An Investigation of the effect of age of onset on amblyopia

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Abstract

This research aimed to investigate if age of onset affects the pathogenesis of amblyopia. Amblyopic subjects were compared with normal controls.

Strabismic amblyopes were assigned to early or late onset groups on the basis of detailed clinical history. Confirmatory histories were obtained from parents where possible.

Contrast Sensitivity (CS) to a 3.2 cyc/deg (cpd) sinusoidal grating pattern was recorded. Monocular pattern appearance visual evoked potentials (VEP) at five contrast levels were recorded. Luminance and chromatic CS, motion onset and colour VEPs were recorded.

Late onset amblyopes showed reduced CS at 3.2 cpd for the amblyopic, but increased CS for the fellow eye compared to normal. Late onset amblyopic eye VEP CII latencies were longer and amplitudes smaller than normal. CII responses in amblyopic and fellow eyes of the early onset group were of shorter latency and smaller amplitude than normal. Late onset amblyopes had reduced luminance CS of the amblyopic compared to fellow eyes. Fellow eye chromatic CS was lower than normal in all eyes. The (M-P)/M ratio was greater in the late onset amblyopic eyes. This ratio was increased in all fellow eyes compared to normal. All amblyopes had shorter N200 latencies for both eyes compared to normal. Late onset amblyopic eye VEP N130 latency was longer than in early amblyopes. Early onset amblyopic eye N130 amplitude was smaller than fellow eye amplitude. Results were independent of visual acuity.

Different patterns of contrast abnormality occur in early and late onset amblyopes. Late onset amblyopes have a greater parvocellular (P) deficit. Magnocellular (M) results suggest an enhancement of this pathway in all amblyopes. Fellow eyes of amblyopes are abnormal. Examining children during treatment for amblyopia would develop these hypotheses. The changes observed in the M and P pathways could be used to develop treatment strategies in the future.

Contents

Tables

Figures

Figure 1. Calibration curve for contrast psychophysics, contrast VEPs, magnocellular psychophysical experiments and motion VEPs.

Figure 2. Calibration curve for parvocellular psychophysical experiment.

Figure 3. Calibration curve for colour VEP.

Figure 4. Box –and –whisker plots for log central CS at 3.2 cpd for early- and late-onset amblyopes and normal control subjects.

Figure 5. Group mean pattern – onset VEPs for early and late amblyopes and normal control subjects.

Figure 6. Latencies and amplitudes of the CII pattern onset VEP responses at different contrast levels for early- and late onset-amblyopes and normal subjects.

Figure 7 (A) Latencies and (B) amplitudes of the CII pattern onset VEP responses at different contrast levels for combined data from early- and late-onset amblyopes and normal subjects.

Figure 8. CII pattern-onset VEP latencies to 100% contrast plotted against logMAR equivalents for (A) amblyopic and (B) fellow eyes.

Fig 9. Box –and –whisker plots for log luminance contrast sensitivity for early- and late-onset amblyopes and normal control subjects.

Fig 10. Box –and –whisker plots for log chromatic contrast sensitivity for early- and late- onset amblyopes and normal control subjects.

Fig 11. Box –and –whisker plots for (M-P)/M ratio for early and late onset amblyopes and normal control subjects.

Fig 12. Group mean Motion Onset VEP traces for early onset amblyopes and normal control subjects.

Figure 13. N200 motion-onset VEP latencies plotted against logMAR equivalents for (A) amblyopic and (B) fellow eyes.

Figure 14. N200 motion-onset VEP amplitudes plotted against logMAR equivalents for (A) amblyopic and (B) fellow eyes.

Fig 15. Group mean VECP traces for early onset amblyopes.

Fig 16. Comparison colour VEP latencies to logMAR acuities.

Fig 17. Comparison colour VEP amplitudes to logMAR acuities.

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Ethics

The ethical committee of Moorfields Eye Hospital, London, approved the psychophysical and electrophysiological studies.

Chapter 1. Introduction

The aim of this research has been to investigate whether the age of onset has an effect on the pathogenesis of amblyopia in humans. If true, this could explain differences in previously noted treatment outcomes. More importantly, it might provide a basis for the development of novel treatment strategies in the future.

Amblyopia is the term used to describe loss of vision when significant interruption of normal visual development has occurred. It is usually classified according to the cause of the abnormality, the main groups being strabismic (i.e. due to a squint), anisometropic (i.e. due to an unequal refractive error) and form deprivation amblyopia (i.e. due to a media opacity) [1]. Amblyopia is derived from the Greek word meaning 'dullness of vision' and has been recognised since antiquity [2]. Despite marked abnormalities of the central visual pathways amblyopia is significant in that it is a potentially reversible condition. The reasons for this potential reversibility are addressed below.

