bubble traversing the chamber from one side to the other, can completely disrupt image acquisition. Bleeding on the surface of the brain from dural vessels create an expanding pool of blood, which also obstructs the illumination of the cortex and blots out any signals resulting from neural activity.

Ideally, the surface of the brain must be in perfect condition before the chamber is filled and sealed. Reopening the chamber and thus releasing the pressure again can seriously deteriorate the quality of the optical signal. Nevertheless, it is of course necessary in some instances when an unforeseen large-scale obstruction (e.g. air bubbles or bleeding) arises in the chamber.

5.2 Illumination and camera setup

With the pressurised recording chamber in place, the cortex must be illuminated and the camera set up for image acquisition. The glass window in the chamber is cleaned using petroleum to remove traces of the oil and any dust particles. Then the camera is focused on the surface of the brain, making sure that roughly all areas in the region of interest are in focus. Two camera objectives are mounted front-to-front to create a macroscope. This permits a relatively narrow depth of focus over a comparably wide area.

For this the cortex is illuminated using two fibre-optic light guides, one on each side of the recording chamber. Green light (546 nm) is used to reveal the vascular bed on the brain surface with high contrast. An image of this blood vessel pattern is captured to later permit the localisation of functional domains through the obtained cortical maps and to target microelectrode recordings.

A rectangular region of interest (RoI) is defined based on this vascularisation image encompassing the exposed cortex. A smaller rectangular zone (the "superpixel") on a particularly clean part of the cortex is also defined, which is later used by the imaging software (VDAQ Server, Imager 2001; Optical Imaging Inc., Mountainside, NJ) for online observation of the signal quality during the data acquisition.

Following the general camera setup and definition of the RoI, the illuminating light is switched to red. A red filter limiting the wavelength of the light entering the camera to a very narrow range (700 $nm \pm 10$ nm, in our experiments) is placed behind the objective.

The camera is focused 500 μm under the surface, which puts the imaging focus within layers II and III of the cortex. With a pseudo-colour image indicating the intensity of reflected light an even illumination of the cortex is achieved by finding the best alignment of the light guides. The intensity of the light must be optimised without permitting any over-saturation, i.e. spots with too strong reflection (leakage of cerebrospinal fluid through perforations in the dura can be especially problematic in this regard).

At this stage of the preparation, once more the need for a stable pressurised environment inside the recording chamber becomes clear. Pulsation of the cortex can often cause fluctuations in the intensity of reflected light. Even a very well-sealed chamber can have some pulsations. Care must be taken to keep the maximal intensity level of such pulsations below the threshold beyond which recording is impossible.

5.3 Other considerations

The final preparations before data collection can begin concern the stimulus generating apparatus. A computer screen is placed in front of the animal. Separate stimulation of the two eyes can be accomplished either through a Wheatstone stereoscope or by means of mechanical eye shutters.

It is of course essential that the animal's eyes focus onto the computer screen. Gas-permeable contact lenses with a small pupil are used to refract the eyes in this manner. The optimal refraction is determined with an ophthalmoscope. The eyelids are kept wide open by topical application of phenylephrine eye drops (which may need to be diluted to prevent excessive increases in heart rate). Furthermore, application of atropine eye drops prevents any accommodation: the ciliary muscle is relaxed and the viewing distance is thus infinity prior to placement of corrective contact lenses.

A neuromuscular blocking agent like gallamine (an acetylcholine receptor antagonist), injected intramuscularly or infused intravenously, is used to paralyse the animal. This eliminates any eye movements that could disrupt a stable retinal stimulation. It also has the advantage that it minimises endogenous respiratory movements, which could influence the state of the subject.

In order to monitor the arousal state of the animal, the electroencephalogram is monitored continuously through silver wire electrodes implanted into the skull anterior to the recording chamber. The trace is displayed on an oscilloscope. A more detailed analysis of the recording is unnecessary, because it is only needed to check for the appearance of slow wave activity, which would indicate too deep anaesthesia, or alternatively for the emergence of high frequency activity, if the anaesthesia was too light. Generally, the level of anaesthetic gas is kept at a minimum in order to permit good visual responses, while nonetheless keeping the subject anaesthetised adequately.

Finally, to avoid any mechanical noise, the entire experimental setup should be mounted on a vibration-free table for optical setups, which allows the fixation of all components either by screw or magnetic foot. Strong vibration in the experimental room should be avoided. Naturally, as intrinsic signal imaging measures reflected light, the room is darkened and must be free of frequently changing light sources.

6

Image acquisition

Images are captured by a slow scan CCD camera at regular intervals. In order to minimise requirements for storage space, a number of video frames are averaged into a single data frame (e.g. in my study, 15 video frames of 40 *msec* were summed into one 600 *msec* data frame). In studies of functional architecture such as the ocular dominance or orientation maps, which are stable properties of the cortex, this poses no problems.

Other means of reducing storage space can be employed, such as averaging (binning) of 2-3 adjacent pixels in the image into one pixel. As the spatial resolution of the imaging system is greater than that of the biological optical imaging signal, this does not lead to a loss of relevant information.

6.1 Stimulation protocol

Due to the small scale of intrinsic signals ($< 1/1000$), a high bit depth is necessary to store the information in digital format for which the resolution of an 8-bit frame grabber ($1/256$) is insufficient. A differential imaging system can circumvent this problem by analogue subtraction of a reference frame from each image frame. Through this approach only the modulation with respect to the reference frame is encoded, which is possible even with 8 bits.

Stimuli typically used in optical imaging experiments mapping primary visual cortex are sinusoidal or square-wave gratings of various spatial frequencies and orientations. Because it is generally of interest to measure responses from as many neurons as possible, high luminance contrast gratings at low spatial frequencies, which evoke the strongest optical maps (Carandini and Sengpiel, 2004), are used to maximise the response. Further,

by differentiating the activation patterns in response to low and somewhat higher spatial frequencies, the boundaries of primary visual cortex and neighbouring area 18 (V2) can easily be delineated, because receptive field sizes of cells in this stage of visual cortical processing are larger and they are on average tuned to lower (wider) spatial frequencies (Ohki *et al.*, 2000).

It is of course imperative to also have a stimulus for a baseline recording of unstimulated cortex, because any response to stimulation is only a relative change of metabolic activity against the spontaneous background activity. A uniform grey blank screen stimulus equiluminant to the stimulus gratings is used for this purpose.

The duration of stimulus presentation should not exceed much more than 5 *sec*, because beyond that time the relatively poorly localised blood flow signal starts to dominate. Concurrently, the inter-stimulus interval should be chosen such that it allows sufficient time for the signal to decay back to baseline levels before beginning with the next stimulation epoch. In order to compromise between a sufficiently long interval and keeping the overall duration of data acquisition relatively short, 7 - 10 *sec* are ideal.

In order to increase the signal to noise ratio, the maps obtained from several trials of stimulus presentations are averaged. Generally 24-32 trials are sufficient to obtain reliable maps. Each trial consists of a full set of stimuli, presented in a pseudorandom order to exclude adaptation or order effects.

In preparations with strong pulsation artefacts, image acquisition and respiration can be coupled with the heartbeat. This way the sequence of data frames is always the same in each cycle and inconsistencies due to the respiratory and cardiovascular pulsation are eliminated.

Other sources of biological noise tend to be very slow ($\sim 0.1\ Hz$). Most predominant is vasomotor noise, cyclic changes in blood volume and changes in oxygen saturation. A 1% change in oxygenation is not pathological for the brain, but it has fundamental consequences for the intrinsic signal used for mapping. Fortunately, such biological noise is usually slower than the duration of stimulus presentation. Therefore, this problem can be addressed by first-frame analysis (Bonhoeffer and Grinvald, 1996): a number of frames are collected prior to the stimulus, and this image is subtracted from the frames collected during stimulation. By this approach the image acquired in response to every stimulus presentation is normalised against the current baseline level.

Another stimulation protocol has been proposed, which circumvents the various sources of cyclic noise, and also promises to reduce the time required for image acquisition

(Kalatsky and Stryker, 2003). Instead of presenting stimuli in a random order, they can be presented in a progression covering the whole set. For each pixel in the image, the frequency of the stimulus change can be extracted from the modulation of the optical signal. If the frequency of stimulation is chosen to be suitably different from that of recurring noise, these sources are filtered out reliably. However, this method is confounded by a number of complications (see Zepeda, Arias, and Sengpiel, 2004, section 2.3.3.3 for a discussion) and it requires a continuous stimulus range. It may be more suitable for relatively simple measures like retinotopic mapping, although it is routinely used in some laboratories for the mapping of orientation domains.

6.2 Offline image processing

After a sufficient number of images has been collected, the maps are processed further offline. The activity maps to each stimulus can be normalised in a number of ways. The first, and perhaps most intuitive, is to divide the response to each stimulus by the response to the blank screen. This way activity patches associated with the stimulus are compared to the absence of patterned stimulation. Blank-normalised maps are the purest and most reliable method of processing the data, because no assumptions are made about the response (other than that they are visually evoked).

Unfortunately, however, using the blank response as baseline leaves the image vulnerable to artefacts and noise from a number of sources, most notably blood vessels, and selective activity patches are generally weak, because there is also a global signal, that is a rather homogeneous, non-selective activation of the whole cortex by visual stimulation.

Therefore, a second approach is to use cocktail-blank normalisation. Instead of using an unstimulated cortex as baseline, the response to each individual stimulus is divided by the summed response to the whole stimulus set. Every pixel in the image is thus calculated relative to the uniform visually evoked activation. This enhances stimulus specific domains (the mapping signal), while the global signal is cancelled out.

The problem with this method is that it introduces assumptions into the analysis, which may not always be fulfilled. Only stimuli from a continuous range can be used, and the whole range of possible stimuli must be used. For instance, if orientation selectivity were to be mapped, a set of different orientations separated from one another in equal steps and covering the whole 180 degrees of angle is necessary. In contrast, a complete range is harder to define, for example, for spatial frequency.

Even when the range of stimuli is appropriate, great care must be taken in analysing cocktail-normalised maps. Choosing a spatial frequency that evokes only localised, weak responses would leave wide parts of the cortex relatively inactive, and this results in spurious patches, if a cocktail-blank of orientation is used as baseline. In fact, it was the analysis of cocktail blank-divided orientation maps which led to the discovery of spatial frequency maps in primary visual cortex (Shoham *et al.*, 1997). A way to test the reliability of cocktail blank-normalised maps is to divide the cocktail blank by the actual blank response. Any patches that appear in these images are a sign of an inhomogeneous representation of the stimulus set in the cortex.

Due to these problems the cocktail blank is only suitable for cortical areas about whose functional architecture a great deal is already known. Fortunately, primary visual cortical maps have been well described allowing us to obtain reliable maps with the cocktail-blank approach in this brain area, even though it should nevertheless be used only alongside with the conventional blank baseline.

Finally, differential maps can be obtained by dividing the response to one visual stimulus by that of another, for example the horizontal orientation map by the vertical, or high and low spatial frequencies. This is the most appropriate way to calculate ocular dominance maps, because by dividing the response of the left and the right eye the response in distinct ocular dominance domains is amplified (and non-specific signal eliminated). Like the orientation-cocktail blank, differential maps make assumptions about the functional architecture and must therefore be interpreted with caution. A pixel showing no response in a differential image can either be the result of a genuine absence of a response or be due to the same response to both stimulus conditions. Inspecting the blank-divided left and right eye response is an essential test to verify any conclusions derived from differential ocular dominance maps.

6.3 Range-fitting and band pass filters

After the maps have been calculated, it is often wise to apply range-fitting and band pass filters. Range-fitting entails the clipping of pixel values at the tails of the pixel distribution. In other words, all the pixels whose values fall outside a specified range (defined by the experimenter to remove overexposure artefacts) are set to the minimum or maximum, respectively, and the values of the remaining pixels are rescaled to cover the full bit depth of the image (0-255 for 8-bit images). This is useful for the preparation of

illustrations in order to achieve good contrast images.

Furthermore, low-pass filters can be applied to smooth high frequency noise (for example, shot noise from random quantal fluctuation of emitted light) that is well above the spatial resolution of the imaged cortical domains. High-pass filters (unsharp masking) can be employed to remove large DC offsets. In many situations, however, this may be undesirable, because a significant source of information about the global cortical response level is lost. It is therefore advisable to analyse both the response of all pixels averaged across the region of interest alongside the higher frequency information obtained after high-pass filtering.

7

Data analysis

The kind of analysis performed on the images depends on the particular question under study. When investigating the ocular dominance map in visual cortex a key interest is the cortical territory occupied by the two eyes. An easy way to do this is by simply counting the pixels passing a criterion response threshold in the left and right-eye differential maps, and calculating the percentage for each (Kind *et al.*, 2002). However, more complex analysis is possible. From the response to each eye in the blank-normalised ocular dominance maps an OD index could be calculated for each pixel. The histogram of these values may give insight as to the degree by which cortical responses are balanced between the two eyes. This approach may prove useful for studying the binocularity of cortical responses, and perhaps even very small scale modulations of the cortical response (e.g. to dichoptic stimulation), but in my experiments it did not seem to yield information beyond that which can be more easily obtained with the conventional calculation of cortical territories.

Orientation single condition maps can be analysed in a similar fashion, by comparing the cortical area representing a particular orientation against that of its orthogonal. When inspecting orientation maps, it is often wise to calculate angle or polar maps by vectorial addition of the iso-orientation maps. In angle maps, orientation preference is pseudo-colour coded. In addition, in polar maps the intensity of a pixel denotes the intensity of the response.

Like with differential maps, it is of utmost importance to cautiously inspect the blank normalised single condition maps before drawing any conclusions on these colour coded images. A dark area in a polar map may represent either a genuinely low response or an equal response to all orientations (which would result in a null vector). Orientation selectivity for each pixel can be calculated by normalising the length of the orientation pref-

erence vector by the sum of all responses. It is also possible to conduct cross-correlation analysis on orthogonal iso-orientation maps, to test if they are complementary (ie. a low correlation coefficient).

Similar approaches are possible for any map obtained throughout the cortex. It is important, however, to be aware of the pitfalls of data processing. The less is known about the functional architecture of the brain area under study, the more caution is advised in analysing brain images. The safest approach is always to inspect blank-divided single condition maps to identify activated domains without making any assumptions. And even when a lot is known about the architecture of a brain area (as is the case for V1) one should always begin one's inspection with the unprocessed data and progress to more filtered, processed data from there.

7.1 Final remarks

Probably, the best approach to studying brain function is the combination of a number of methodologies thus overcoming the caveats of each method and bringing together several pieces of information that are unique to each. Comparing the results obtain with the different techniques allows more reliable conclusions to be drawn on the whole. Intrinsic signal imaging permits relatively precise spatial mapping of cortical architecture, but it lacks the temporal resolution to study reliably the tuning characteristics, interactions, and temporal evolution of neuronal responses. Therefore, it is beneficial to conduct electrophysiological recordings.

A quick and simple measurement to be taken is the visual evoked potential, in order to test the gross responsiveness of the cortex. This allows both for confirmation of the optical imaging results in terms of overall responsivity, as well as a physiological estimate of visual acuity (Berkley and Watkins, 1973; Freeman, Sclar, and Ohzawa, 1983). Further, with single-unit electrophysiological recordings, in which a microelectrode is inserted into the cortex, the discharges of one or more cells in the vicinity of the electrode tip can be distinguished. This technique allows the sampling of neurons in deep layers of the cortex that are inaccessible by optical imaging. More importantly, due to the excellent temporal resolution of unit recordings, peristimulus time histograms can be plotted, which give detailed insight in the distribution of action potentials across the time domain. Further, because this technique is the most direct measure of neuronal spiking it is extremely sensitive to subtle differences in responsivity, which enables it to distinguish mild modulations of the

response that cannot be visualised with optical imaging, and more exact measurements of tuning sharpness. The combination of unit recordings with optical imaging permits the targeted recording of locations of interest on the brain map instead of blindly sampling neurons from all over the cortex.

Recent developments allow optical imaging at a single cell resolution (Ohki *et al.*, 2005; Hübener and Bonhoeffer, 2005; Mrsic-Flögel *et al.*, 2006). The cortex is bulk loaded with a calcium dye (a marker of neuronal spiking activity), which is then taken up by neurons in the vicinity. Through two-photon microscopy high resolution images of the cortex are captured and the changes in fluorescence associated with the activity of individual neurons can be measured. With this approach very precise mapping of neuronal responses is possible, permitting insights that could previously only be inferred from the population statistics based on unit recordings. For example, it enabled the first direct demonstration that pinwheel centres in the orientation map contain a highly organised arrangement of sharply selective cells (Ohki *et al.*, 2006). It is clear that two-photon imaging opens up whole new avenues for neuroscience research, leading to a greater understanding of the functional organisation of the brain.

Anatomical, histological, and microbiological studies can also be performed on the tissue. After the functional imaging and electrophysiological observations have been made, the imaged brain area can be excised and the tissue analysed by microscopy or proteomic analysis. It can even be possible to remove only single cortical columns identified on the maps, in order to investigate subtle molecular differences between functional domains.

8

Choice of animal model

While much of the earlier research on ocular dominance plasticity focussed predominantly on felines, primates, and ferrets recent years have opened an avenue for rodent models of ocular dominance plasticity. The murine primary visual cortex is not segregated into ocular dominance columns, does not show an arrangement of orientation domains, and because of the small binocular visual field only a very moderate portion of cortex receives input from both eyes. Generally speaking, visual quality in these species is far below that of predators or primates, and they rely far more on their tactile and olfactory senses than on vision. Most importantly for studies on visual cortical plasticity, monocular deprivation does not cause true amblyopia in rodents, but only a moderate decrease (of approx. 30%) of the visual acuity in the deprived eye (Prusky and Douglas, 2003).

There is of course value in rodent models of OD plasticity, partly because of the efficiency for breeding them, the short period of development, and also (in mice) because of the possibility of genetic modifications. Further, optical imaging of intrinsic signals can be very easily applied in mice, as it is often possible to image through the thinned skull bone, making a craniotomy unnecessary (Hübener, 2003). Nonetheless, the fundamental differences between rodents and higher mammals underline the necessity for research on felines or even primates. Only a visual system akin to the human one can serve as a valid model for human visual function and for testing potential treatments for the impairment caused by deprivation. The feline visual system shares enough similarities with the primate one to make cats ideal models for these manipulations.

Chapter III

Experimental Chapter

9

Introduction

The mammalian cerebral cortex displays remarkable experience-dependent plasticity, in particular during a critical period early in life. Wiesel and Hubel (1963; 1965) showed in their classic experiments that an early period of monocular deprivation (MD) causes neurons in the primary visual cortex (V1) to become driven almost exclusively by the open, nondeprived eye. This physiological effect is mirrored anatomically by shrinkage of the deprived eye's ocular dominance (OD) columns (Shatz and Stryker, 1978). Vision through the deprived eye is severely degraded or lost altogether (Giffin and Mitchell, 1978; Mitchell, 1988) in this animal model of deprivation amblyopia (Mitchell, 1989).

While it is economical to allot more of the available neuronal resources to the processing of information from the good eye instead of wasting half on the deprived one, such dramatic changes to cortical function and visual capability after only transient periods of MD would appear to be maladaptive. Transient conditions such as injury or infections of lid margins or conjunctiva could have disastrous consequences, effectively rendering one eye useless. While even periods of deprivation as short as 6 hours can cause significant shifts in ocular dominance (Frank, Issa, and Stryker, 2001), it was recently shown that following a period of continuous monocular exposure, recovery is rapid (within a few days) and substantial, if the deprived eye is simply re-opened and the subsequent binocular experience is concordant (Mitchell, Gingras, and Kind, 2001; Mitchell and Gingras, 1998; Kind *et al.*, 2002). I asked here how successive periods of monocular and binocular vision each day are weighted in terms of their influence on visual cortical function. If all types of visual experience were equally instructive, then one would expect to observe cortical deprivation effects of graded severity proportional to the ratios of monocular and binocular visual exposure. Recent behavioural studies indicated that this might not be the case.

Mitchell and colleagues (2003; 2006) found that two hours of binocular experience per day allowed kittens to develop normal grating acuity for both eyes despite the animals receiving five hours of monocular vision each day. In this context, it made no difference whether the period of daily mixed monocular and binocular experience followed a month of dark-rearing from birth, or whether it was preceded by a month of normal binocular visual experience, which presumably led to the establishment of a normal, predominantly binocular V1. Moreover, in monkeys reared with similar regimens the deprivation-induced loss of grating acuity and contrast sensitivity was greatly reduced when a period of unrestricted, binocular vision was intercalated between much longer daily phases of monocular deprivation by a diffusing contact lens (Wensveen *et al.*, 2006).

The intriguing behavioural consequences of mixed normal and abnormal experience pose questions concerning its neural basis and, in particular, the role of visually driven activity in development of the visual cortex. In an earlier single-cell study (Olson and Freeman, 1980), kittens that had experienced daily regimens of 4 *h* of monocular and 14 *h* of normal binocular vision exhibited, perhaps unsurprisingly, normal ocular dominance distributions. A recent study investigating the ocular dominance changes when MD alternates between the two eyes (similar to the alternation of which eye is fixating found in some strabismic monkeys; Horton, Hocking, and Adams, 1999) reported that only if the duration of MD in one eye is over an order of magnitude greater than in the other a significant loss of OD by the less used eye is found (Mower, 2005). Yet, while intriguing, this paradigm is very abnormal and leads to an almost complete loss of binocularity in cortical responses.

I therefore set out to determine the minimum requirements for maintaining normal V1 function. Kittens were reared with various regimens of daily periods of monocular and binocular exposure. The V1 ocular dominance architecture was assessed through optical imaging of intrinsic signals, a technique that gathers information from a very large number of cells and thus overcomes the problem of sampling bias inherent to single-cell recordings (Bonhoeffer and Grinvald, 1996; Zepeda, Arias, and Sengpiel, 2004). I appraised the relative cortical area of ocular dominance domains dedicated to the two eyes, as well as visually evoked potentials as a physiological measure of grating acuity (Berkley and Watkins, 1973; Freeman, Sclar, and Ohzawa, 1983) and single-unit electrophysiological recordings to characterise the properties of visual cortical neurons.