Prevalence and consequences of amblyopia

The reported prevalence of amblyopia varies between 1% and 5%, depending upon definition of amblyopia, methods of examination, age at testing and ethnicity. The ALSPAC study found a prevalence of amblyopia of 2% in children who did not attend pre school screening and 1.1% in those children that did attend screening [3]. The Cambridge vision screening programmes initially used photorefraction and subsequently videorefraction to detect infants between 6 and 9 months with strabismus

and significant refractive error [4], [5]. Those at risk were randomised to treatment or no treatment with a partial refractive correction. Combining data from control subjects and untreated hyperopes at 4 -5 years of age gives an estimated prevalence of amblyopia 1 – 2% [6]. The prevalence of amblyopia in adults over 40 years in Victoria State, Australia has been reported to be 3% [7]. Screening has been shown to produce a substantial reduction in the prevalence of amblyopia in target populations. In Sweden a comprehensive screening program for eye disease and visual function has been in place for 20 years. As a result of screening and treatment of amblyopia the prevalence of severe amblyopia (visual acuity < 6/18) has been reduced from 2.0% in 1970 to 0.2% in 1992 [8].

A significant proportion of jobs in Great Britain have a minimal visual requirement [9]. A recent study identified all individuals in the UK with unilateral amblyopia (acuity worse than 6/12) who had newly acquired vision loss in the non-amblyopic eye. This study found that only 35% of subjects who lost vision in their good eye were able to continue in paid employment. This was in part due to subjects no longer having vision within the driving standard. Thus treatment of amblyopia during childhood is a potentially valuable strategy to prevent incapacitating vision loss later in life [10].

Untreated amblyopia is very important to the relatively small number of people affected by loss of their fellow eye, but in addition the psychological impact of untreated amblyopia on all amblyopes and their families should not be underestimated [11]. In a study of amblyopes without strabismus, 50% felt that amblyopia affected their lifestyle, including their self-image and friendships [12].

Natural history of amblyopia and the effect of treatment in children

Two studies have been published reporting the natural history of untreated amblyopia in children not complying with treatment [13], [14]. Both found no spontaneous improvement in the acuity of the amblyopic eye in the absence of treatment. The first and second Cambridge screening programmes found that giving partial spectacle correction to significant hyperopes or anisometropes at age 9 – 11 months (i.e > = 4.0 DS in any axis or > 1.50 DS difference between corresponding axes) significantly reduced the incidence of amblyopia and non - accommodative strabismus at 4 – 6 years [6], [15]. Visual outcome has been shown to be stable for at least 10 years following treatment [16]. Cleary [17] compared outcomes over a one year period in sixty-nine strabismic amblyopes who complied with both spectacles and occlusion to seventeen children who complied only with spectacles. Those using spectacles alone had poorer acuities than those using both occlusion and spectacles, although acuities of the amblyopic eyes improved in both groups. Moseley et al also showed that spectacles alone improved acuity in amblyopic eyes [18]. In those studies spectacle correction must be considered a form of treatment since it removes the cause of visual deprivation and restores a sharp retinal image. In a retrospective study of 246 strabismic and anisometropic amblyopes, a good visual outcome was achieved in all pure anisometropic patients [19]. Esotropic patients were more likely to have a poor outcome particularly if non–compliant, if also anisometropic and if they had a low visual acuity at the start of treatment. It may be that spectacle correction and patching are correcting two different components of amblyopia, and that these are present in differing proportions in different patients.

Given good compliance, occlusion therapy can be effective in strabismic and anisometropic amblyopes even when initiated after 7 years of age [20]. The possibility that the effect of occlusion therapy may be enhanced by a subsequent period of sleep is suggested by an animal study in which sleep appeared to increase the effects of a short period of prior deprivation [21]. Nevertheless, it is evident that patients of the same age with apparently similar amblyopia respond differently to the same treatment. Using an objective measure of occlusion it has been suggested that factors other than compliance are likely to affect outcome [22].

Visual pathways in the brain

The majority of retinal ganglion cells project to the two lateral geniculate nuclei (LGN). Their axons terminate at synapses with LGN cells, which are arranged in layers or laminae. The LGN of each hemisphere represents the contralateral half of the visual field and is composed of 6 principal layers of neurons. Layers 1,4 and 6 receive input from the contralateral eye and layers 2,3 and 5 from the ipsilateral eye. Two major relay populations of neurons that project on to the visual cortex have been identified. The largest cells are the magnocellular (M) cells and are found in the two lower laminae, layers one and two. Smaller parvocellular (P) neurons are found in the upper four laminae, layers three to six. The axons of the LGN cells terminate at synapses mainly in layer IVc in the striate cortex [23] where M and P cells terminate in separate sub layers, IVCα and IVCβ respectively. In layer IVc cells respond to a stimulus presented to one eye only, just as cells in the LGN do. In other layers cortical cells have binocular fields; they respond to the same optimal stimulus if it is presented in either eye. Even so, cells in the visual cortex often respond more strongly to a stimulus presented to one eye

rather than the other and are said to show ocular dominance. Cells sharing the same ocular dominance are grouped together into columns or bands. These columns form an alternating pattern of right and left eye bands running across the cortex and can be made visible by appropriate staining techniques [24]. Beyond the striate cortex (V1 and V2) the extrastriate area segregate into two main pathways. A dorsal pathway, which runs via V3 and V3A to the middle temporal lobe (also known as MT or V5), to the medial superior temporal (MST) area and finally to area 7a in the parietal lobe [25], [26]; and a ventral pathway which runs to V4, then to the posterior and anterior area in the temporal lobe [27].