10

Materials and Methods

10.1 Rearing details

All procedures were approved by local ethical review and covered by UK Home Office licenses. Fifty-two cats were used in this study. Selective rearing was carried out for about 3 weeks, starting at the peak of the critical period between postnatal days 30 and 35. Subjects were subdivided into two cohorts: 23 subjects were permitted 7 *h* of visual exposure per day, the other group of 26 subjects only 3.5 h. An additional three subjects received just 0.25 *h* visual exposure, all of which was binocular. For the remainder of the day animals were kept in complete darkness, together with their mother. The room in which they were reared contained cardboard boxes, toys, and furniture for environmental enrichment. Animals were encouraged to play to keep them awake and active during the period of visual exposure.

Each animal was assigned to one of various rearing regimens, which determined the order and duration of MD and binocular exposure (BE) it received on a daily basis (see Figure 10.1). The period of BE was 0 *h*, 0.25 *h*, 0.5 *h*, 1 *h* or 2 *h* and either preceded or followed the period of MD. Six subjects were permitted two binocular periods of 0.25 *h* which flanked the monocular period. Four subjects in each cohort served as controls and received binocular vision only. Finally, two subjects were reared with 1 *h* of discordant binocular exposure followed by 2.5 *h* of MD. Discordant vision was achieved by means of binocular prism goggles inducing either divergent (Fig. 10.1P) or convergent (Fig. 10.1Q) artificial squint of 8° (4° in each eye). Deprivation was carried out by means of completely opaque eye patches made from surgical face masks that were fastened with Velcro bands.

During the period of visual exposure the animals were monitored regularly to readjust

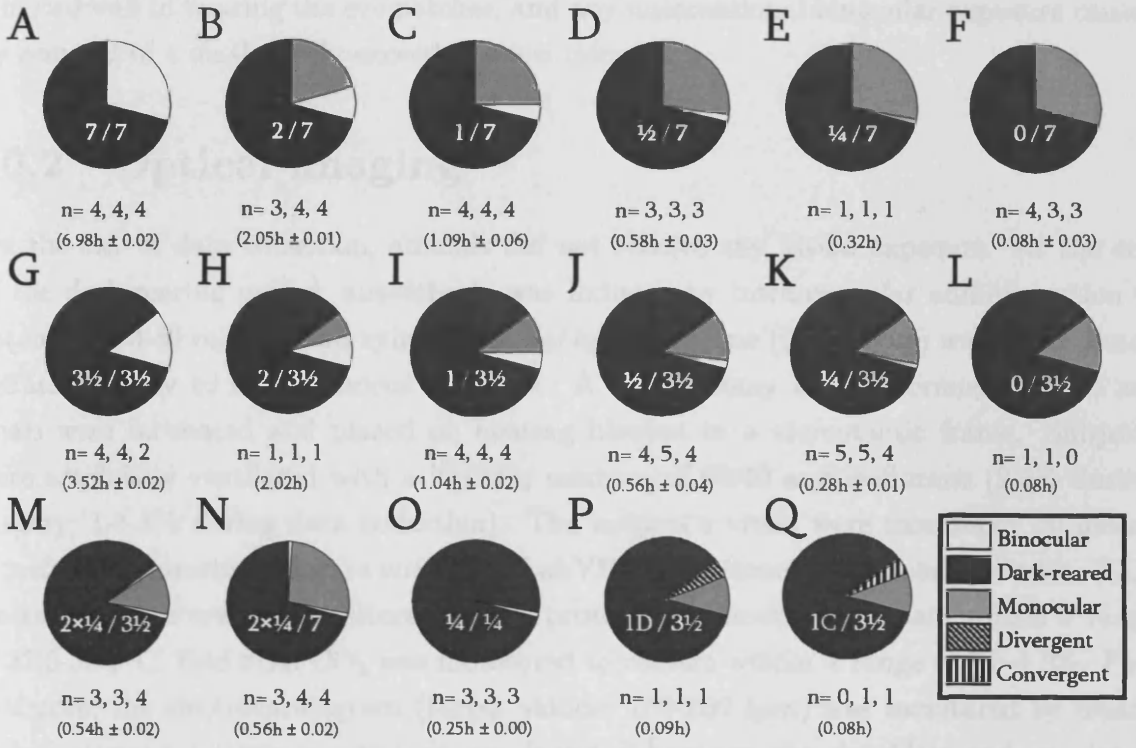


Figure 10.1: *Selective rearing regimens.* Iconic representations of all the mixed experience regimens used in this study (cf. legend). The fraction shown within each icon depicts the duration of binocular exposure out of the overall visual exposure animals were permitted per day. Underneath each icon is the number of subjects, n, in each experimental group (shown separately for optical imaging recordings, visual evoked potentials, and single-unit recordings, respectively). In parentheses the group averages (± standard error) of daily binocular exposure are indicated. (**A-F**) cohort with 7 h of daily vision. (**G-L**) cohort with 3.5 h of daily vision. In either cohort the binocular period could precede or follow monocular deprivation, however, only the BE-after conditions are shown here. (**M-N**) Split binocular exposure groups with 3.5 h (**M**) and 7 h (**N**) overall visual exposure. (**O**) 0.25 h BE only controls. (**P-Q**) Two animals were reared wearing divergent (**P**) and convergent (**Q**) prisms to induce artificial strabismus for one hour, followed by 2.5 h of monocular deprivation by eyepatch.

the masks, if necessary. Whenever a mask slipped and permitted binocular experience this was recorded and the average maximal time of binocular experience was calculated for each subject at the end of the rearing period. After the first few days, subjects usually adapted well to wearing the eye patches, and any unintentional binocular exposure caused by removal of a mask rarely exceeded a few minutes.

10.2 Optical imaging

On the day of data collection, animals did not receive any visual exposure. At the end of the dark-rearing period, anaesthesia was induced by intramuscular administration of ketamine (20-40 mg/kg) and xylazine (4 mg/kg). Atropine (0.2 mg/kg) was also injected intramuscularly to reduce mucus secretion. A tracheotomy was performed and the animals were intubated and placed on heating blanket in a stereotactic frame. Subjects were artificially ventilated with a N_2O:O_2 mixture of 60:40 and isoflurane (2-3% during surgery; 1-1.5% during data collection). The subject's vitals were monitored by means of patient monitoring software written in LabVIEW (National Instruments, Austin, TX). Rectal temperature was monitored with a probe thermometer to remain within a range of 37.5-38.0°C. End tidal CO_2 was monitored to remain within a range of 3.5-4.0%. Furthermore, the electrocardiogram (target values: 150-200 bpm) was monitored by means of two electrodes (syringe canulas), one inserted between the shoulders and one laterally on the abdomen. If any of the values diverged from the described target values adequate actions were taken. As a simple measure of the animal's state of arousal, the electroencephalogram was also monitored throughout the experiment with two silver wire electrodes inserted into the skull at two points anterior to the recording chamber (one near the midline and another approximately 1 cm lateral from that position). Ophthalmic atropine (1%) and phenylephrine (2%) eye drops were administered to the eyes, which were fitted with gas-permeable contact lenses, to protect them and to focus the animal's vision onto the stimulus display.

An intravenous (i.v.) catheter was inserted in one of the hind legs for administration of drugs and for a continuous infusion of 4% glucose in saline at a rate of 3 $ml/kg/h$; the infusion solution also contained dexamethasone (Dexafort, Intervet, UK; 0.2 $mg/kg/h$) for prevention of cortical oedema, and gallamine triethiodide (Sigma, UK; 10 $mg/kg/h$) for prevention of eye movements. The posterior portion of the lateral gyrus, containing the central visual field representation of the primary visual cortex, was exposed in both

hemispheres through craniotomy. For some animals the dura was also removed, because its opacity and vascularisation would have compromised data collection. The cortical surface was carefully cleared and kept free from any traces of blood using Sugi sterile swabs (Kettenbach, Eschenburg, Germany). A titanium chamber was cemented to the skull and sealed on the inside with dental wax. The chamber was filled through an inlet with silicone oil and closed with a cover slip (Bonhoeffer and Grinvald, 1996).

Initially, the exposed brain was illuminated with green light and a reference image of the surface vascular pattern was taken. Subsequently, the cortex was illuminated with red light at 700 nm. Intrinsic signals were recorded using an enhanced differential imaging system (Imager 2001, Optical Imaging Inc., Mountainside, NJ), with the camera focused approx. 500 μm below the cortical surface. Images were therefore obtained primarily from layers II/III, which are thought to play a key role in initiating cortical plasticity in response to altered visual experience (Trachtenberg, Trepel, and Stryker, 2000; Trachtenberg and Stryker, 2001). The imaged area subtended about 12 mm by 9 mm.

Using an ophthalmoscope to determine the focal distances, the animal's eyes were focused on a 21" computer monitor (distance, 33 cm) on which stimuli were displayed by a visual stimulus generator (VSG Series 3; Cambridge Research Systems, Rochester, UK). They consisted of high contrast sinusoidal or square-wave gratings (0.1 - 0.6 $cycles/°$; mean luminance 38 cd/m^2) of four different orientations (0°, 45°, 90° and 135°) drifting at a temporal frequency of 2 Hz, randomly interleaved with trials in which the screen was blank. Activity maps were analysed using IDL (RSI, Boulder, CO) and MatLab software (The Mathworks, Inc.). Single-condition responses (averages of 48-64 trials per eye and orientation) were divided (a) by responses to the blank screen, and (b) by the sum of responses to all four orientations ("cocktail blank"; Bonhoeffer and Grinvald, 1996) to obtain iso-orientation maps. Orientation preference maps were calculated by vectorial addition of four blank-divided iso-orientation maps and pseudo-colour coded.

Stimuli were presented to the two eyes separately in randomised sequence by means of shutters placed in front of the animal. Ocular dominance maps were calculated by dividing all responses to one eye by the responses to the other. The actual signal used for subsequent quantitative analysis was reflectance change ($\Delta R/R$) for each pixel, given at 16-bit precision. For analysis of the relative strength of responses through the two eyes, images were only low-pass filtered (smoothed). For analysis of areas responding preferentially through one or the other eye (see below), images were additionally high-pass filtered well above the periodicity of ocular dominance domains (cut-off, 200 pixels =

7.8 *mm*) to level the image intensity across the region of interest. For illustrations, signals were range-fitted such that the 1.5% most responsive (least responsive) pixels were set to black (white), and Gaussian averaging over 6 pixels was applied to remove high-frequency noise. Signal amplitude was displayed on an 8-bit grey-scale.

To quantify cortical territory occupied by the two eyes, for each hemisphere a region of interest (RoI) was defined. I manually excluded blood vessel and other artefacts, using an image of the cortical surface taken under green-light illumination for guidance. Based on differential responses to gratings of high (0.4-0.6 *cycles*/°) and low (0.1-0.2 *cycles*/°) spatial frequency, analysis was restricted to V1 (Bonhoeffer *et al.*, 1995). In order to minimise subjectivity in defining the RoI, MatLab software shifted the RoI by ± 10 pixels in x- and y-coordinates and calculated mean results across all shift conditions. The ratio of the numbers of pixels responding more strongly to the left and the right eye, respectively, were calculated. Finally, the percentages of pixels responding to the deprived eye and nondeprived eye were averaged across both hemispheres.

Iso-orientation maps were analysed by calculating an orientation selectivity index (OSI) for each pixel, which represents the length of the orientation preference vector normalised against the response strength (Kind *et al.*, 2002):

$$OSI = \frac{\sqrt{(R_0 - R_{90})^2 + (R_{45} - R_{135})^2}}{R_0 + R_{45} + R_{90} + R_{135}} \tag{10.1}$$

where R_x denotes the response measured during stimulation with orientation x. The mean OSI across all the pixels in the region of interest was then calculated separately for each eye, and the percentage of the mean OSI through the deprived eye relative to that through the nondeprived eye was taken as a measure of the deprivation-induced change in orientation selectivity.

10.3 VEP recording

After imaging data acquisition was completed, the chamber was reopened and the silicone oil replaced with saline for visual evoked potential (VEP) recording. A silver ball electrode was placed on the surface of the primary visual cortex near the representation of the area centralis (approximate Horsley-Clarke coordinates, P4 L2; Horsley and Clarke, 1908). The recorded signal was amplified by a factor of 20,000 and low-pass filtered (cut-off, 300 *Hz*). Usually, four recordings were made, recording from each hemisphere and stimulating

each eye separately. Stimuli were displayed on a computer screen at a distance of 100 *cm* (which was longer than in the optical imaging experiments because at very high spatial frequencies square-wave gratings would otherwise produce aliasing effects) and consisted of high contrast phase-reversing square wave horizontal gratings that varied only in spatial frequency, typically from 0.14-2.26 *cycles/°*. For animals, which showed a very pronounced deprivation effect, an additional set of low frequencies (0.05-0.4 *cycles/°*) was used to test the deprived eye. Gratings reversed contrast at a rate of 1 Hz and drifted upwards at a velocity of 0.1 *cycles/sec*. Moreover, a blank screen was used to measure the baseline response. Stimuli were presented to the left and right eye separately for 3 *sec*, corresponding to six contrast reversals, with inter-stimulus intervals of 3 *sec*. Responses were averaged across the six contrast reversals per presentation and across 10-20 presentations of each stimulus using software written in LabVIEW (National Instruments, Austin, TX). The resulting signals therefore constituted the responses to 60-120 contrast reversals each per stimulus. The total amplitude of the VEP signal was defined as the difference in voltage between the signal peak and the subsequent trough within a 500 *msec* window.

As a physiological measurement of visual acuity, the VEP cut-off point was determined from the VEP amplitude vs. spatial frequency curve in two different ways. First, as a completely computerised method, a straight line was fitted through the final 3-4 descending data points, and the spatial frequency of its intersect with a line corresponding to the blank response plus standard error was calculated (Schwarzkopf *et al.*, 2007). However, these findings may have been confounded by the large standard error in some traces that nonetheless showed a good visual evoked response: in subjects showing a strong deprivation effect the spatial frequency-response curve is near horizontal, which makes the fitting procedure difficult.

Therefore, I calculated the noise level for each stimulus by averaging the data across 5 bins as opposed to the 6 bins according to contrast reversal. The amplitude of this noise was extracted as described above, and the mean noise amplitude across all stimulus presentations was defined as the baseline level from which the cut-off was extrapolated by visual inspection of the stimulus-response curves. To preclude subjective bias inherent to such an analysis, all VEP data were analysed blind to the experimental condition of the animal by hiding the experiment ID and conducting the analysis in a randomised order.

Further, a ratio of the VEP amplitudes through the two eyes was calculated by dividing the sum of the amplitudes in response to the 4 lowest spatial frequencies for the deprived

eye by the same sum obtained for the nondeprived eye.

10.4 Single unit recording

Single-cell recordings were carried out using an array of four tungsten-in-glass microelectrodes (Ainsworth, Welford, UK), controlled by a stepping motor (Alpha-Omega, Nazareth Illit, Israel). Penetration sites were chosen on the basis of the ocular dominance map such that a representative sample from patches dominated by both eyes was obtained. Usually, two penetrations were made in each cortical hemisphere.

The discharges of individual neurons were isolated offline with Brainware software (TDT, Alachua, FL) based on their spike waveforms (defined in a multidimensional feature space using characteristics such as the 1^{st} peak to amplitude ratio, the peak to peak ratio, and the peak latencies), and the spike count per stimulus presentation was recorded for each neuron. A Wheatstone stereoscope was placed in front of the subject in order to stimulate both eyes independently. Left- and right-eye responses to drifting gratings (of optimum spatial frequency) of 16 different directions in 22.5° steps were averaged over 5 trials of 1.5 *sec* duration (inter-stimulus interval, 2.5 *sec*). In order to determine the optimal spatial frequency for each neuron, spatial frequency curves were obtained with stimuli from 0.1-4.52 *cycles*/° in half octave increments.

For each neuron, Gaussian curves were fitted to the orientation tuning curves for the left and right eye, respectively, and the half width at half height (hWhH) of the fitted Gaussian was calculated as a measure of tuning sharpness. An ocular dominance index, ODI, was calculated as,

$$ODI = \frac{DE - NE}{DE + NE} \tag{10.2}$$

where DE is the mean response to the deprived eye and NE that to the normal eye. This yields values between -1 and 1 with negative (positive) numbers indicating a dominance of the nondeprived (deprived) eye. This range was further divided into seven equal bins, which were used to assign an ocular dominance category akin to the classification by Hubel and Wiesel (1962). Thus, neurons in category 1 were driven exclusively by the nondeprived eye, cells in category 7 by the deprived eye, and those in category 4 received balanced input from both eyes. The numbers of cells were pooled across experimental conditions and ocular dominance histograms were plotted. Finally,

similar to how it was previously described (Murphy and Mitchell, 1986) a binocularity index, BI, was calculated for each subject,

$$BI = \frac{N_4 + \frac{2}{3}(N_3 + N_5) + \frac{1}{3}(N_2 + N_6)}{N} \tag{10.3}$$

where N_x indicates the number of cells in OD category x and N the total number of cells recorded.

For most cells in the 3.5 h cohort, and a number of cells in the 7 h cohort, relative interocular phase disparity tuning curves were recorded by presenting the optimal grating stimulus at 8 different phases (from -135° to 180° in 45° steps) to the non-dominant eye, while the dominant eye viewed a grating with its optimal orientation and spatial frequency. In addition, a monocular response was recorded with only the dominant eye stimulus, and a blank screen being presented to the other eye. Finally, for a number of subjects in the 3.5 h cohort, responses to rivalrous stimuli were collected: the dominant eye was again presented with an optimal stimulus, while the other eye viewed a grating rotated ± 90°, ± 60°, ± 30°, ± 20°, ± 10° or 0° relative to the optimal. Monocular responses for each eye were also recorded separately.

Phase disparity tuning was assessed using a method described by Ohzawa and Freeman (1986b): a sine-wave was fitted to responses to the eight disparity stimuli (averaged across trials) and a binocular interaction index, BII, was calculated as the ratio of the sine amplitude and the mean response to all disparity stimuli. In addition, a signal-to-noise ratio, S/N was calculated by dividing the sine amplitude by the residual root mean square error of the fit. Based on visual inspection of neurons by the experimenter, only neurons that showed a BII > 0.25 and a S/N > 1 were classified as phase-selective.

The data from the rivalry stimulation protocol were analysed by visual inspection as follows: if the peak binocular response was more than one standard error greater than the monocular response to the dominant eye, the neuron was defined as *selective-facilitative.* If most of the binocular responses were more than one standard error below the monocular response, the neuron was defined as *selective-suppressive.* If all the dichoptic stimuli elicited a response below the monocular one, the cell was classified as *suppressive-only.* Finally, if there was no consistent difference between the binocular and monocular responses, the neuron was defined as showing no binocular rivalry effects.

After all data collection was completed, the animals were sacrificed with an i.v. overdose of barbiturate.

11

Results

11.1 Ocular dominance maps

The relative representation of the two eyes in V1 as a function of daily binocular exposure was determined from ocular dominance maps generated by optical imaging. Figure 11.1A shows typical examples from the first cohort that was permitted 7 *h* of total daily visual experience. The top row of maps shows responses through the deprived eye (dark patches); the bottom row of maps from the same animals shows responses through the nondeprived eye. For each animal, both visual cortex hemispheres are shown (occipital pole at bottom). It is immediately apparent that the longer the period of daily binocular exposure (from left to right, 0 *h*, 0.5 *h*, 1 *h*, 2 *h*) the larger was the area responding to deprived-eye stimulation. Conversely, in the animal with 0 *h* binocular exposure most of the imaged cortex responded exclusively to nondeprived eye stimulation but this over-representation decreased dramatically in the 0.5 *h* condition and further still in the 1 *h* and 2 *h* conditions. This is illustrated more clearly in Fig. 11.5A, which shows the deprived eye domains in three of these animals highlighted with red outlines.

The subject displayed from the 0 *h* condition (i.e. no binocular exposure) exhibited an ocular dominance pattern typical of a kitten monocularly deprived by lid suture (Faulkner, Vorobyov, and Sengpiel, 2005), with the deprived eye dominating only 21.1% of the cortical surface (9.7% of cortical territory in the hemisphere ipsilateral to the deprived eye and 32.5% in the contralateral hemisphere). In total, 5 animals were raised without any binocular exposure (see Fig. 10.1). In these animals, the deprived eye dominated, on average, 20.3% (± 3.3% SEM) of the V1 surface, compared with 16.2% reported previously in animals of similar age deprived by lid suture (Faulkner, Vorobyov, and Sengpiel, 2005).

If anything, the patching regimen appeared to have a slightly less (but not significantly so) detrimental effect on the cortical representation of the affected eye than monocular lid suture.

The animal that was permitted 0.5 hours of BE exhibited a slightly reduced representation of the deprived eye as compared with the other eye, which was dominant for 35.7% of the cortical surface. However, in marked contrast, the two subjects that received 1 or 2 hours of BE per day, respectively, exhibited ocular dominance maps typical of normally reared kittens. A contralateral bias was evident in both hemispheres of the latter animals (i.e. the left hemisphere being dominated by the right eye and vice versa), and the deprived eye dominated, respectively, 46.7% and 53.5% percent of the cortical surface. Therefore, a relatively short daily period of normal visual experience was sufficient to completely offset a much longer period of abnormal, monocular vision (6 *h* and 5 *h*, respectively).

In order to assess whether the absolute amount of daily binocular vision or the ratio of binocular to monocular experience was the critical factor for the physiological outcome, I examined a second cohort of animals that were allowed 3.5 *h* of daily vision. Figure 11.1B shows representative maps from subjects that received, respectively, 0.25 *h*, 0.5 *h*, and 1 *h* binocular exposure. While the first exhibited a marked effect of monocular deprivation on OD architecture (with only 23.3% of the cortex dominated by the deprived eye), the maps from the latter two animals appeared normal or close to normal despite the brevity of daily binocular vision (0.5 *h* BE, 35.8% deprived-eye territory; 1 *h* BE, 52.6% deprived-eye territory). By comparison, animals reared with only 0.25 *h* BE per day, but no MD (n = 3) exhibited normal ocular dominance maps (Figure 11.2A-B) with roughly equal cortical territory representing the two eyes (46.8 $\pm$ 2.0% left-eye territory, compared to 46.7 $\pm$ 6.1% and 51.2 $\pm$ 4.0% in controls, which received, respectively, 3.5 *h* and 7 *h* of daily binocular exposure).

Quantitative analysis of images from all animals confirmed that brief daily periods of binocular vision offset much longer periods of monocular vision so that the latter had virtually no effect on OD representation in V1. Analysis of surface area data for each cortical hemisphere separately revealed the same principal result; 0.5 *h* of daily BE were sufficient to maintain a share of territory that was not significantly different from those for the contralateral or ipsilateral eye, respectively, in normal animals (Fig. 11.3A-B). Of course, because of the contralateral bias in the cortical representation of the two eyes, absolute values differed considerably between the two hemispheres. For further analysis,

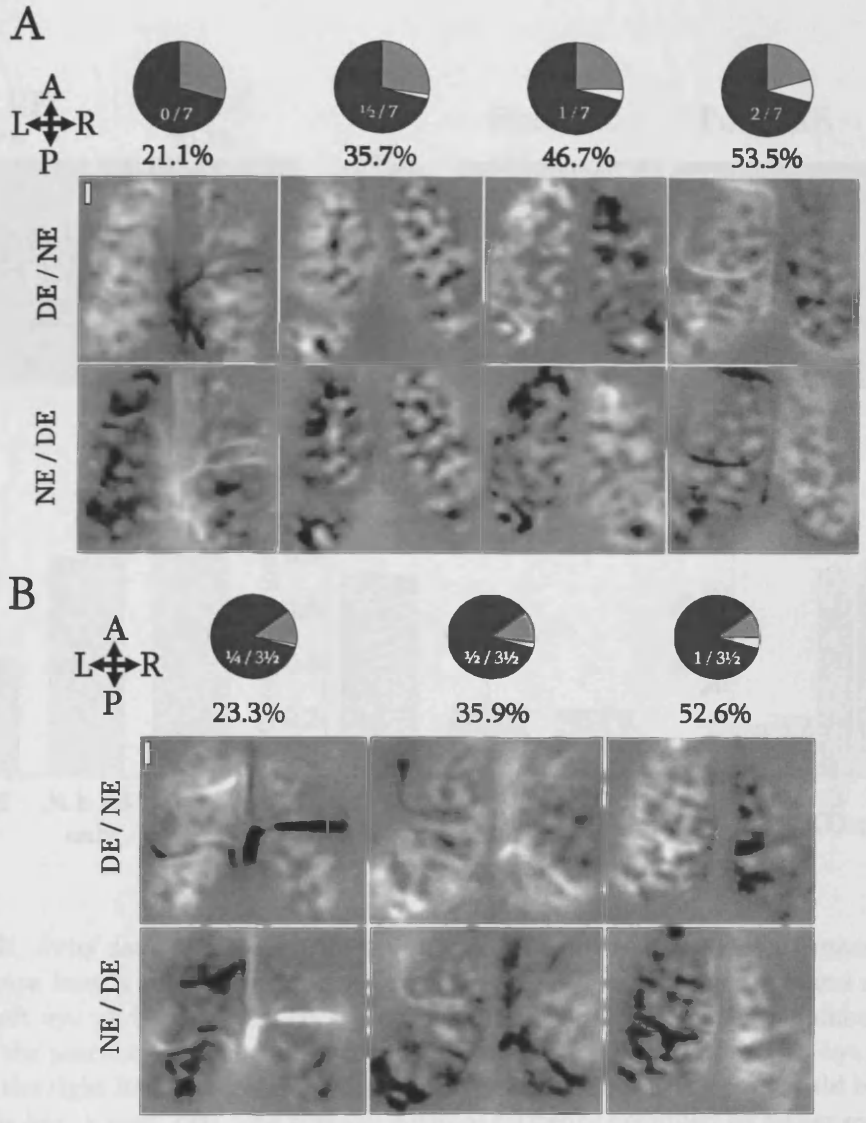


Figure 11.1: *Representative ocular dominance maps.* Differential ocular dominance maps of subjects from various rearing conditions. For each subject, the experimental condition is denoted by the icon above the pair of ocular dominance maps obtained from V1 for left- and right-eye stimulation, respectively. The numbers indicate the percentage of cortical territory dominated by the deprived eye. In the row of maps labelled DE/NE, dark areas correspond to cortical domains activated by the deprived (left, DE) eye, in the row labelled NE/DE dark areas represent cortical areas responding to the nondeprived (right, NE) eye. Because of the way the OD maps are calculated (see Methods), the images in the two rows are "negatives" of each other; they are both shown in order to facilitate by-eye comparisons between the effects of the different rearing conditions. (**A**) Subjects from the cohort with 7 h of total daily visual exposure. (**B**) Subjects from the cohort with 3.5 h of daily vision. Scale bar, 1 *mm*.

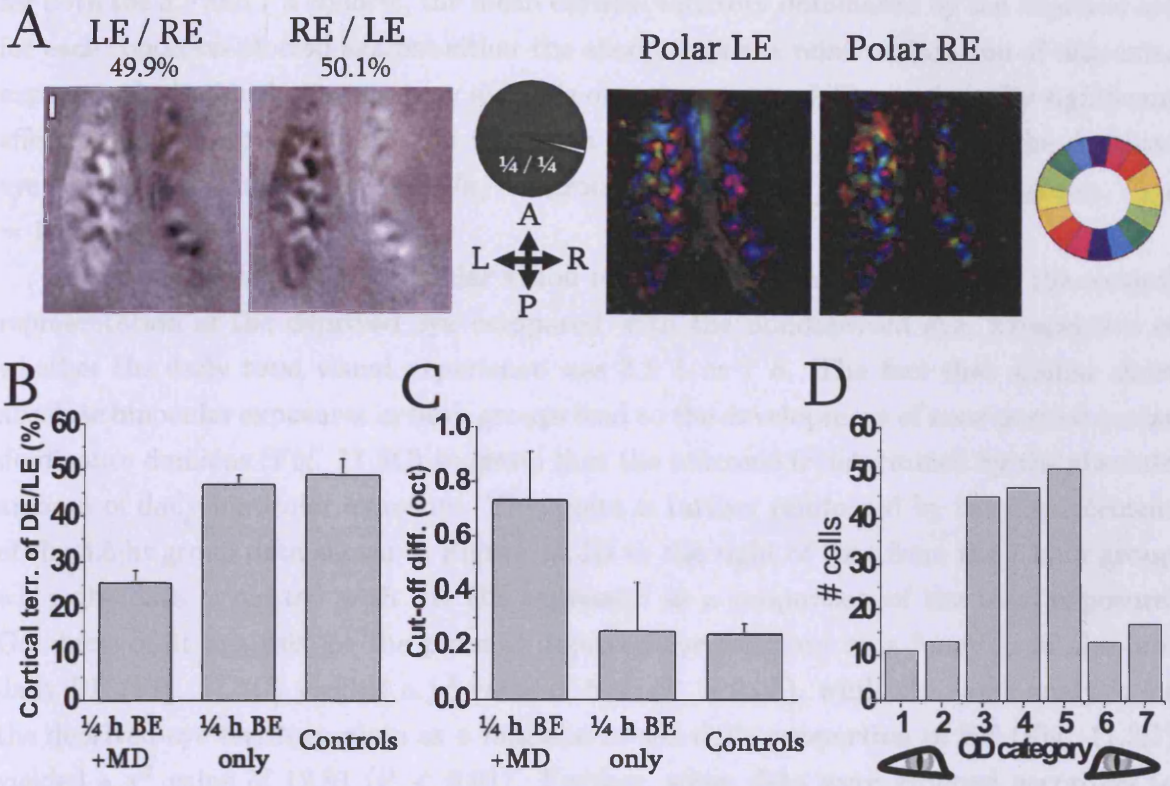


Figure 11.2: *Brief daily binocular experience results in normal cortical responses.* (**A**) Ocular dominance maps from a subject with only 0.25 h binocular exposure per day (and no monocular exposure). Left eye and right eye ocular dominance map are shown. The number above each map denotes the percentage of cortical territory representing the left and right eye, respectively. Note that on the right hemisphere, only the medial part of the lateral gyrus could be exposed for imaging. Scale bar, 1 *mm*. (**B**) The average cortical territory occupied by either eye in 3 kittens that had 0.25 h of BE only (and no other, monocular experience) was close to 50% just like in 8 control animals that had 3.5 h or 7 h of BE. In contrast, for 6 animals that had experienced 0.25 h BE and either 3.25 h or 6.75 h of MD (BE + MD, see Fig. 10.1), the deprived eye occupied on average 25.5% of cortical territory. (**C**) The average VEP cut-off difference for the same experimental groups as shown in B. In contrast to animals that showed a marked deficit after having received 0.25 h BE paired with monocular deprivation, kittens with only 0.25 h BE exhibited normal VEPs. (**D**) Pooled ocular dominance histogram from 3 kittens with only 0.25 h of daily binocular vision but no monocular deprivation. The distribution is normal with the majority of cells being binocular and small number of monocular cells driven by only the left or right eye, respectively.

the results from both hemispheres were averaged for each animal. Figure 11.3C-D shows for both the 3.5 and 7 *h* cohorts, the mean cortical territory dominated by the deprived eye for each condition plotted against either the absolute or the relative duration of binocular exposure. In both cohorts, one-way analysis of variance revealed a statistically significant effect of experimental condition on the mean cortical territory occupied by the deprived eye across both hemispheres (AnoVa, 7 *h* group, $F_{4,10} = 7.52$, $p < 0.01$; 3.5 *h* group, $F_{4,10} = 10.2$, $p < 0.01$).

As little as 0.5 *h* of daily binocular vision resulted in only a small deficit in the cortical representation of the deprived eye compared with the nondeprived eye, irrespective of whether the daily total visual experience was 3.5 *h* or 7 *h*. The fact that similar short absolute binocular exposures in both groups lead to the development of near-normal ocular dominance domains (Fig. 11.3C) suggests that the outcome is determined by the absolute amount of daily binocular exposure. This point is further reinforced by the displacement of the 3.5 hr group data shown in Figure 11.3D to the right of that from the 7 hour group when the data is plotted with the BE expressed as a proportion of the total exposure. Goodness-of-fit analysis for the plots of deprived-eye territory as a function of absolute daily BE (Fig. 11.3C) yielded a χ^2 value of 5.59 ($P > 0.05$), while the same analysis for the deprived-eye territory plots as a function of the daily proportion of BE (Fig. 11.3D) yielded a χ^2 value of 12.81 ($P < 0.01$). Further, when data were grouped according to their absolute daily BE no significant difference between cohorts (2-way AnoVa, $F < 1$) was found, but data grouped by their relative daily BE differed significantly between cohorts (2-way AnoVa, $F_{1,20} = 8.72$, $p < 0.01$). Taken together this suggests that the absolute amount of binocular exposure is critical in terms of the resulting cortical ocular dominance. I therefore pooled data from the 7 *h* and 3.5 *h* cohorts and plotted deprived-eye territory against absolute daily BE (Fig. 11.4A). The data were well fitted with an exponential function ($r^2 = 0.94$), which allowed extrapolation of the amount of BE needed to reduce the deprivation effect by 50% ($t_{50} = 0.42$ h) and by 95%, respectively ($t_{95} = 1.79$ h).

There was no significant difference (2-way AnoVa, $P > 0.1$) in terms of cortical territory occupied by the deprived eye between animals in which the daily period of binocular exposure had preceded the period of monocular deprivation and those in which it had followed it (Fig. 11.4B).

Since it could be argued that the first effect of brief or intermittent MD on cortical ocular dominance is a weakening of responses through the deprived eye rather than

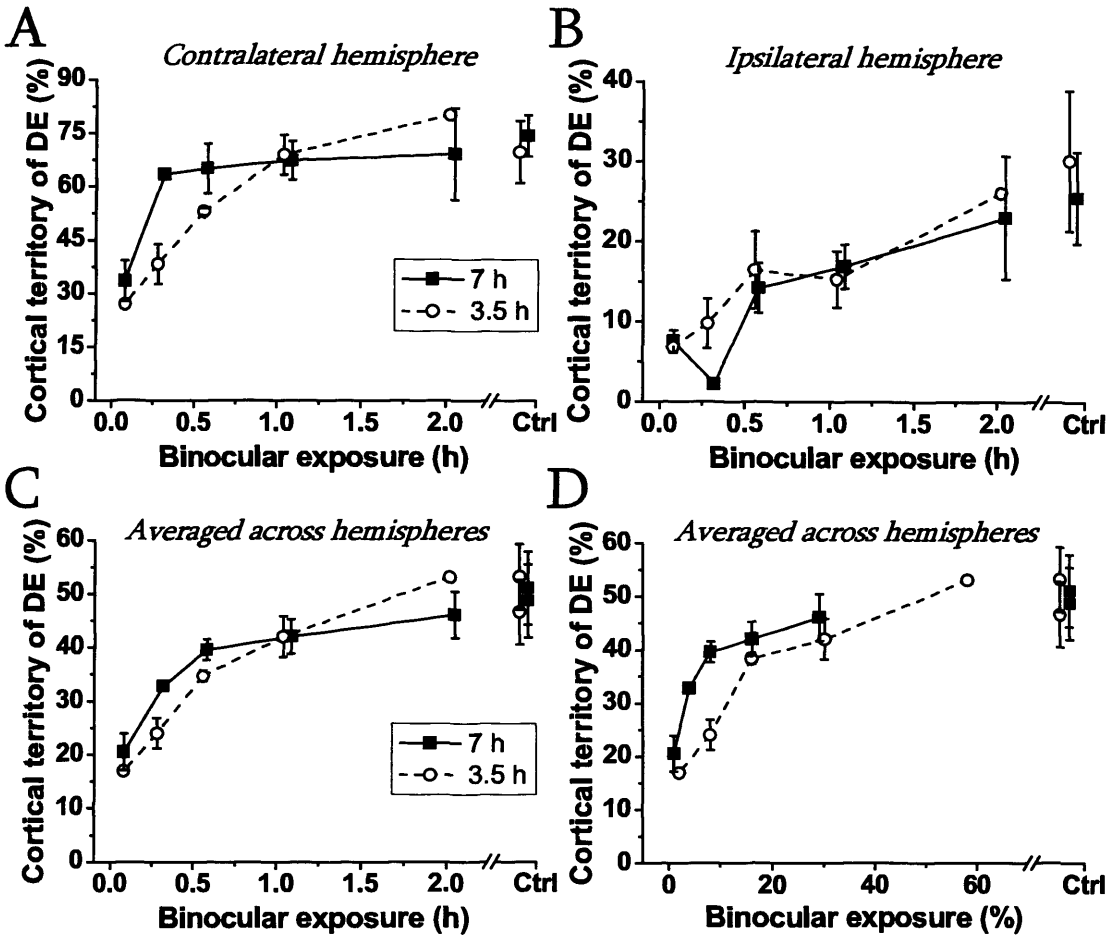


Figure 11.3: *Ocular dominance balance depends on daily binocular experience.* The percentage of cortical area dominated by the deprived eye (DE) in each experimental condition is plotted against the amount of binocular vision provided. (**A**) shows data for the hemispheres contralateral to the DE, (**B**) those for the ipsilateral hemispheres. Data from control animals, which did not receive any monocular experience, are displayed as the mean of two hemispheres obtained when the left or the right eye were taken as the deprived one. Panels (**C**) and (**D**) depict the mean values averaged across both cortical hemispheres. In (**C**), data are plotted against the absolute duration of daily binocular vision; in (**D**), data are plotted against the percentage of binocular vision relative to total daily vision. Data from control animals are plotted separately for the left or the right eye, because none of the eyes was deprived. Filled squares: 7 h cohort. Open circles: 3.5 h cohort. All data represent mean $\pm$ 1 standard error; absence of error bars indicates n = 1.

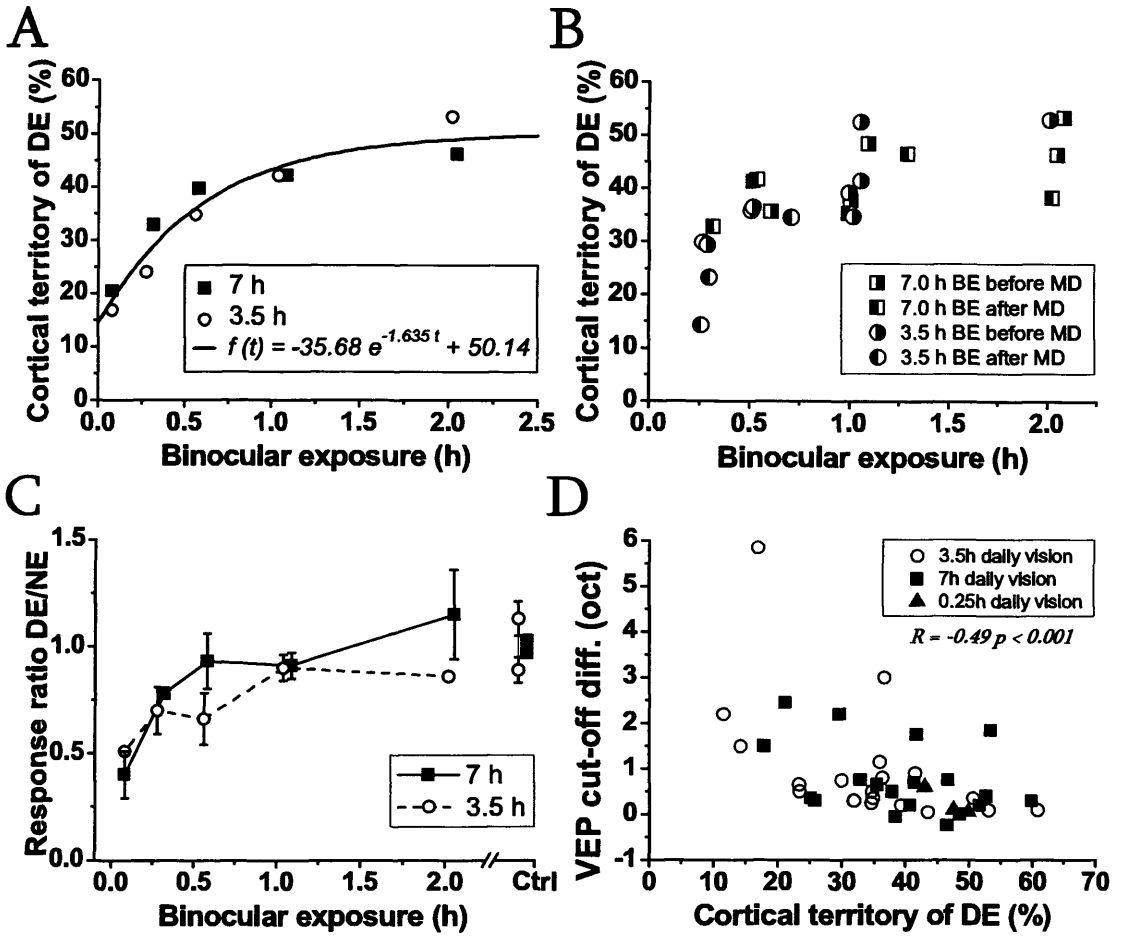


Figure 11.4: *Further analysis of ocular dominance maps.* (**A**) Pooled data from the 7 h and 3.5 h cohorts are plotted against the absolute duration of daily binocular vision, and an exponential function (see inset) is fitted to the data (solid line). In (**B**), data from all individual animals from both 7 h and 3.5 h cohorts are shown that received mixed daily binocular and monocular vision, with the shading of symbols indicating whether binocular experience preceded or followed monocular deprivation (see inset). (**C**) Ratio of absolute strength of responses through the deprived and nondeprived eyes for the 3.5 h cohort (open circles, dashed lines) and the 7 h cohort (filled squares, solid lines), as determined from the regions of interest in unfiltered images, averaged across both cortical hemispheres (see text). All data represent mean ± 1 SEM; absence of error bars indicates n = 1. (**D**) Correlation of visual acuity deficit, as determined from the difference in high-frequency cut-off points between deprived and nondeprived eye in each animal, and cortical territory occupied by the deprived eye, as determined from optical imaging maps. Animals from the cohort that received 3.5 h of total daily visual experience are represented by open circles, animals from the 7 h cohort by filled squares. Three animals that received just 0.25 h of BE a day are shown as filled triangles.

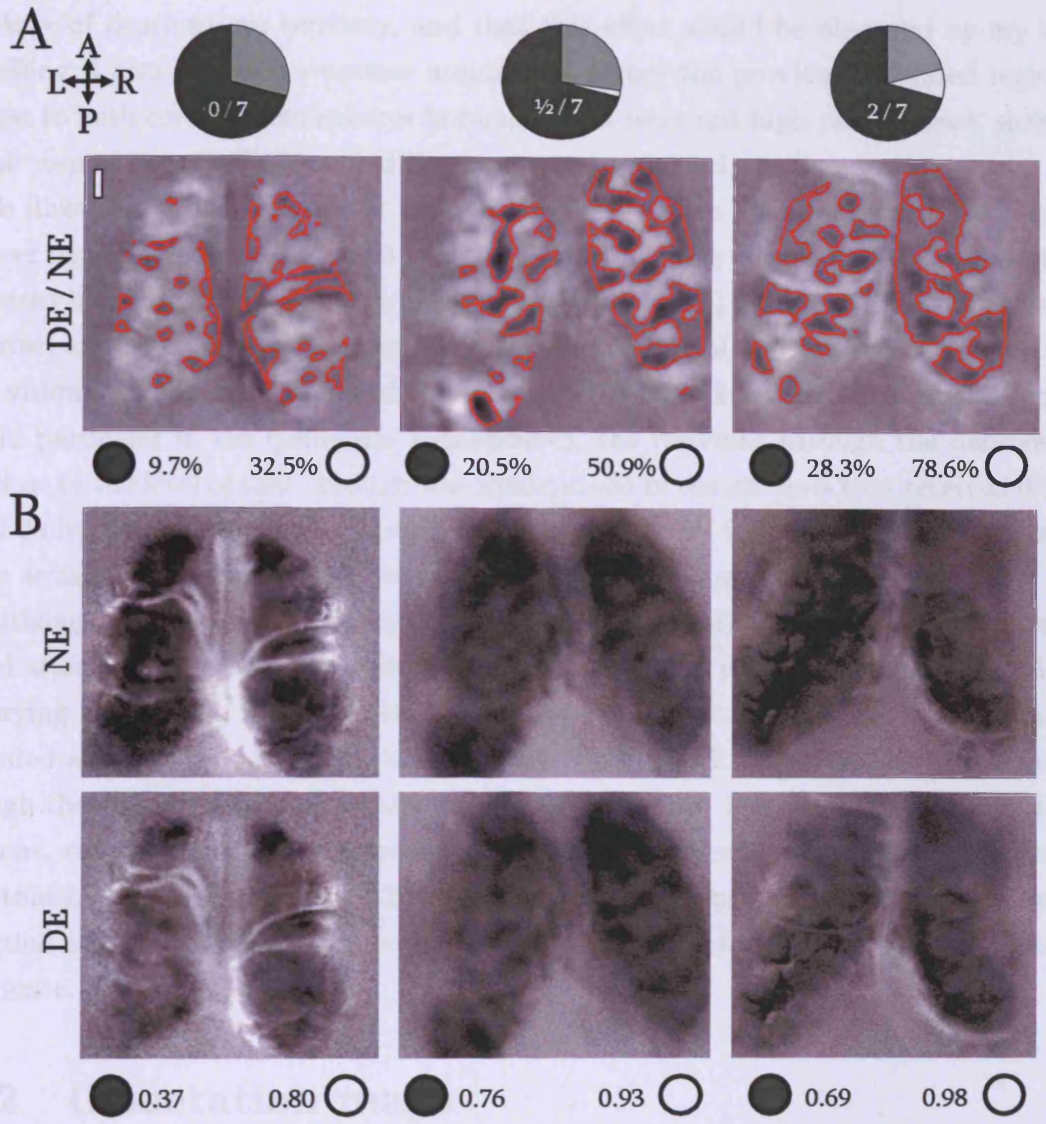


Figure 11.5: *Comparison of filtered and unfiltered maps.* (**A**) The same deprived eye ocular dominance maps from Figure 11.1A highlighting the deprived eye dominated patches with red outlines. The percentage of cortical territory representing the deprived eye is denoted by the numbers beneath each map for the ipsilateral (left, filled circles) and contralateral (right, open circles) hemispheres separately. (**B**) Unfiltered activity maps through the nondeprived eye (top row) and deprived eye (bottom row) divided by the blank response. Note that these images are range-fitted 8-bit illustrations of the actual data used to calculate the response ratio. The numbers at the bottom indicate the response ratio for the deprived over the nondeprived eye maps for the ipsilateral (left, filled circles) and contralateral (right, open circles) hemispheres separately.

shrinkage of deprived eye territory, and that this effect would be obscured by my image processing, I also analysed response amplitudes across the previously defined regions of interest in both cortical hemispheres in images that were not high-pass filtered, such that overall responsiveness (DC level) differences were preserved.

To illustrate this, in Figure 11.5 the differential ocular dominance maps of deprived eye over nondeprived eye responses (Fig. 11.5A) from three subjects are compared to the unfiltered blank divided single-condition maps (Fig. 11.5B) through the nondeprived eye (top row) and deprived eye (bottom row). While the animal deprived for the whole 7 *h* of daily vision showed a much reduced (i.e. less dark) cortical response through the deprived eye (in particular in the ipsilateral hemisphere), the response through the deprived eye was close to the level of that through the nondeprived in the animals that received 0.5 *h* or 1 *h* of daily binocular exposure (note that these images are range-fitted 8-bit illustrations of the actual floating point data used to calculate the response amplitude).

Although population results inevitably displayed greater variability than those obtained after high-pass filtering because a large part of the inter-animal variability is due to varying DC offsets, the trend confirmed the results obtained from the area analysis presented above (Fig. 11.4C). In both the 3.5 *h* and the 7 *h* cohort, the responsiveness through the deprived eye was within the normal range for 1 *h* BE or 2 *h* BE. In the 3.5 *h* cohort, reduced strength of responses through the deprived eye was observed for animals that had experienced only 0.25 *h* or 0.5 *h* of daily binocular vision; a more marked reduction in responsiveness was seen in both cohorts in the absence of any binocular experience.

11.2 Orientation maps

The effects of my selective rearing regimen on the ocular dominance maps were reflected by similar changes to orientation preference maps obtained through the deprived eye (Fig. 11.6). While full-time deprived subjects or those receiving just 0.25 *h* of binocular vision showed little to no orientation-selective responses through the deprived eye (Fig. 11.6C), in animals with 0.5 *h* of binocular exposure orientation maps for the deprived eye were relatively normal, albeit weaker than through the fellow eye (Fig. 11.6B), and in most animals with 1 *h* or more of daily binocular vision the maps for the two eyes were qualitatively indistinguishable (Fig. 11.6A).

In order to quantify this I calculated orientation selectivity indices for all pixels in the

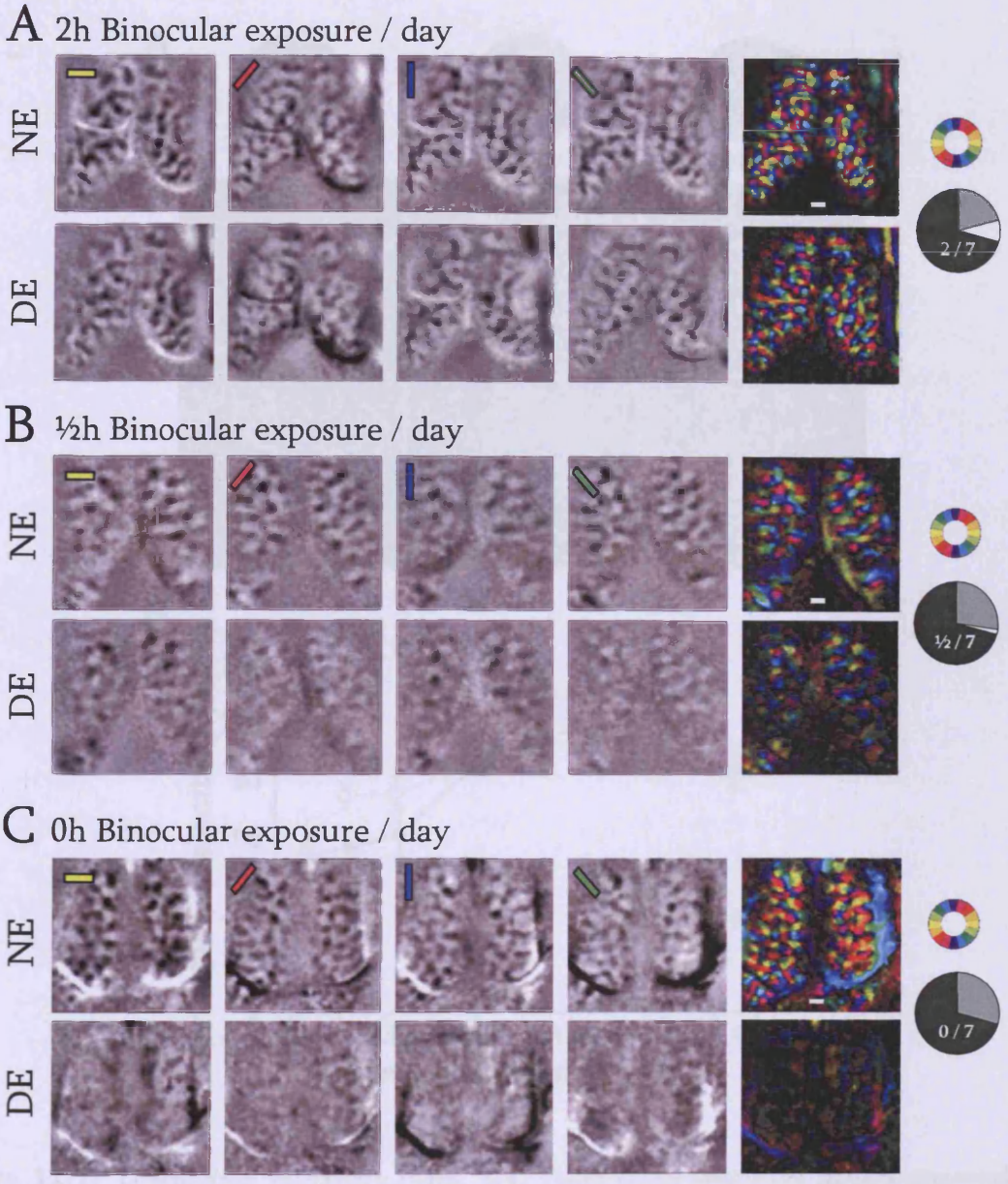


Figure 11.6: *Representative orientation maps.* Orientation maps from three subjects that received 2 h (**A**), 0.5 h (**B**), and 0 h (**C**) of daily binocular exposure, respectively. The first four columns of maps show single-condition, cocktail-blank divided iso-orientation maps obtained by stimulation with the orientation depicted by the coloured bar in the top left corner. The right-most column shows "polar" maps in which orientation-selective domains are colour-coded (see colour key) and response strength is indicated by intensity. The top row of maps in each figure panel shows maps obtained through the nondeprived eye, the bottom row shows maps obtained through the deprived eye. Scale bar, 1 *mm*.

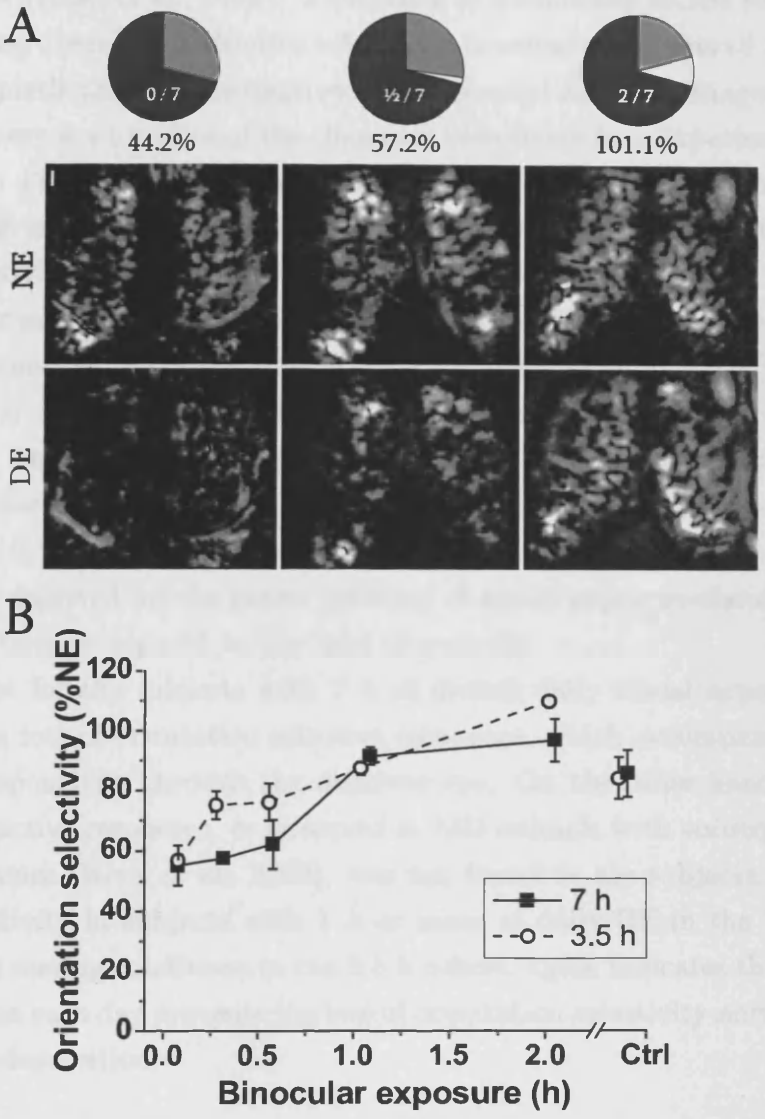


Figure 11.7: *Orientation selectivity maps.* (**A**) From single-condition images grey-scale orientation selectivity maps were calculated (see Exp. Methods). Bright areas represent high selectivity. Note that for illustration in these images the contrast was digitally enhanced; this does not affect the results. The data shown here are from the same three animals as in Fig. 11.6. The numbers above each pair of maps denote for each animal the mean orientation selectivity index for the deprived eye expressed as a percentage of that through the nondeprived eye. (**B**) Population averages of deprived eye orientation selectivity, relative to the nondeprived eye, plotted against daily binocular exposure for the 3.5 h cohort (open circles, dotted line) and the 7 h cohort (filled squares, solid line).

region of interest (Kind *et al.*, 2002). This index is normalised to the response strength to all orientations, therefore it denotes selectivity irrespective of overall amplitude. The mean across all pixels through the deprived eye expressed as a percentage of that through the nondeprived eye is a measure of the change in selectivity (see Experimental Methods).

The maps in Figure 11.7A illustrate the effect of rearing conditions on orientation selectivity in the same three subjects as in Figure 11.6. They suggest a reduction of orientation selectivity to only 57% relative to the nondeprived eye in the animal with 0.5 *h* daily binocular exposure, and a further reduction to 44% in the cat which had not been permitted any binocular exposure. The population averages (Fig. 11.7B) showed a main effect of binocular exposure on orientation selectivity in the 7 *h* cohort (AnoVa, $F_{5,13} = 6.67$, $p < 0.01$), but not in the 3.5 *h* cohort ($p < 0.1$). For the former, a marked loss of orientation selectivity in deprived eye maps was observed in animals with binocular experience up to 0.5 *h* per day, but stable in those with 1 *h* or 2 *h* BE. In the 3.5 *h* cohort only the subject deprived for the entire duration of visual exposure showed a decrease in orientation selectivity compared to the level of controls.

Thus, at least for the subjects with 7 *h* of overall daily visual exposure, there also appeared to be a loss of orientation selective responses, which accompanied the weakening of overall responsivity through the deprived eye. On the other hand, persistence of strong but unselective responses, as observed in MD animals with subsequent discordant binocular experience (Kind *et al.*, 2002), was not found in my subjects. The conserved orientation selectivity in subjects with 1 *h* or more of daily BE in the 7 *h* cohort, and throughout most rearing conditions in the 3.5 *h* cohort, again indicates that a brief period of binocular vision each day prevents the loss of orientation selectivity normally associated with monocular deprivation.

11.3 Visually evoked potentials

I was interested in establishing whether mixed normal and abnormal visual experience has a similar effect on visual function as it does on cortical ocular dominance. I recorded visually evoked potentials (VEPs), since there are several reports (Berkley and Watkins, 1973; Freeman, Sclar, and Ohzawa, 1983; Campbell, Maffei, and Piccolino, 1973; Harris, 1978) which indicate that they provide an adequate electrophysiological estimate of visual acuity. Figure 11.8 displays VEP sample traces and signal amplitudes over a range of spatial frequencies for two individual animals. As expected, for a subject monocularly

deprived for the whole of the daily 7 *h* period of visual experience, large differences in VEP amplitude and cut-off point between responses through the two eyes were observed (Fig. 11.8B,D); in fact, in the hemisphere contralateral to the deprived eye, no significant responses could be elicited through that eye at any spatial frequency. In contrast, for an animal that was permitted just 2 hours BE in a total of 7 *h* of visual exposure per day, these differences were minimal (Fig. 11.8A,C).

The difference in VEP cut-off frequency between the two eyes is the most appropriate physiological measure of the acuity deficit in the deprived eye. I found that there was a good correlation overall between cortical territory dominated by the deprived eye and the difference in acuity between the eyes as estimated from the VEP recordings ($r = 0.49$, $p < 0.001$; Fig. 11.4D). Population data for both cohorts of animals (7 *h* and 3.5 *h* per day total visual experience, respectively) are plotted against the absolute and relative daily binocular exposure in Figure 11.9A-B. As was found for cortical ocular dominance, very brief daily epochs of 0.5 *h* to 1 *h* normal binocular vision were sufficient to nearly eliminate the effects of much longer periods of monocular deprivation. While for both 3.5 *h* and 7 *h* cohorts, only the groups without any BE (0 *h* BE) differed significantly from the control group in Tukey-Kramer *post-hoc* analysis ($p < 0.05$), the 0.25 *h* BE group in the 3.5 *h* cohort also showed significantly reduced acuity in the deprived eye compared with the control group in a 1-tailed t test ($p < 0.05$). This is in contrast to animals reared with a mere 0.25 *h* BE, but no monocular deprivation, in which the cut-off frequencies were similar through both eyes (Fig. 11.2C). There was a statistically significant main effect of the duration of binocular exposure on the severity of the impairment of the deprived eye for both the 7 *h* cohort (AnoVa, $F_{5,15} = 5.97$, $p < 0.01$) as well as the 3.5 *h* cohort ($F_{5,14} = 13.1$, $p < 0.001$). These findings mirror those our lab previously reported when the same data were analysed using a computerised fitting procedure for extrapolating the cut-off point (Schwarzkopf *et al.*, 2007) and there was a very high correlation between the results from the two procedures ($R = 0.84$, $p < 0.0001$).

Moreover, the VEP signal amplitudes reflected the findings from my image analysis. I plotted the ratio of nondeprived and deprived eye amplitudes against the absolute (Fig. 11.9C) and relative (Fig. 11.9D) duration of binocular exposure. These data showed a statistically significant main effect of binocular exposure for the 3.5 *h* cohort (AnoVa, $F_{5,14} = 8.50$, $p < 0.001$) and the 7 *h* cohort ($F_{5,13} = 5.01$, $p < 0.01$). Only subjects without any binocular exposure in the 7 *h* cohort and subjects with 0 *h* BE or 0.25 *h* BE in the 3.5 *h* cohort showed responses to stimulation of the deprived eye that were significantly

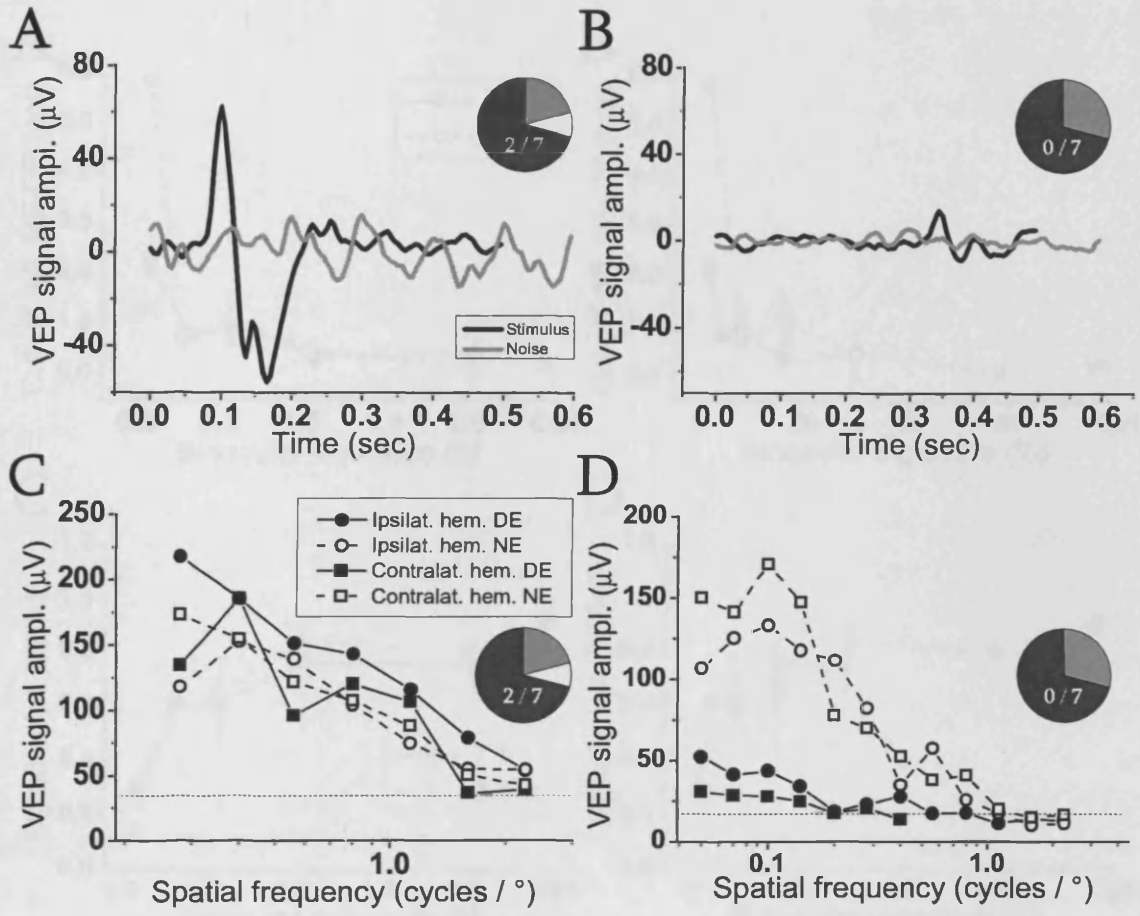


Figure 11.8: *Visual evoked potentials (VEPs) from two subjects.* Panels (**A-B**) show for two subjects the average VEP response through the deprived eye (black line) to a phase-reversing vertical grating with a spatial frequency of 0.28 cycles/°, and the noise level from the same sweep (grey line; cf. Exp. Methods). Panels (**C-D**) show the VEP signal amplitude for the same two subjects shown in (**A**) and (**B**) plotted against the spatial frequency of the stimulus. The subject in (**A,C**) received 2 h binocular vision and 5 h of monocular vision each day. The animal in (**B,D**) underwent monocular deprivation for the whole 7 hours of visual exposure per day. The mean noise level is indicated by the dotted horizontal line. Filled symbols: Deprived eye (DE) responses. Open symbols: Nondeprived eye (NE) responses. Circles: Ipsilateral hemisphere. Squares: Contralateral hemisphere.

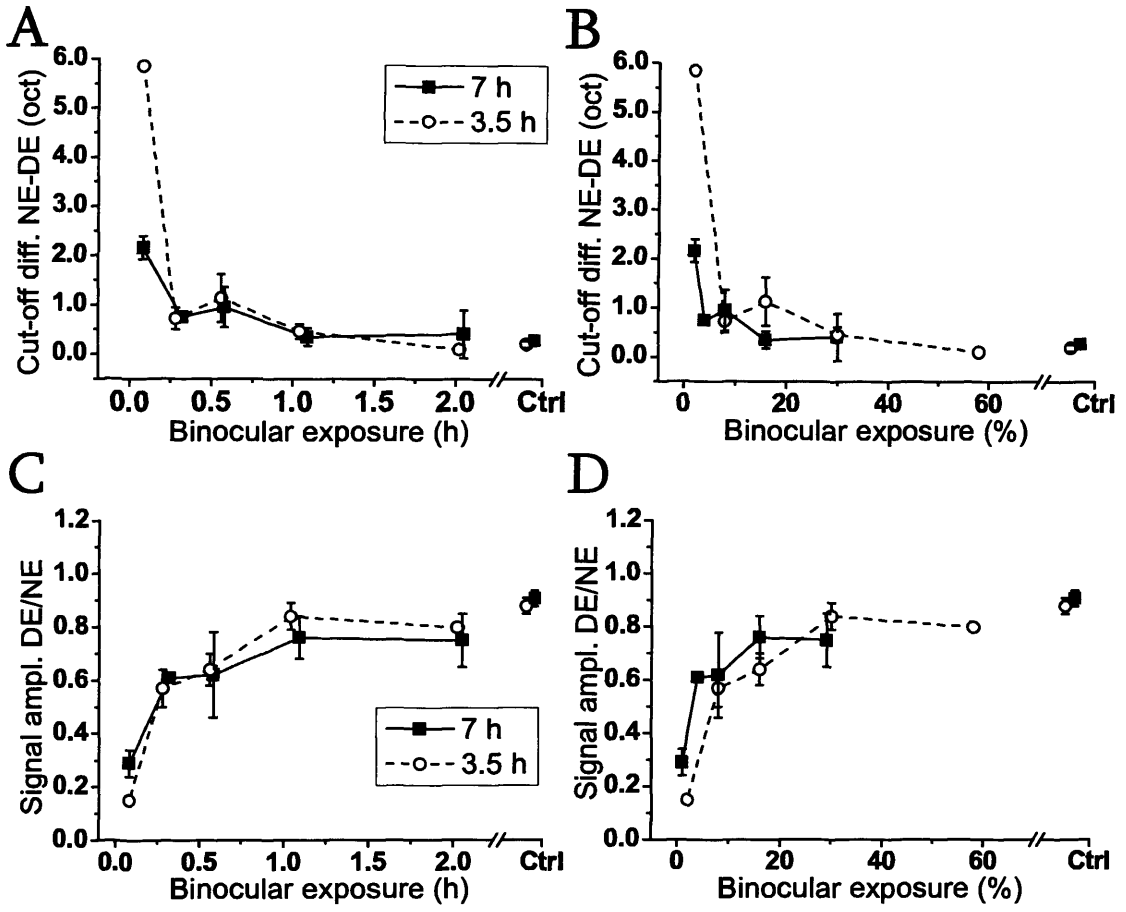


Figure 11.9: *Visual acuity based on visually evoked potentials (VEPs).* (**A-B**) Difference in spatial-frequency cut-off points between the two eyes (in octaves) is plotted against binocular exposure. In (**A**), binocular exposure is plotted in terms of absolute duration of daily binocular vision and in (**B**) as percentage of total daily visual exposure. (**C-D**) Interocular VEP amplitude ratios plotted against binocular exposure. In (**C**), binocular exposure is plotted in terms of absolute duration of daily binocular vision, and in (**D**), as percentage of total daily visual exposure. For control animals, which did not receive any monocular experience, the eye eliciting the weaker response was defined as the deprived one. Filled squares: 7 h cohort. Open circles: 3.5 h cohort. All data are mean ± 1 SEM; absence of error bars indicates n = 1.

(Tukey-Kramer *post-hoc* analysis, $p < 0.05$) reduced compared with those through the normal eye.

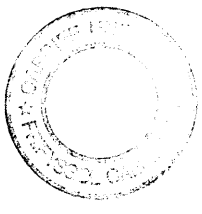
As was the case for the OD data, it appears to be the absolute amount of daily BE that determines the visual acuity deficit and VEP amplitudes through the deprived eye (Fig. 11.9A,C), as indicated by the displacement of the 3.5 *h* group data to the right of those for the 7 *h* group when the data are plotted with the binocular exposure expressed as a proportion of the total exposure (Fig. 11.9B,D).

11.4 Split binocular exposure periods

To assess whether the period of binocular exposure must be continuous to prevent deprivation-induced effects or whether it can be accumulated within a 24-h period, I imaged 6 animals that received 0.25 *h* (15 *min*) BE both before and after the period of MD, giving a total 0.5 *h* BE per day. The results are illustrated in Figure 11.10. The OD maps from kittens that received 2 × 0.25 *h* BE per day exhibited a pronounced deprivation effect. Thus they were comparable to those from animals that were given a single binocular period of 0.25 *h* per day, rather than to those that were given 0.5 *h* BE resulting in relatively normal OD maps. This is more clearly seen in Fig. 11.10B, which shows subjects from each of these three rearing conditions in the 3.5 *h* cohort. In the 7 *h* cohort (Fig. 11.10A) only one animal received 15 min BE paired with monocular deprivation. In the contralateral hemisphere of this subject a relatively large cortical area (63.4%) remained dominated by the deprived eye even though the overall responsivity was significantly reduced: the DE/NE response ratio for this hemisphere in unfiltered images was only 0.95, compared to 1.2 of contralateral hemispheres in controls (t-test, $t(3) = 4.6$, $p < 0.02$).

More importantly, however, in the animal which received two 15 *min* epochs of BE flanking the monocular period only 22.8% of the cortex was dominated by the deprived eye, which is very representative for this group (on average, 24.5 ± 0.9%). In other words, like the similar rearing regimen in the 3.5 *h* cohort, split binocular exposure resulted in a considerably greater deprivation effect than 30 *min* of continuous binocular vision.

Because no differences were observed between the 7 *h* and 3.5 *h* cohorts, data from the two cohorts were pooled (Fig. 11.10C). One-way analysis of variance comparing the 0.25 *h* continuous BE, the 2 × 0.25 *h* split BE, and the 0.5 *h* continuous BE conditions revealed a significant main effect of experimental condition on the cortical territory occupied by the deprived eye ($F_{2,16} = 6.2$, $p = 0.01$). Tukey-Kramer *post-hoc* analysis showed a significant



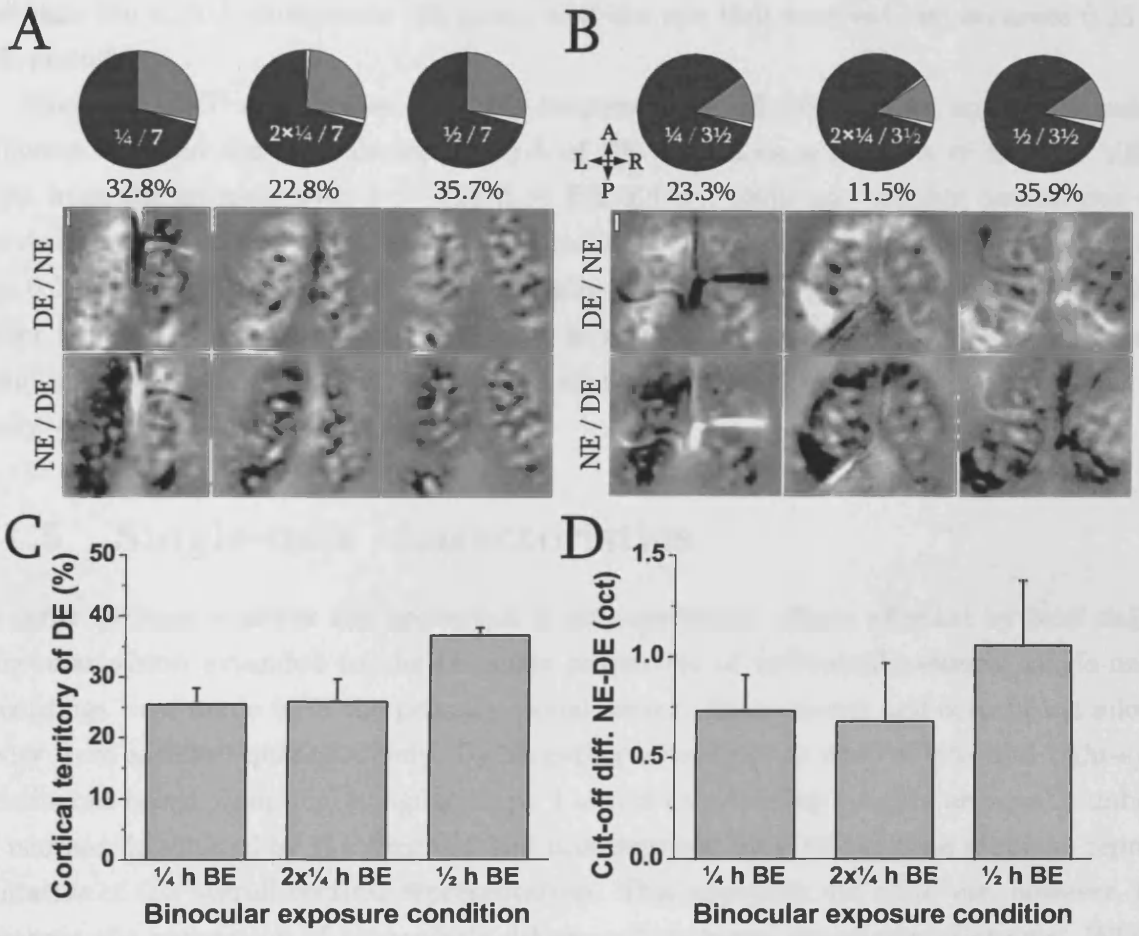


Figure 11.10: *Ocular dominance in split binocular exposure conditions.* (**A-B**) Ocular dominance maps from subjects that received 0.25 h continuous binocular exposure, 2 periods of 0.25 h binocular exposure flanking a period of MD, and 0.5 h continuous binocular exposure paired with monocular deprivation, respectively (as indicated by the icons above). Maps in the top row, labelled DE/NE, show ocular dominance domains of the deprived eye as dark patches, whereas maps in the bottom row, labelled NE/DE, show nondeprived eye domains as dark patches. The number above each pair of maps denotes the percentage of cortical area dominated by the deprived eye. Subjects in (**A**) received 7 h of daily visual exposure. Subjects in (**B**) received 3.5 h of daily vision. (**C-D**) Cortical territory occupied by the deprived eye (**C**) and VEP high spatial frequency cut-off (**D**) in three experimental groups (data combined from 7 h and 3.5 h cohorts), having either 0.25 h or 0.5 h of continuous binocular exposure, or two periods of 0.25 h binocular vision flanking the monocular period. All data represent mean ± 1 SEM.

difference ($p < 0.05$) between the 0.5 *h* BE group and the other two, but no difference between the 0.25 *h* continuous BE group and the one that received two separate 0.25 *h* BE periods.

Since the VEP amplitudes and high-frequency cut-off points were not significantly different between the animals with 0.25 *h* of BE and those with 0.5 *h* of BE, the VEP data from the animals with 2 × 0.25 *h* of BE did not allow any reliable conclusions to be drawn. The pooled data for the VEP cut-off points are shown in Figure 11.10D. In the 0.5 *h* BE condition the deficit was actually insignificantly ($F < 1$) greater than in the other two conditions, but one has to keep in mind that none of these rearing regimens resulted in the marked VEP deficit found after monocular deprivation for the whole of daily visual exposure (see Fig. 11.9).

11.5 Single-unit characteristics

In order to learn whether the protection from deprivation effects afforded by brief daily binocular vision extended to the response properties of individual neurons, single-unit recordings were made from the primary visual cortex. Binocularity and orientation selectivity were assessed quantitatively. By targeting recordings at sites of left- and right-eye dominance based on optical imaging maps, I aimed at collecting roughly an equal number of neurons dominated by the deprived and non-deprived eyes, rather than numbers representative of the overall cortical representation. This approach did allow me, however, to compare the proportion of binocularly driven cells between experimental groups. While the animals that were permitted 2 *h* BE out of 7 *h* of overall daily vision (Figure 11.11D) displayed an OD distribution typical of normally reared kittens with most neurons in categories 3-5, the shorter the daily binocular exposure, the more pronounced was the ocular dominance shift towards the nondeprived eye (despite the non-random sampling, which resulted in the slight bias towards the left eye in controls; Fig. 11.11E). More importantly, there was a prevalence of monocular or only weakly binocular cells in these animals. This loss of binocularity is illustrated in Figure 11.11F, which plots the binocularity index, BI, for each experimental group against the daily duration of binocular exposure. While controls and animals that had been given 1 *h* or 2 *h* BE per day exhibited high binocularity, for the 7 *h* cohort this declined with the amount of daily BE. This was not observed for the 3.5 *h* cohort where all groups that had undergone any monocular deprivation showed reduced binocularity compared to controls. As was the case for cortical maps and the

VEP recordings, animals with only 0.25 *h* BE, but no MD, had a normal ocular dominance distribution with a high proportion of binocular cells (BI = 0.61) and no obvious bias towards representation of any eye (Fig. 11.2D).

In order to investigate this in greater detail, the mean and median ocular dominance index (see Experimental Methods) across all neurons from an experimental group were calculated. They are plotted in Figure 11.12 as a function of daily binocular exposure. The more negative the index the greater the shift of the population towards the nondeprived eye. Unlike for the binocularity index, there is a clear trend visible in both the 7 *h* cohort (Fig. 11.12A) and the 3.5 *h* cohort (Fig. 11.12B). While animals with no or very brief binocular exposure showed an ocular dominance shift towards the nondeprived eye, in animals with 2 *h* BE out of 7 *h* overall exposure and 1 *h* BE out of 3.5 overall exposure, ocular dominance was comparable to controls. Note that there was only one subject that received 0.25 *h* continuous BE in the 7 *h* cohort, and only one that was permitted 2 *h* BE in the 3.5 *h* cohort. Given the biased sampling (see above), it is unsurprising that data from single animals can produce outliers far from the trend.

It is immediately apparent that there is a divergence of the mean and median values, which is dependent on the rearing condition. This suggests a non-normal distribution of the data as values become more scattered in animals with a strong ocular dominance shift. Therefore, non-parametric statistical testing revealed a significant main effect of binocular exposure on the ocular dominance index for the 7 *h* cohort (Kruskal-Wallis test, $\chi^2_{5,1009}$ = 77.34, $p < 0.001$), but for the 3.5 *h* cohort this did not reach statistical significance ($\chi^2_{4,545} = 8.47$, $p = 0.076$).

Another way to display these data is to plot the cumulative percentage of ocular dominance indices for each condition. These plots are shown for the 7 *h* and 3.5 *h* cohorts in Figure 11.12C and D, respectively. The steeper the curves for ocular dominance indices below zero, the stronger is the ocular dominance shift. In the 7 *h* cohort most curves are relatively close. Only the curve for subjects that received 2 *h* daily BE (grey dotted line) is clearly below the others, suggesting a more normal ocular dominance distribution.

To analyse the effect of rearing on orientation selectivity at the single cell level, I assessed the tuning widths of the orientation stimulus-response curves. Two neurons (from a control with 7 *h* daily binocular exposure) with very different orientation selectivity are shown in Figure 11.13. While the neuron in 11.13A is very sharply tuned, the cell in 11.13B exhibits only broad orientation selectivity.

For most binocular neurons (OD categories 3-5) the orientation preference through

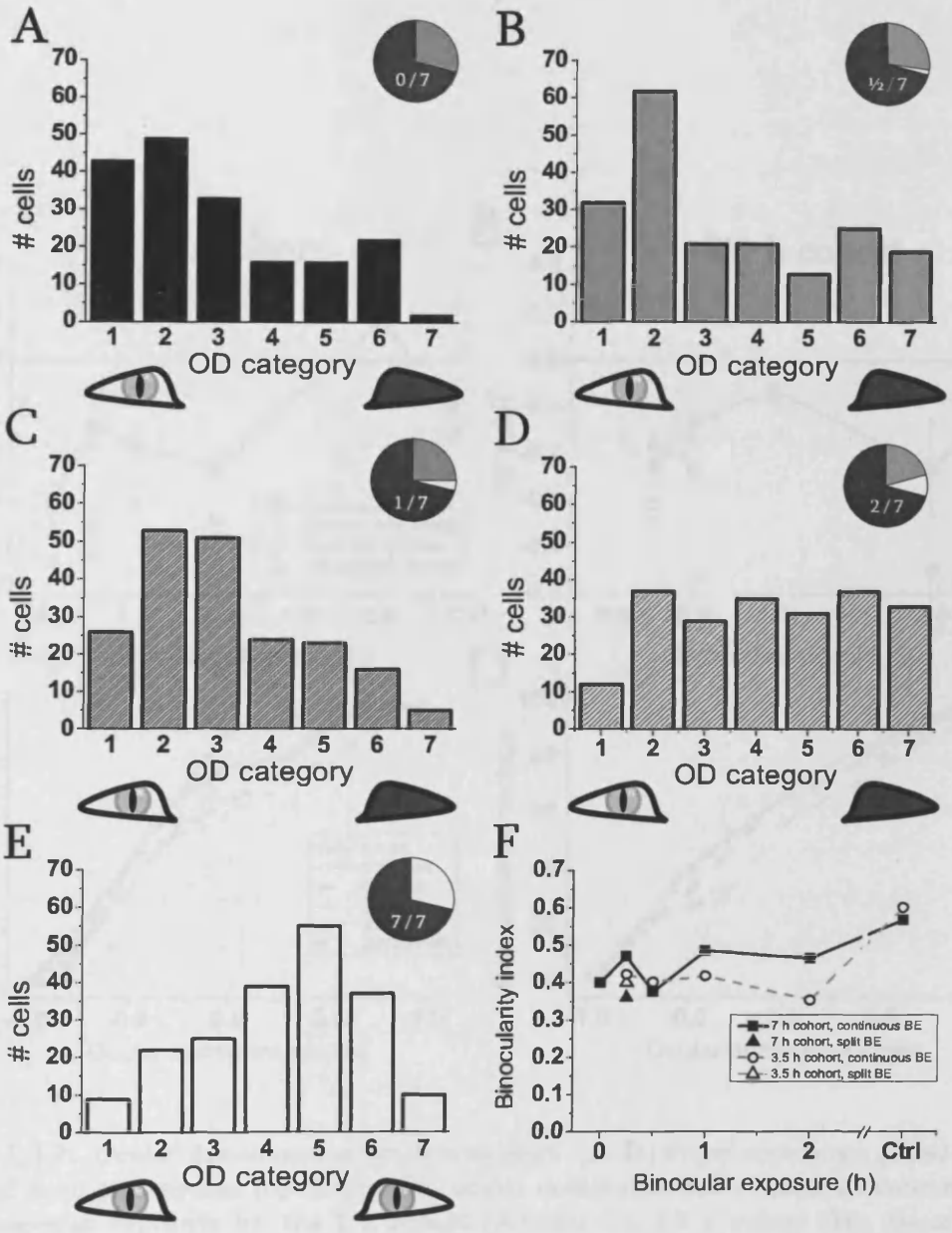


Figure 11.11: *Effect of rearing regimens on OD distribution.* (**A-E**) Ocular dominance histograms from 5 experimental groups in the cohort with 7 h of daily vision, using a seven bin system akin to that employed by Hubel and Wiesel (1962). Category 1 represents monocular NE (in controls: right eye) cells, category 7 monocular DE (left eye) cells. Rearing conditions are depicted by the icon in the upper right hand corner of each panel. (**F**) Binocularity index (cf. Exp. Methods) for each experimental group plotted against daily binocular exposure. Squares: Continuous binocular exposure. Triangles: Split binocular exposure. Filled symbols: 7 h cohort. Open symbols: 3.5 h cohort.

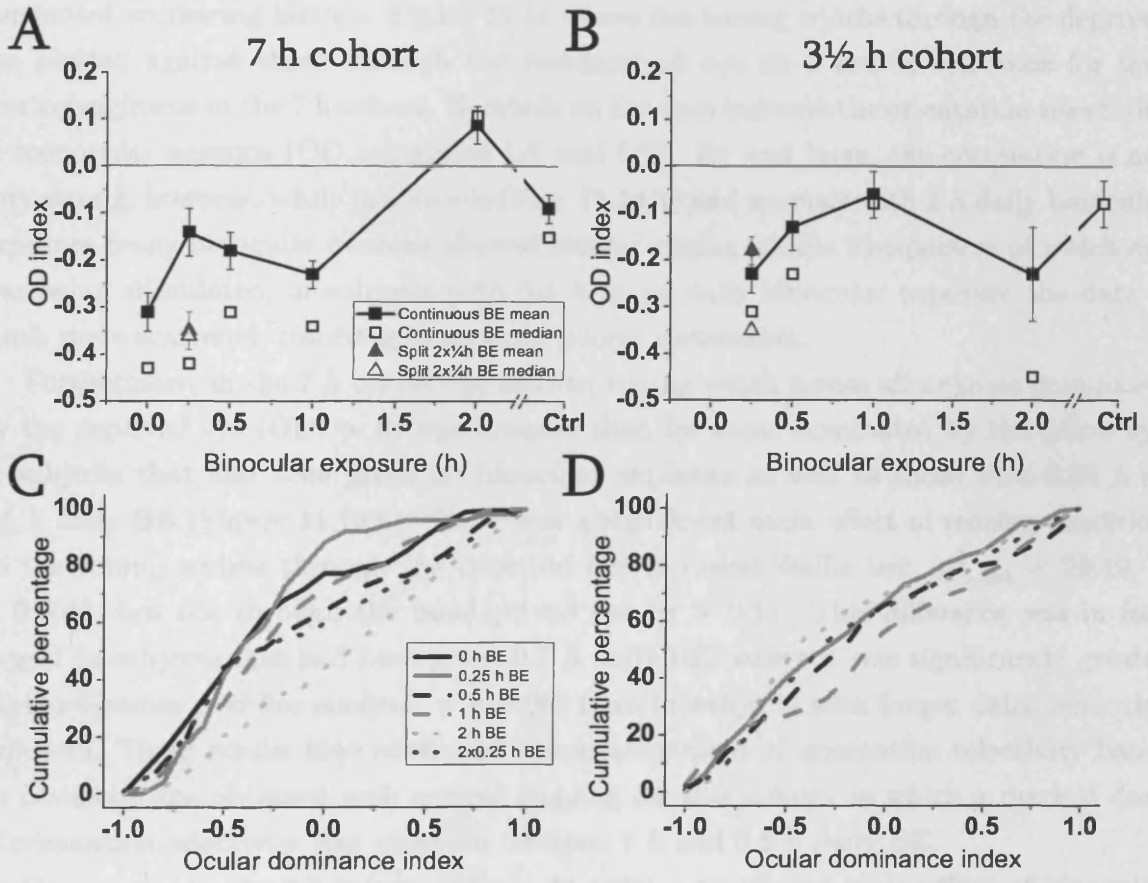


Figure 11.12: *Ocular dominance at single-unit level.* (**A-B**) Population mean (filled symbols) ± standard error and median (open symbols) ocular dominance index across all neurons plotted against binocular exposure for the 7 h cohort (**A**) and the 3.5 h cohort (**B**). Squares: Continuous binocular exposure. Triangles: Split binocular exposure condition. (**C-D**) Cumulative percentage of neurons plotted against ocular dominance index for the various rearing regimens (see legend) in the 7 h cohort (**C**) and the 3.5 h cohort (**D**).

the two eyes, extrapolated from the fitted Gaussians, was highly correlated, as expected in controls (Fig. 11.13C). Even in animals with no binocular exposure the correlation remained strong (Fig. 11.13D), albeit somewhat weaker than in most other conditions.

However, the tuning widths through the two eyes in these cells became more discrepant, dependent on rearing history. Figure 11.14 shows the tuning widths through the deprived eye plotted against those through the nondeprived eye on a cell-by-cell basis for four rearing regimens in the 7 h cohort. Symbols on the axes indicate the orientation selectivity of monocular neurons (OD categories 1-2 and 6-7). By and large, the correlation is not very strong, however, while in controls (Fig. 11.14A) and animals with 2 *h* daily binocular exposure many binocular neurons showed similar tuning widths irrespective of which eye was being stimulated, in subjects with 0.5 *h* or no daily binocular exposure the data is much more scattered, resulting in an even poorer correlation.

Furthermore, in the 7 *h* cohort the median tuning width across all neurons dominated by the deprived eye (ODI > 0) was broader than for those dominated by the fellow eye in subjects that had been given no binocular exposure as well as those with 0.25 *h* or 0.5 *h* daily BE (Figure 11.15A). There was a significant main effect of rearing condition on the tuning widths through the deprived eye (Kruskal-Wallis test, $\chi^2_{5,424} = 29.19$, $p < 0.001$), but not through the nondeprived eye ($p > 0.1$). This difference was in fact largest in subjects that had been given 0.5 *h* daily BE, where it was significantly greater (Tukey-Kramer *post-hoc* analysis, $p < 0.05$) than in subjects with longer daily binocular exposure. These results thus confirmed the measurement of orientation selectivity based on cortical maps obtained with optical imaging for this cohort, in which a marked drop of orientation selectivity was observed between 1 *h* and 0.5 *h* daily BE.

Conversely, in the 3.5 cohort (Figure 11.15B) a significant main effect of binocular exposure on neuronal tuning width was only found for the nondeprived eye ($\chi^2_{4,340} = 15.25$, $p < 0.01$), but not the deprived eye ($p < 0.1$). This was due to a significant difference between the subjects with 0.25 *h* BE and the single animal that had received 2 *h* daily BE. These findings again support the data from orientation maps of this cohort, in which at most a moderate reduction in orientation selectivity was observed.

Finally, I also set out to assess binocular interactions of neurons. It was previously reported that even after relatively prolonged monocular deprivation functional connections remain from the deprived eye to cells driven by the nondeprived eye (Freeman and Ohzawa, 1988). While these cells do not respond to a stimulus shown to the deprived eye alone, they are nonetheless influenced by dichoptic stimulation, for example they show selectivity for

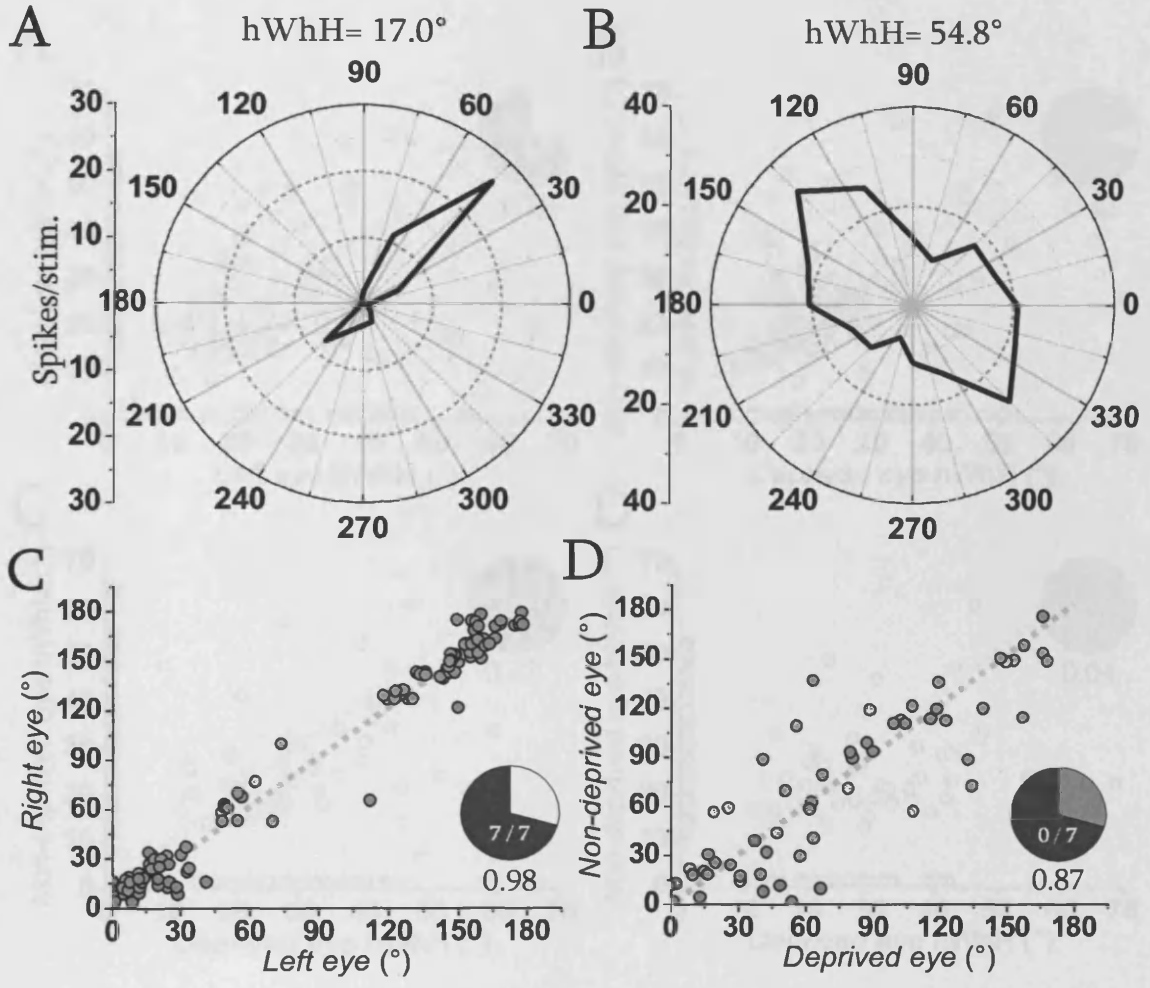


Figure 11.13: *Orientation preference of neurons.* (**A-B**) Polar plots of orientation tuning for two neurons from an animal that received 7 h binocular exposure per day. (**C-D**) Interocular comparison of orientation preference. The neuron in (**A**) was very sharply tuned and direction selective, while the neuron in (**B**) showed only broad orientation preference. The half width at half height (hWhH) of the fitted Gaussian is shown in the top of each panel. The scatter plot in (**C**) compares the orientation preference in the two eyes for binocular neurons in control animals with 7 h daily binocular exposure, whereas in panel (**D**) the same comparison is shown for animals with continuous monocular deprivation. The numbers underneath the icons representing the rearing conditions denote the correlation coefficients.

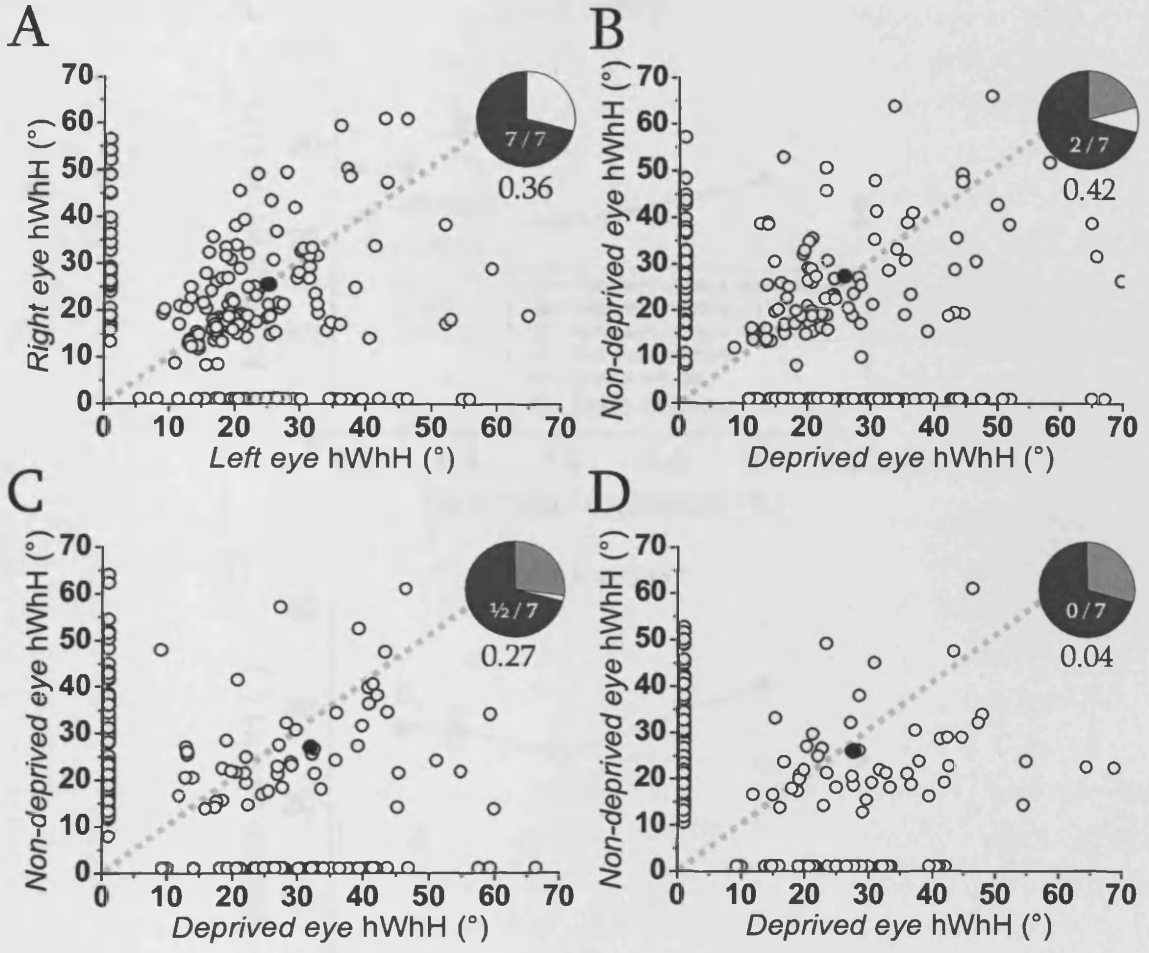


Figure 11.14: *Reliability of orientation selectivity between the eyes.* Scatter plots showing orientation selectivity for each neuron (measured in half width at half height of fitted Gaussian) through the nondeprived (in (**A**): right) eye, plotted against the deprived (left) eye. Monocular neurons (OD category 1-2 and 6-7) are depicted by the symbols on the axes. The black filled circle denotes the mean hWhH for nondeprived eye dominated cells plotted against the mean of deprived eye dominated cells. (**A**) Control animals with 7 h of daily binocular exposure. Panels (**B-D**) show plots for animals with 2 h, 0.5 h and no binocular exposure, respectively. The correlation coefficient for the binocular neurons (OD categories 3-5) is denoted in the upper left-hand corner underneath the icon for the rearing condition.

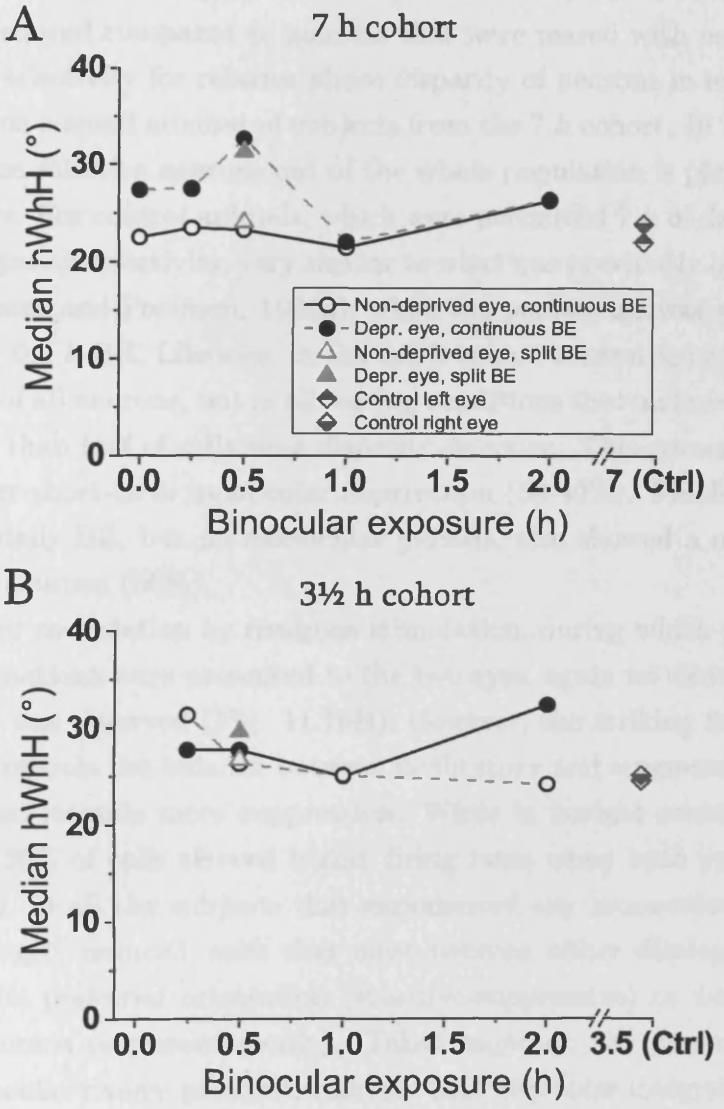


Figure 11.15: *Orientation tuning through each eye.* Median orientation selectivity, measured as half width at half height of fitted Gaussian, across all deprived eye dominated (filled symbols) and nondeprived eye dominated (open symbols) neurons, plotted against daily binocular exposure. Circles: Continuous binocular exposure. Triangles: Split binocular exposure. Diamonds: Controls (see legend). Panel (**A**) shows data from the 7 h cohort, panel (**B**) data from the 3.5 h cohort.

the relative interocular phase disparity of gratings (Fig. 11.16A) or response modulation to rivalrous stimuli (Fig. 11.16D-F). However, the overall proportion of disparity-tuned neurons may be reduced compared to animals that were reared with normal experience.

I assessed the selectivity for relative phase disparity of neurons in most subjects from the 3.5 *h* cohort and a small number of subjects from the 7 *h* cohort. In Figure 11.16C the proportion of phase selective neurons out of the whole population is plotted against daily binocular exposure. For control animals, which were permitted 7 *h* of daily vision, 59% of cells exhibited disparity-selectivity, very similar to what was previously observed in normal control cats (Ohzawa and Freeman, 1986a), while the percentage was greatly reduced in animals with only 0.5 *h* BE. Likewise, in the 3.5 *h* cohort control animals showed phase-selectivity in 68% of all neurons, but in all rearing conditions that included any period with the eye patch less than half of cells were disparity-selective. This percentage is similar to what is found after short-term monocular deprivation (30-40%). Finally, animals reared with only 0.25 *h* daily BE, but no monocular periods, also showed a normal proportion of phase-selective neurons (56%).

In terms of their modulation by rivalrous stimulation, during which gratings with various different orientations were presented to the two eyes, again no clear trend dependent on rearing history was observed (Fig. 11.16H). However, the striking finding was that in comparison with controls the balance between facilitatory and suppressive neuronal populations was shifted towards more suppression. While in normal controls from previous experiments[1]over 50% of cells showed higher firing rates when both eyes saw stimuli of similar orientation, in all the subjects that experienced any monocular deprivation this population was largely reduced, such that most neurons either displayed reduced firing rates to all but the preferred orientation (selective-suppressive) or were suppressed by any binocular stimulus (suppressive-only). Taken together, the results from the phase disparity and binocular rivalry protocols indicate that binocular integration may be more susceptible to monocular deprivation than visual acuity or ocular dominance.

[1]Note that no data were collected with this paradigm in the 3.5 *h* control condition. Therefore, I could only compare the results to previously collected data from normal animals, which had been reared with unrestricted vision on a conventional 12:12 *h* light:dark cycle.

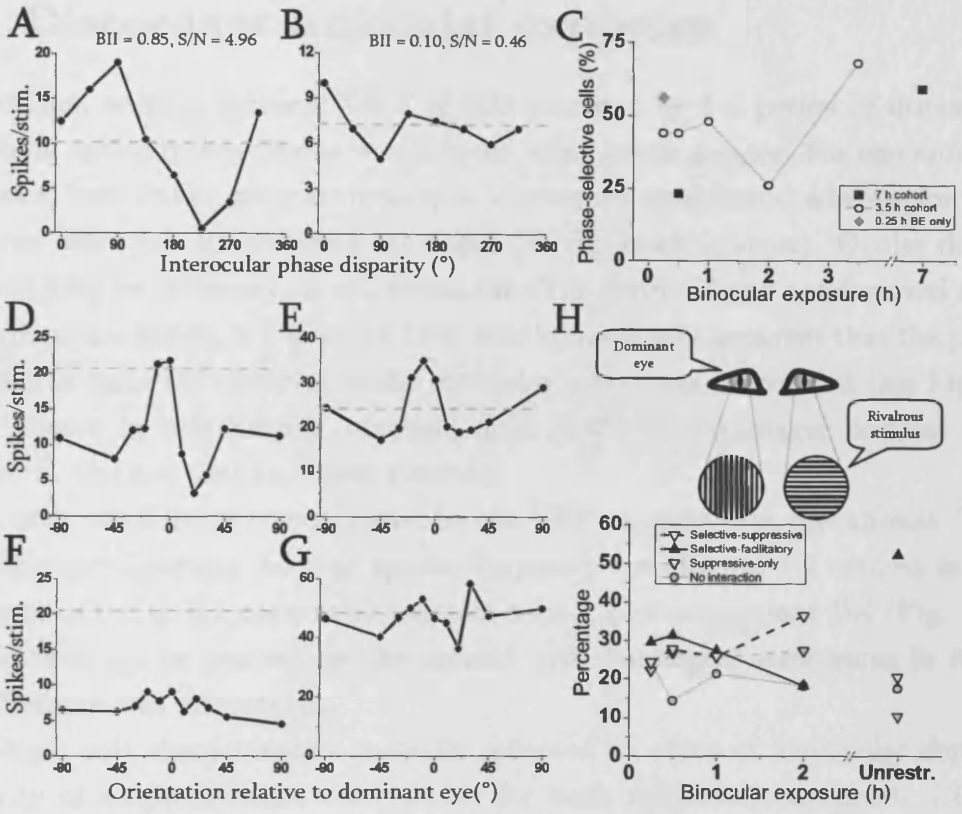


Figure 11.16: *Binocular interactions of neurons:* Interocular phase disparity (**A-C**) and orientation rivalry (**D-H**) are affected by rearing condition. (**A-B**) Two typical neurons showing phase disparity-selective responses (**A**) and poor disparity-selectivity (**B**), respectively. The average response is plotted against the phase offset between the stimuli presented to the two eyes. The dotted line indicates the mean response across all binocular stimuli. The BII and S/N for each cell (cf. Exp. Methods) are shown in the top of each panel. (**C**) Percentage of phase disparity-selective neurons plotted against daily binocular exposure. Open circles: 3.5 h cohort. Filled squares: 7 h cohort (there were representative samples for only two groups in this cohort). (**D-G**) Stimulus-response curves for typical neurons classified as selective-suppressive (**D**), selective-facilitatory (**E**), suppressive-only (**F**), or showing no binocular interaction (**G**). The average response is plotted against the orientation seen by the non-dominant eye, while the dominant eye was presented with the optimal stimulus. The level of the monocular response to the dominant eye is indicated by the dotted line. (**H**) Top: Schematic showing the binocular rivalry protocol (see Methods). While the dominant eye was presented with the optimal stimulus, the other eye viewed gratings at various orientations relative to the optimum. Bottom: the proportions of neurons with selective-suppressive (open triangles), selective-facilitatory (black triangles), suppressive-only (grey triangles), and without interaction (grey circles) are plotted against daily binocular exposure in animals that received 3.5 h of daily visual exposure. Note that no data were collected in the control condition of this cohort, therefore data from normal controls that received unrestricted binocular vision are shown instead.

11.6 Discordant binocular exposure

Two additional animals received 2.5 *h* of MD preceded by 1 *h* period of discordant BE during which optically strabismus was induced using prism goggles. For one animal these were oriented base-out to generate esotropia (convergent strabismus) whereas for the other subject they were base-in inducing exotropia (divergent strabismus). Ocular dominance maps could only be obtained for the latter cat. The deprived and nondeprived eye maps for this animal are shown in Figure 11.17A. It is immediately apparent that the protective effect of 1 *h* of daily BE observed when binocular vision was concordant (see Fig. 11.1B) was not present in this subject. Instead only 25.4% of the imaged cortical area was dominated by the eye that had been patched.

This result was further corroborated by the VEP recordings in this animal. The interocular difference between the high spatial frequency cut-offs was 1.2 octaves as opposed to the mean of 0.4 $\pm$ 0.1 octaves in animals with 1 *h* of concordant BE (Fig. 11.17B). This result was not as marked for the animal with convergent strabismus in which the cut-off difference was 0.7 octaves.

The single unit characteristics partially reflected an effect of monocular deprivation. Binocularity of single neurons was reduced for both subjects (convergent: BI = 0.38, divergent: BI = 0.35, concordant: BI = 0.42). The OD histogram for the animal with convergent binocular experience showed a strong shift of the distribution towards the nondeprived eye (Fig. 11.17D). On the other hand, the subject with divergent experience retained a relatively large number of cells monocularly driven by the deprived eye (Fig. 11.17C).

The orientation tuning widths showed the greatest discrepancy between the two animals (Fig. 11.17E). While there was no difference in tuning widths through the two eyes for the animal with divergent vision, the tuning through the deprived eye was clearly wider in the subject with convergent binocular experience.

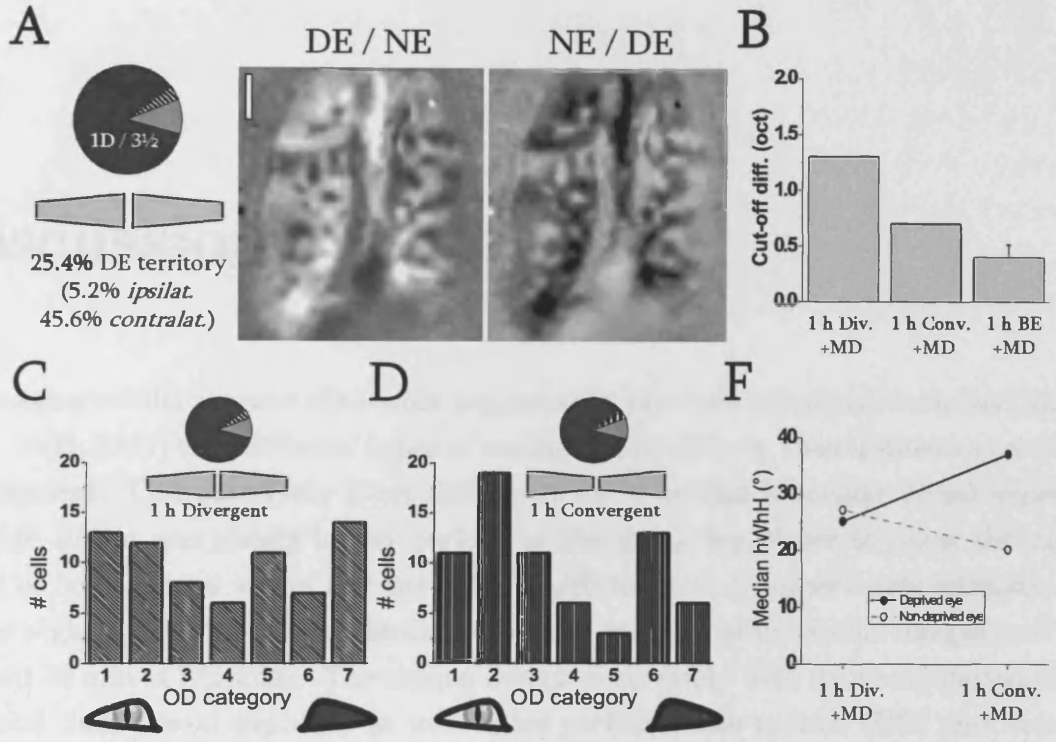


Figure 11.17: *Effects of discordant binocular vision.* (**A**) Ocular dominance maps from an animal that received 1 h of divergent vision followed by 2.5 h of monocular deprivation per day. The map on the left shows deprived eye domains as dark patches, the one on the right shows nondeprived eye domains as dark patches. Underneath the icon on the left a schematic of the prisms worn by the animal as well as the percentage of cortical territory dominated by the deprived are shown. (**B**) Interocular high spatial frequency VEP cut-off difference for the animal shown in (**A**), the animal with 1 h of daily convergent vision followed by 2.5 h of MD, and the mean across all subjects with 1 h of continuous, concordant binocular exposure (paired with MD) per day. (**C-D**) Ocular dominance histograms for the two subjects that wore prisms inducing optical strabismus. The schematic below each icon denotes the alignment of the prisms. The subject in (**C**) received divergent vision and the subject in (**D**) received convergent vision. (**F**) Median half width at half height for deprived eye dominated (filled symbols) and nondeprived eye dominated (open symbols) neurons in the two cats with discordant visual experience.

12

Discussion

My imaging results support the notion suggested by previous behavioural studies (Mitchell *et al.*, 2003; 2006) that different types of sensory input differ in their influences on cortical development. Comparatively short daily periods of normal binocular visual experience override almost completely longer periods of abnormal experience to allow the maintenance of both normal vision and normal V1 architecture. A conservative estimate based on the slightly different figures obtained from the analyses of functional images and VEPs is about 30 min of BE a day. The unique design of my study with its manipulation of both the total daily visual exposure as well as its partition into normal (BE) and abnormal (MD) components, allowed us to address an additional important issue, namely whether the outcome was dictated by an absolute amount of binocular exposure as opposed to the proportion of the total daily exposure that was binocular. The finding that the absolute amount of daily binocular experience has a greater bearing on the cortical territory occupied by the two eyes supports the conclusion that a certain minimum of daily normal vision is necessary and sufficient to maintain a normal V1 architecture. This binocular exposure period must be continuous, since the effect of two short epochs flanking the monocular period accumulates neither in terms of the protection against deprivation-induced changes to visual cortex nor of the behaviourally measured acuity (Mitchell *et al.*, 2006). Finally, it appears that the daily binocular experience must be concordant (ie. allowing matched inputs from the two eyes) to prevent acuity loss (Mitchell *et al.*, 2003), which is in accord with earlier findings that binocular recovery after prolonged MD is ineffective when subjects are strabismic (Kind *et al.*, 2002). The results from my two animals reared with one hour of optically induced strabismus pitted against a longer period of MD point in the same direction.

Two previous studies reported a beneficial effect of daily binocular experience: the first employed a single predominantly binocular exposure paradigm (Olson and Freeman, 1980), which could not address the question of how many hours of daily unrestricted vision can counteract the effects of monocular deprivation.

In a more recent study on monkeys (Sakai *et al.*, 2006), electrophysiological recordings revealed a striking reduction of the ocular dominance shift in those animals which had received binocular vision paired with monocular deprivation. However, their subjects had been permitted three years of unrestricted vision from the time when selective rearing had been completed until physiological recording was conducted. During this period recovery of cortical organisation may have taken place, which complicated the interpretation of the data.

More importantly, their limited number of data points was insufficient to determine the threshold amount of daily unrestricted vision below which considerable deprivation-induced cortical changes occur. The minimum amount of daily binocular exposure their subjects were permitted was 1 hour, which already resulted in a near to normal ocular dominance distribution. This is illustrated in Figure 12.1, which plots the severity of the cortical deprivation effects, expressed as a ratio relative to control values, in the monkeys studied by Sakai and colleagues (Sakai *et al.*, 2006) as well as the two cohorts of kittens used in the present study, against daily binocular exposure. For the former study the measure of the ocular dominance shift was the imbalance in the OD histogram. In the present study it was the cortical territory of the deprived eye as obtained through optical imaging.

As expected, both studies show a pronounced loss of cortical representation by the deprived eye in those animals which did not receive binocular exposure. For the electrophysiological data in monkeys the severity is much stronger, however, this is related to the different methods used to obtain the data: while electrophysiological classification of ocular dominance at the single cell level frequently finds a very drastic loss of deprived eye dominated neurons, optical imaging of intrinsic signals is more likely to measure a response even through the deprived eye, albeit a reduced one (see Fig. 11.5), especially after only relatively short periods of deprivation as in my study. Functional connections from the deprived eye to many neurons have been shown to remain, even when stimulation of the deprived eye on its own cannot elicit a cortical response (Freeman and Ohzawa, 1988). Since optical imaging measures the response of a large number of cells, and it is a metabolic rather than direct electrophysiological technique, a degree of cortical activation

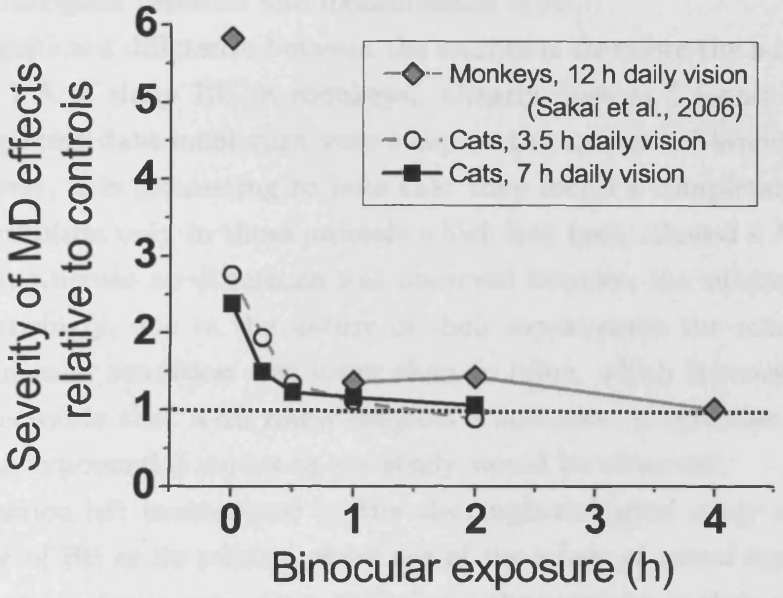


Figure 12.1: *Interspecies comparison of the effects of rearing with mixed experience.* The severity (relative to controls) of the ocular dominance shift in cat visual cortex, measured by optical imaging of intrinsic signals in the present study, and by single unit recordings in monkeys in the experiments by Sakai *et al.* (2006), are plotted against the daily binocular exposure animals were permitted. Data from cats with 7 h overall daily vision are shown by filled squares, data from cats with only 3.5 h daily vision are denoted by open circles. Data from monkeys, which were allowed 12 h of daily visual exposure, are depicted by grey diamonds. The dotted black horizontal line indicates 1, that is the level of controls without monocular deprivation.

still occurs even through the deprived eye.

Furthermore, it is very apparent that between 1 *h* and 2 *h* of daily unrestricted vision both studies found at most a moderate deprivation-induced shift in ocular dominance towards the nondeprived eye. The deficit in these conditions is slightly worse in monkeys than in my cats, however, note that there appears to be very little difference between these two conditions in monkeys and the difference to my results is well within what could be expected from biological variance and measurement error.

The most significant difference between the studies is therefore the lack of data points between 0 and 1 *h* of daily BE in monkeys. Clearly, just as I found in my cats, the curve describing their data must turn very steep as the amount of binocular exposure is reduced. Moreover, it is interesting to note that they found a completely normal ocular dominance distribution only in those animals which had been allowed 4 *h* of unrestricted binocular vision, whereas no difference was observed between the subjects with 2 and 1 *h* BE. Not surprisingly, due to the nature of their experiments the number of subjects in their study in each condition was lower than in mine, which increases measurement error. It is conceivable that with more subjects a smoother progression in their results more akin to the exponential curves in my study would be observed.

Another question left unanswered by the electrophysiological study was whether the absolute amount of BE or its relative share out of the whole of visual exposure is critical in determining ocular dominance. On a daily basis the monkeys in their study were given 12 *h* in the light in total. Therefore, if plotted against relative proportion of BE their data in Figure 12.1 would be shifted slightly leftwards, which would bring it closer to the data from my cohort of cats with 7 *h* of daily visual exposure. While I established that for my result the absolute amount of binocular vision appeared to be more critical, it may be possible that this does not hold true for longer periods of daily vision. If employed during very brief periods of overall visual experience (e.g. 1 hour), monocular deprivation may no longer be effective in inducing an ocular dominance shift. The first measurable changes to neuronal response properties can be observed after only a few hours of monocular experience (Freeman and Olson, 1982; Mioche and Singer, 1989; Frank, Issa, and Stryker, 2001), however, MD epochs lasting mere minutes may not be sufficient even when given on a daily basis. Perhaps at 3.5 *h* of daily vision the weighting of binocular periods is therefore increased relative to 7 or 12 *h* of illumination.

However, a number of reasons make this unlikely. First, as I have shown here, 3.5 *h* of continuous monocular deprivation are quite sufficient to induce a typical ocular dominance

shift and the associated loss of visual acuity in the deprived eye, very similar to what I observed in animals deprived for 7 *h* every day. Therefore, the effectiveness of monocular deprivation is not diminished at this duration of exposure. Second, 15 *min* of normal binocular vision alone result in normal ocular dominance and visually evoked responses, which proves the efficacy of very brief binocular experience, much shorter than 3.5 *h*. This underlines the fact that the loss of cortical deprived eye representation when 15 *min* were pitted against much longer periods of MD was not due to the trivial fact that such brief binocular epochs cannot exert any effect at all, but that it was inadequate for counteracting the effects of monocular experience. Third, at least in terms of ocular dominance maps, the ocular dominance shift in rearing conditions with 0.5 *h* BE or less appears to be slightly greater in the cohort with 3.5 *h* of daily vision (at least on the cortical hemisphere contralateral to the deprived eye). If the binocular periods had greater weight during the shorter duration of overall visual experience, the opposite would be expected.

On the other hand, at the single-unit level I found a strong ocular dominance shift correlated with the amount of daily binocular exposure only for the 7 *h* cohort, and the same applied to the loss of orientation selectivity, both at the neuronal level and as measured by optical imaging. It could be argued that this was due to a stronger protective effect of binocular exposure in the 3.5 *h* cohort compared to the 7 *h* cohort. However, these electrophysiological data are confounded by the biased sampling method used in my experiments, aimed at collecting data from roughly equal numbers of neurons dominated by the two eyes, which resulted in an over-sampling of deprived eye cells than what would be representative for the whole cortex. If homeostatic mechanisms bring about potentiated responses of those cells strongly dominated by the deprived eye (Mrsic-Flögel *et al.*, 2006), one might also expect orientation selectivity to be unaffected (or even enhanced) after monocular deprivation, and a certain proportion of monocular DE cells to be retained. Regardless of these questions, however, as far as the results from ocular dominance maps and visual evoked potentials are concerned, there certainly appears to be no difference between the two cohorts.

In the absence of any data from monkeys with short amounts of daily binocular vision, no definite conclusions can be drawn about the similarity of the results obtained by Sakai *et al.* (2006) with mine. Of course, any discrepancies between the two studies may reflect interspecies differences between the feline and primate visual systems. Recovery from the effects of monocular deprivation is generally less effective in monkey and humans than

it is in cats (Blakemore, Vital-Durand, and Garey, 1981). The time constant for the protective effect of brief binocular periods may be somewhat longer in primates than I describe here. Finally, it must be noted that the monkeys received their binocular periods interspersed in between two daily monocular phases, which may have had additional unanticipated effects. As my work and the associated behavioural study (Mitchell *et al.*, 2006) showed with split binocular exposure regimens, two shorter separate epochs of binocular vision are not equivalent to the same duration of continuous BE. Possibly, this may also apply to the inverted situation, i.e. a split monocular period as employed by Sakai *et al.* (2006). Despite all these issues, there is close agreement between the results obtained in monkeys and those from cats presented here. This similarity is also mirrored by the behavioural measurements of acuity and contrast sensitivity (Mitchell *et al.*, 2006; Wensveen *et al.*, 2006). In summary, brief binocular periods strongly outweigh the effects of monocular deprivation.

From a teleological perspective, the sort of input integration I observed is highly beneficial since it ensures that rather minor and transient impairments of vision in one eye do not compromise vision in the longer term. Only when patterned visual input is lost altogether to one eye does an ocular dominance shift occur. The preferential weighting of binocular over monocular experience may in fact reflect the "inertia" of the visual cortex to change a previously established functional architecture. It is now quite clear that at the onset of visual experience, the visual cortex is not a *tabula rasa*; on the contrary, ocular dominance and orientation columns of an adult-like periodicity are already present (Crowley and Katz, 1999; Crowley and Katz, 2000; Horton and Hocking, 1996; Crair, Gillespie, and Stryker, 1998; Crair *et al.*, 2001). Even dark-rearing up to the beginning of the critical period (around P20 in cats) has little effect on binocularity in V1, and orientation selectivity is reduced only slightly, in particular through the ipsilateral eye (Crair, Gillespie, and Stryker, 1998). Since my selective visual exposure started only at P30-35, one could argue that a "normal" functional architecture had been stabilised by the preceding period of binocular vision. However, an earlier study (Mitchell *et al.*, 2003) showed that kittens dark-reared from birth up to the start of the period of selective visual exposure (at 4 weeks of age) were no more susceptible to monocular deprivation and required no more daily binocular vision to maintain normal visual acuity through the deprived eye than their light-reared litter mates.

My results have important implications both in terms of the general learning mechanisms at play in the visual cortex and in terms of the time course of synaptic events

presumed to underpin visual cortical plasticity. First of all, I have to reject a purely instructive role of visual experience, since different types of input clearly differ in their effects on cortical development. The functional architecture of V1 is, at least to some extent, selective for concordant binocular input, such that even a small amount of normal visual experience allows ocular dominance patterns and binocularity to be stabilised, regardless of the nature of visual experience during the remaining time. Indeed, a binocular V1 may be considered the default state. Second, the compensation of long periods of monocular deprivation by brief periods of binocular vision suggests a much slower time course for the synaptic depression of deprived-eye inputs, which is widely believed to be the initial response to MD during the critical period, than for the potentiation induced by re-opening the deprived eye. Some of these processes could correspond to NMDA receptor-mediated LTD and LTP, respectively (Frenkel and Bear, 2004; Roberts, Meredith, and Ramoa, 1998), although direct evidence for involvement of these processes is still lacking. Recent work has shown NMDA receptor-mediated plasticity to be bidirectional, and more importantly, the observed time constants are in good agreement with my findings. Moving a dark-reared rat into the light causes very rapid changes in synaptic transmission, within less than 2 hours, while dark-rearing of a previously light-reared animal induces much slower changes, taking days (Quinlan *et al.*, 1999; Quinlan, Olstein, and Bear, 1999). The molecular basis of this experience-dependent plasticity is a change in NMDA receptor subunit composition. Visual experience decreases the proportion of receptors containing NR2B and increases the number of those containing NR2A, while visual deprivation exerts the opposite effect; both effects are reversible (Philpot *et al.*, 2001; Philpot, Cho, and Bear, 2007).

An alternative mechanism by which altered experience shapes cortical functional architecture may be related to changes in molecular signalling cascades triggered by visual experience, which are independent of actual synaptic activity. The observation that the absolute duration of BE appears to be more critical in driving ocular dominance plasticity, and that the period of BE must be continuous to be more effective, could be seen as an indication for such processes. The effects of monocular deprivation have been shown to depend on cortical activity (Jha *et al.*, 2005). It would therefore be interesting to test whether the considerable protective effect of 1 *h* of daily binocular vision against the effects of deprivation would occur if synaptic activity in the cortex is pharmacologically silenced during this time. Likewise, it should be investigated whether this effect requires cortical protein synthesis similar to the plasticity induced by monocular deprivation (Taha

and Stryker, 2002).

It is worth noting that similarly rapid recovery (within 0.5-2 *h*) following re-opening of an eye deprived by lid suture has been reported for ferret V1, albeit only for the hemisphere contralateral to the deprived eye; in the ipsilateral hemisphere, recovery took about 4 days (Krahe *et al.*, 2005). The same study also showed that the rapid form of recovery is independent of protein synthesis. In light of the evolution of the binocular field representation this is interesting, as it must have involved an invasion of the ipsilateral eye inputs into part of the visual cortex, and the ipsilateral afferentation may be evolutionarily younger and less stable. Some evidence for this may be the fact that at their first emergence (by two weeks of age in cats) ocular dominance maps show a severe contralateral bias such that the ipsilateral eye domains are strongly innervated by the contralateral eye (Crair *et al.*, 2001).

My finding that the absolute amount of daily binocular experience appears to be a stronger driving force of ocular dominance plasticity than its relative share of the overall experience is corroborated by earlier studies on the recovery from MD. Such recovery primarily depends on the absolute level of visual evoked activity in deprived-eye afferents, and not on competition between the afferents from the two eyes: mere hours after reopening of the deprived eye there is substantial recovery of vision in that eye (Mitchell and Gingras, 1998). The initial speed of recovery is even greater when visual experience is binocular than when the experienced fellow eye is closed (Mitchell, Gingras, and Kind, 2001). Similarly rapid recovery of vision following surgical treatment has been observed in human infants who had been deprived of patterned visual input by congenital cataract (Maurer *et al.*, 1999).

Thus, while the afferents arriving from the deprived eye appear to slowly lose synaptic weight during monocular viewing, high activity levels in cortical neurons when binocular vision is restored can quickly and effectively reverse this change. The daily binocular episodes in my paradigm can be regarded as very brief recovery periods, which immediately counteract the deprivation effect during the preceding period of monocular viewing. Alternatively, one could argue that each daily period of binocular experience leaves a memory trace that enhances the effectiveness of similar inputs on subsequent days, analogous to the recently reported effects of repeat MD in mouse visual cortex (Hofer *et al.*, 2006) and of selective exposure to stripes of only limited orientation (O'Hashi and Tanaka, 2006). It is worth noting that neither my study nor the behavioural study by Mitchell *et al.* (2003) found evidence of an order effect: it appears to make no difference

to the eventual cortical architecture and visual acuity whether the daily period of binocular exposure follows that of monocular exposure or vice versa. This confirms an earlier study of alternating monocular occlusion (Freeman and Olson, 1980). Sleep has been proposed to consolidate experiences into memory (Stickgold, James, and Hobson, 2000) and may also consolidate the effects of monocular deprivation (Frank, Issa, and Stryker, 2001; Jha *et al.*, 2005). Therefore, the type of exposure that occurs at the end of the day might be expected to be more effective in driving plasticity. Interestingly, a recent study reported that the recovery from monocular deprivation is not influenced by sleep (Dadvand, Stryker, and Frank, 2006), which may explain why the order of binocular and monocular exposure did not seem to affect my results.

However, the absence of any order effect in my data does not preclude the possibility of a consolidating property of sleep, as it may be the overall balance of binocular and monocular experience in a day which is consolidated during sleep. Moreover, I did not observe the animals' sleep patterns and cannot, therefore, be sure whether they were more likely to sleep in the beginning of the dark period than at other times. Regardless, my results do indicate that the second exposure period in my rearing regimen is no more capable of driving cortical plasticity than the first one.

Interestingly, one data point showing great variability in my study was the rearing condition with 0.5 h of BE, which appears to be close to the threshold below which binocular vision seems only inefficient to protect against monocular deprivation. Of course, any variation in the experience pattern of these animals, whether from sleeping patterns, physiological conditions of the animal, or disturbances of the rearing procedure, would be expected to have the most marked effects.

It has been argued that VEP cut-off frequencies provide a physiological measure of visual acuity (Berkley and Watkins, 1973; Freeman, Sclar, and Ohzawa, 1983), an interpretation that has been reinforced by the similarities in the estimates found in normal animals with those measured by use of behavioural techniques. The somewhat lower estimates obtained from my VEP data than those behaviourally measured acuities in animals reared under identical exposure conditions (Mitchell *et al.*, 2003; 2006) may be a consequence of a number of factors that include the possibility that the VEP data may not have sampled the activity of the most sensitive neurons in young kittens that mediate behaviourally determined acuity values. Nevertheless, the conclusions that can be drawn from the VEP data mirror closely those obtained earlier on the basis of behavioural measurements. Importantly, short periods of daily binocular experience outweighed far longer

periods of monocular experience to lead to the development of normal acuities in both eyes, regardless of whether acuity was determined from VEP data or from behavioural measurements.

In apparent contrast to my results, the earlier behavioural measurements (Mitchell *et al.*, 2003) found a longer daily binocular exposure (up to 2 hours) necessary to protect against the effect of monocular deprivation. However, ocular dominance architecture and VEP deficits are unlikely to show a perfect correlation with visual acuity. The reduced size of deprived-eye domains compared to the nondeprived eye's territory, and the reduction in VEP amplitudes through the deprived eye, reflect the numbers of neurons responding to low spatial frequencies. Conversely, the behavioural measurement of grating acuity is more likely to depend on the "best" cells, i.e. those responding to the highest spatial frequencies. It is possible that those are more vulnerable to monocular deprivation.

Similarly, the VEP cut-off is probably the pooled activity of a number of neurons passing a certain threshold at which the signal-to-noise ratio is adequate (especially under anaesthesia), whereas the number of cells that process and convey information about spatial frequencies at the behavioural detection threshold may be very small.

Alternatively, of course, behavioural performance may depend on the response characteristics of neurons beyond V1, at a stage where the representation of visual stimuli is integrated into a behavioural response, because the read-out of visual information is likely to occur at higher cortical stages of visual processing (Paradiso, Carney, and Freeman, 1989). While behavioural assessments of course measure the functional interplay of the entire brain, my results merely reflect the deprivation-induced changes of primary visual cortex. Certainly, as far as the effects of binocular visual deprivation are concerned, primary visual cortical function alone is insufficient for reliable visual perception. In patients recovering from the restoration of bilateral congenital ocular defects, visual evoked responses are often better than the actual visual capabilities (Ackroyd, Humphrey, and Warrington, 1974; Carlson, Hyvärinen, and Raninen, 1986). While there is of course a close relationship between functional ocular dominance and the severity of amblyopia in patients (Goodyear, Nicolle, and Menon, 2002), it is likely that the hierarchy of visual processing beyond V1 is also affected by monocular deprivation to a degree.

Finally, stereopsis is a property of vision extremely vulnerable to the effect of monocular deprivation. Neither the present study nor previous investigations tested the stereoacuity of animals reared with such mixed experience regimens. Considering that stereopsis is still impaired after even the most successful eye-patching treatments for amblyopia in

humans (Birch *et al.*, 1993) and part-time reverse occlusion in cats (Mitchell, Ptito, and Lepore, 1994), it is likely that moderate deficits in binocular interactions between the eyes are present even in those animals in my study which received binocular exposure sufficient for normal ocular dominance and visual acuity to develop. The electrophysiological study using similar rearing regimens in monkeys (Sakai *et al.*, 2006) suggested that binocular integration at the single cell level was affected by monocular deprivation even in those rearing conditions which resulted in normal visual acuity of the deprived eye and a balanced ocular dominance distribution.

The results from my electrophysiological investigation of binocular interactions lend some support to this notion. I found that the population of neurons selective for relative phase disparity, which is likely to play an important role in stereopsis, is reduced in all conditions that included a daily period of monocular deprivation. Stereopsis in cats (Timney, 1981; Mitchell, 1989), as well as disparity-sensitivity of single neurons (Pettigrew, 1974), first emerge at around postnatal day 30-35, in other words at around the peak of the critical period for ocular dominance plasticity, and mature very rapidly to reach adult levels over the next two months. Hence, my selective rearing period (approx. P35 to P60) fell precisely into the period for developing stereopsis.

The proportion of neurons exhibiting facilitation when both eyes viewed similar orientations was also reduced in all conditions tested in my study. However, the absence of data for the animals without any monocular deprivation within 3.5 h of overall daily visual exposure prevents me from drawing any conclusions about whether this kind of binocular interaction is diminished specifically by monocular deprivation, or generally by the brevity of overall exposure. This data was also not collected for the animals permitted 0.25 h of daily BE only, which would shed more light on this issue. All things considered, my limited single unit data cannot answer this question with any certainty and thus the minimum amount of daily visual experience required for the development of stereo vision remains unknown.

It would be ideal to develop protocols for measuring these effects by means of optical imaging as it would circumvent the sampling bias of single cell electrophysiology. Imaging of intrinsic signals seems unsuitable for this purpose, probably because of the slow time course of the signal. It may be possible to study binocular integration by means of optical imaging with voltage sensitive dyes (Shoham *et al.*, 1999) although it remains to be confirmed whether there is a clearly delineated functional architecture of binocular interactions, in particular since earlier electrophysiological inves-

tigations failed to reveal such clustering of disparity tuning (LeVay and Voigt, 1988; DeAngelis *et al.*, 1999; see also p. 26). A great number of cells do not display disparity-selective modulations. If these neurons are scattered throughout primary visual cortex irrespective of the binocular properties of their neighbours, the optical signal would be diluted thus preventing functional mapping. A recently devised method of two-photon calcium imaging, allowing the functional imaging at single cell resolution (Ohki *et al.*, 2005), may be superior at revealing these interactions, and there has been a preliminary report of functional architecture for relative phase disparity tuning (Kara, 2006).

12.1 Conclusions

The present study demonstrates that visual cortical development in early life is biased towards a normal outcome supporting binocular vision. Even brief periods of binocular experience can outweigh the effects of much more prolonged monocular deprivation, at least if vision has developed normally until vision in one eye becomes compromised. While remarkable plasticity exists in the postnatal brain, this does not come at the cost of economically sensible development. The kind of stimulation most likely to occur under normal circumstances is favoured by the visual system. In this context, it may be important that binocular experience is presented within an enriched environment in order to provide maximal sensory stimulation and therefore to ensure maximal effectiveness (Cancedda *et al.*, 2004). However, this also applies to the monocular deprivation period since I was interested in the effectiveness of both kinds of exposure and I therefore aimed at providing as much visual experience through the nondeprived eye as possible. More generally, environmental enrichment in rodents can only be a poor comparison to a domesticated species like the cat, as the conventional laboratory housing of mice and rats is a very confined environment in comparison to their natural habitat.

Taken together, my findings allow a hopeful outlook for the treatment of ocular defects in infants as brief amounts of daily binocular exposure may be sufficient for normal visual development. In fact, patching regimens similar to those employed in the present study are now routinely used in human patients (Mitchell and MacKinnon, 2002). Assuming that mechanisms of plasticity are similar in the human and cat visual cortices, my results suggest that in children who need to wear a patch over one eye for a longer period of time, normal vision will be maintained in that eye if the patch is removed for about an hour a day to permit normal binocular visual experience. Similarly, the development of

anisometropic amblyopia can likely be prevented if the child wears corrective lenses for only a short period during the day.

Chapter IV

General Discussion

Recent studies have shown that ocular dominance columns in striate cortex form independent of visual experience (and even in the absence of eyes) and are present prior to the onset of the plasticity (Crair *et al.*, 2001; Crowley and Katz, 1999; Crowley and Katz, 2000). However, this fact in itself certainly does not indicate different efficacy for different forms of visual experience, nor does it mean that experience cannot be completely instructive in reorganising the cortex. The observations from my study, together with the behavioural research by Donald Mitchell and his colleagues (Mitchell *et al.*, 2003; 2006), for the first time suggest an innate bias towards attaining a normal organisation of the visual brain during its postnatal development. While experience can have dramatic effects in determining the eventual function of the visual system, it merely appears to act by gradually adapting the predefined circuitry to the circumstances of the individual.

In light of the clinical management of amblyopia, these findings indicate that a small amount of daily binocular experience can counteract the deleterious effects of unilateral eye patching, which supports the patching regimens employed in modern treatments (Mitchell and MacKinnon, 2002) and will hopefully lead to the further refinement thereof. While the many lines of research into the cellular and molecular underpinnings of ocular dominance plasticity have great potential for the eventual development of a cure for amblyopia in adulthood, great care must be taken in these investigations. The closure of the critical period is clear evidence of the maturation of the central nervous system in childhood and adolescence. Before any of the molecular and physiological mechanisms are manipulated to restore plasticity in the adult organism, it must be asked what other processes are associated with this maturation of the system, and how interfering with them will affect the whole organism.

Therefore, it is fundamentally important to continue the current practise of treating amblyopia conventionally as early as it is medically possible, and for finding ways to counteract the effects of monocular visual impairments during the critical period. Research may lead to a cure for adult amblyopia within the next decades, but circumventing the need for such treatment in future generations is even more desirable.

13

Archetypal visual cortical organisation?

Research into ocular dominance shifts can of course only play a small part in elucidating the development of the visual system. While it has great behavioural relevance and medical potential, it is nonetheless important to keep in mind that many aspects of vision undergo plastic changes, and that some of them may very well be much more vulnerable to abnormal visual experience. A comparison of my data on cortical changes induced by monocular deprivation with Mitchell's behavioural results (Mitchell *et al.*, 2006) suggests that even acuity is more vulnerable to abnormal experience than the size of OD columns. Moreover, cases of patients recovering from long-term visual deprivation by bilateral eye defects, like Gregory and Wallace's SB (Gregory and Wallace, 1963) or the much more recent case of MM (Fine *et al.*, 2003), are a testament to complex perceptual capabilities whose development is crucially dependent on experience, such as understanding perspective cues in a two-dimensional image. Patient SB appeared to only recognise visual objects once he had had the opportunity to explore them tactically and could thus associate a haptic representation with the visual image (Gregory and Wallace, 1963). This lends support to the empiricist notion expressed by Molyneux and Locke (Locke, 1694) that a formerly blind man would not be able to recognise objects without touching them.

However, even shortly after his vision was restored, MM's abilities were superior to those of many other patients, perhaps due to the three years of normal visual experience he had had as a child (Fine *et al.*, 2003). Brain imaging studies on congenitally blind subjects have shown that mental imagery of object shape activates the same areas that

are considered to be involved in shape processing in sighted individuals (De Volder *et al.*, 2001). This does not mean that blind people possess visual images, but it indicates that certain systems may be predestined for the processing of particular aspects of the sensory world, regardless of the modality to which they are tuned. In other words, while the abnormal absence of visual input may have strengthened or biased the wiring of auditory or somatosensory areas into the object recognition circuits, the presence of these circuits is independent of the environment. In elegant experiments, Mriganka Sur and colleagues "rewired" the visual afferents of ferrets into auditory cortex (Sur, Angelucci, and Sharma, 1999; Sharma, Angelucci, and Sur, 2000) and found that visual input can drive neuronal responses and is even capable of mediating visual behaviour (von Melchner, Pallas, and Sur, 2000). However, the functional organisation of this rewired cortex is more disorderly than in normal visual cortex as it did not completely disrupt the archetypal circuitry of auditory cortex. Similarly, the adaptation of primary visual cortex seen in blind subjects compared to the normally sighted (Sadato *et al.*, 1996; Röder *et al.*, 2002) is probably not a complete rewiring of the visual system, but reflects the enhancement of already present connections from those sensory modalities to the high definition processing system in V1.

13.1 Binocular vision

The crucial question here is how much binocular visual experience would be sufficient for the establishment of a normal visual system, or more specifically, for a normal functional organisation of the various visual areas. Mixed experience rearing regimens such as those I employed in my study could be adapted to the study of binocular deprivation. In a sense, the experimental group in my study that received a mere 15 *min* of daily binocular experience per day but were kept in the dark for the remaining time, is a first step in that direction. Even such a small amount of visual exposure resulted in completely normal visual cortical function. This is in line with previous reports that visual experience triggers a very rapid reconfiguration of NMDA receptor composition after dark-rearing (Quinlan *et al.*, 1999).

More importantly, it must be reiterated that dark-rearing and binocular deprivation through diffuser lenses or eyelid suture are not equivalent. Dark-rearing, that is the absence of any visual input, delays and slows down the course of the critical period for ocular dominance plasticity (Mower, Christen, and Caplan, 1983; Mower and Christen, 1985). Conversely, diffuse visual experience without contour or texture information is compa-

rable to the rudimentary light sensitivity of subjects with severe bilateral cataracts or corneal opacity. The majority of neurons in early visual areas are detectors for luminance contrasts or edges, due to the typical antagonistic structure of their receptive fields. In this situation, when luminance information reaches the retina but the eyes are incapable of *useful* vision, a more significant reorganisation of the visual system takes place.

Interestingly, while prolonged binocular deprivation leads to behavioural blindness (Mitchell, 1988), the short-term effects of binocular deprivation on the response characteristics of neurons in primary visual cortex are relatively small in comparison to those of monocular deprivation (Wiesel and Hubel, 1965). While there is an increase in the number of visually unresponsive cells, the overall shape of the ocular dominance distribution remains normal. Similarly, visual evoked potentials in many sight-recovery patients are comparably close to normal levels, but these are not indicative of visual quality (Ackroyd, Humphrey, and Warrington, 1974; Carlson, Hyvärinen, and Raninen, 1986). Taken together, these findings show that a relatively normal functionality of V1 after binocular deprivation is insufficient for good vision. It is of course possible that it is this population of neurons whose responses are lost after BD which are crucial for vision, or as I described earlier (p. 118) that higher visual areas are more strongly affected by binocular than monocular deprivation. Evidence for the latter notion has been reported with regard to global motion perception (Ellemberg *et al.*, 2002). Either way, in studies pitting binocular pattern deprivation against normal unrestricted vision it would therefore be imperative to measure the visual acuity of subjects behaviourally alongside any neurophysiological studies.

13.2 Motion perception

The effects of abnormal or restricted visual experience have also been studied for other tuning characteristics of V1 neurons and there is a distinct possibility that experience is instructive in shaping these capacities. For instance, while a map for orientation preference seems to be innate, selectivity for the direction of motion appears to only emerge in V1 cells after visual experience (Li, Fitzpatrick, and White, 2006). Selective exposure to a particular directional stimulus for several hours subsequent to dark-rearing leads to the formation of direction domains for only this stimulus (Li, White, and Fitzpatrick, 2006), suggesting a role for cortical activity in the establishment of the direction-selective system. A ready interpretation of this finding would be to suggest a purely instructive

role of experience in the establishment of direction selectivity.

However, drawing such a conclusion may be premature. Visual experience is clearly very significant in calibrating and fine-tuning the motion processing system. The neuronal circuitry underlying direction selectivity may already be present, but it may only be activated by visual stimuli that traverse space in a coherent fashion. One indication for this idea is the speed with which direction selectivity develops after eye opening (Li, Fitzpatrick, and White, 2006) and, even more drastically, when a "training stimulus" is presented to an anaesthetised animal (Li, White, and Fitzpatrick, 2006). The cortical area of the orientation domains coding for the orientation of the exposed stimulus does not become enlarged, and the direction domains emerging after the training are constrained to these orientation columns. Finally, in this light it is also important to note that in humans the normal development of motion perception takes several years, while the system is only susceptible to deprivation within a very short window after birth (Lewis and Maurer, 2005). Again, the essential test here will be how much normal experience is necessary for the system to develop properly or to counteract the deleterious effects of an abnormal motion environment (Cynader and Chernenko, 1976).

13.3 Face processing

But archetypal circuitry may not be limited to low level functions such as eye dominance or motion sensitivity. Recent research on higher brain areas suggests that there may be an inherent architecture for more complex faculties of visual perception. One example is a brain area located in the fusiform gyrus, which has been proposed to be involved in the processing of faces (Kanwisher, McDermott, and Chun, 1997) as opposed to the more basic processing of basic shape and object identity in other parts of inferotemporal cortex. There are good reasons to suspect an advantageous role of face stimuli: faces are of crucial importance for the identification of individuals, and in particular in primates facial expressions convey a large amount of information about their emotional state and are involved in non-verbal communication. It would therefore not be surprising, if a specific face processing system was innate to the visual system. Single-cell and optical imaging recordings established that selective face detector neurons are also present in the brains of non-human primates in comparable brain regions as face selective areas in humans (Tsao *et al.*, 2003; Tsao *et al.*, 2006; Wang, Tanifuji, and Tanaka, 1998). Cells responding selectively to faces have even been found in sheep (Kendrick and Baldwin,

1987) and therefore may be common to many species.

This prevalence of face-selective neurons could of course be explained more trivially by overtraining of such behaviourally relevant stimuli and therefore the idea of a specialised face processing system is not uncontested (Tarr and Gauthier, 2000). However, prosopagnosic patients are often capable of learning very fine visual object discriminations despite irredeemable deficits in face recognition (Kanwisher, 2000). Certainly, in light of the phylogeny a predestined face perception system is a feasible idea. Through the course of evolution it would not be surprising if stable aspects of the sensory environment would become hard-wired in the brain.

Of course, it is a relatively safe assumption that face selective neurons require a great amount of input to be calibrated and become sensitive to subtle differences in facial identity. Moreover, after a prolonged absence of any visual input it would be economically viable to redistribute these resources such that other senses can carry out this function more reliably, which would explain the poor judgement of facial identity and emotional expressions in many sight-recovery patients (Gregory and Wallace, 1963; Fine *et al.*, 2003). But nonetheless it is not far-fetched to suspect that the basic neural machinery for encoding faces is predetermined. Natural experience with faces is likely to be binary: the individual either has it or they lack useful visual input altogether. Subjects are never raised in an environment where they see only inverted or highly distorted faces. An interesting question in this regard is whether feral children, who grew up in a presumably normal visual environment but without being exposed to (human) faces would exhibit deficits in face perception. However, the complications with these cases make such investigations very difficult and naturally the well-being and successful integration of the individual must always be the foremost priority.

14

Higher cognitive functions

As I discussed on the first pages of this thesis, early experience likely plays a significant role in the development of many higher perceptual and cognitive faculties, and the behaviour and personality of an individual in general. One of the most prominent examples of experience-dependent development is the acquisition of language. However, while lack of early exposure to human speech in feral children hampers the development of normal speech capabilities (Leiber, 1997; Schneider, 2003), there is increasing evidence that there exist brain systems which are predestined to analyse and encode human language. While recent studies have shown a benefit of active social contact for developing language skills by children (Kuhl, 2004), the learning of speech skills follows a similar course for children throughout the world across different cultures and all human languages may have more commonalities than is generally appreciated (Pinker, 1994).

In his recent book *"The Blank Slate"* Pinker delivered a harsh criticism of the notion of an indefinitely malleable brain (Pinker, 2003). In his view, an empiricist doctrine has pervaded scientific and philosophical thinking since the dawn of the Enlightenment. But now increasing scientific evidence is mounting, which suggests that many functions are not learned from scratch through experience, but that organisms are biologically predisposed for the learning of these capabilities. Others scientists have gone even further, like Wolf Singer who sparked great controversy when he espoused a clear-cut determinism, calling into question the concept of free will (Singer, 2004).

Of course, I mean to make no such far-reaching claims based solely on the results of the research I present here. After all, the postnatal plasticity observed in early visual areas is a comparably low level function and can be merely a small part of a much larger picture. Regardless, my study lends further support to the notion that the brain is not sculpted

purely by experience, but that in development there exists a preferential weighting of normal environmental input. By taking advantage of such innate biases for other aspects of postnatal development that may be identified in the future, it will hopefully be possible to improve the quality of life and the development of those found growing up in abnormal environmental conditions (be it through illness or neglect).

Returning to the case of Kaspar Hauser, we will most likely never know the reason for his relatively successful (albeit short-lived) integration into society compared to other wild children. The details of his imprisonment are far too sketchy, and even the accounts of his known life are very limited (von Feuerbach, 1833). However, there is a distinct possibility that he received an amount of normal upbringing prior to his sad fate, which permitted the groundwork to be laid for learning to walk and speak as a teenager.

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