Central Nervous System changes in visual deprivation

The pioneering work of Hubel and Wiesel demonstrated that visual function in kittens was affected by various kinds of abnormal visual experience [28]. Monocular deprivation caused a reduction in the proportion of striate cortical neurons responsive to stimulation through the deprived eye. Artificially induced strabismus caused a reduction in the proportion of binocularly driven cells [29]. Deprivation in kittens was shown to affect both the visual cortex and the lateral geniculate nucleus (LGN). For deprivation to have an effect it must occur during the sensitive period of visual development i.e. the sensitive period of visual development is the duration of time in which deprivation can have an effect on visual function. [30]. The closure of one eye has a much greater disruptive effect on the visual pathways than closure of both eyes. This would suggest that the changes produced by visual deprivation are due at least in part to a competitive interaction between the pathways related to the two eyes [31]. The concept of

competition was subsequently established and extended in the cat by Guillery [32], [33], [34].

Subsequent studies in the monkey showed that anatomically demonstrated ocular dominance columns in layer IV of the primary visual cortex which received input from the deprived eye shrank, whereas those receiving connections from the fellow eye expanded [35], [36], [2]. These changes were associated with changes in ocular dominance.

Evidence for two periods of sensitivity during visual development

The major changes in cortical ocular dominance columns described in the monkey only occur within the first three months of life. Because of the discrepancy between this and the 7-year sensitive period in humans it has been suggested that these animal models are not valid for clinical application to children, [37].

Data from primate LGN studies show that there is a second distinct period of developmental sensitivity. During this second period, the nature of the interaction between the two eyes is different and the conditions for recovery are also differ [38]. During the early sensitive period from birth to 3 months of age, reverse suture, equivalent to patching, is effective in re-expanding the ocular dominance columns in the primate visual cortex, but performed after this age has no effect [36], [39]. At later ages the relative sizes of cortical ocular dominance columns for each eye are unaffected by visual deprivation [36] although it still causes major changes in cell size in the LGN [40]. The LGN changes after late monocular deprivation follow a different pattern to those seen with early deprivation. Late deprivation selectively affects the neurones in

the P LGN laminae and affects cells receiving input from both the deprived and undeprived eyes. Recovery of LGN cell size in this later phase occurs after simply opening the deprived eye [41].

If two comparable sensitive periods are present in humans the corresponding ages for these two sensitive periods are probably from approximately 4 months of age to 18 months of age and from 18 months to 7 years of age. This is based on the generally acknowledged premise that one week of age in the monkey is equivalent to one month in humans, together with an addition to allow for the anatomical evidence that the human visual system is substantially less mature at birth than that of the monkey [42], [38]. Primate studies of strabismic and anisometropic amblyopia have further elucidated abnormalities in V1 neurons [43]. In addition, despite deficient excitatory interactions there is a relative sparing of inhibitory, suppressive interactions particularly in strabismics [44], [45]. It has also been suggested that the suppressive spatial interactions in amblyopic vision extend over larger distances than in normal foveal vision, similar to peripheral vision of non-amblyopic observers [46].

It is probable that changes of ocular dominance columns in the first sensitive period are an important correlate of amblyopia, but not in the later period. This latter point has been demonstrated in two post mortem case reports. In the first a human anisometropic amblyope was found to have no changes in their cortical ocular dominance columns [47]. A similar lack of abnormality of the ocular dominance columns was also shown in a late onset strabismic amblyope [48]. These findings suggest that shrinkage of ocular dominance columns does not occur in humans with amblyopia of late onset as the ocular dominance columns are probably no longer susceptible to shrinkage.

Primate studies have also demonstrated that different conditions were required for recovery of LGN cell size following monocular deprivation at different ages. Whereas reverse suture, equivalent to total occlusion, was required to revert changes following early deprivation, after late deprivation recovery occurred after simply reopening the eye. [38] In humans there is evidence that simply correcting refractive errors in anisometropic amblyopes probably of late onset allows considerable improvement of acuity in both eyes before patching is initiated [49]. This improvement with glasses alone rather than in conjunction with patching suggests that different conditions may be required for recovery from amblyopia dependant upon age of onset in humans. It may also explain some of the treatment failures and differing opinions with regard to the efficacy of screening in humans

Contrast sensitivity – normal development and amblyopia

Psychophysical data

Sensitivity to contrast is approximately 10 times worse in the newborn primate compared with the visually mature adult. Primate studies have shown that the contrast sensitivity function (CSF) shifts both to both higher spatial frequencies and higher contrast sensitivity (CS) [50], [51]. CS (CS) in human infants shows a rapid increase to near adult levels during the first year of life [52], [53]. The form of the CS function does not remain constant during postnatal development. Older infants, like adults, are more sensitive to medium compared to low spatial frequencies. Infants under 2 months of age do not show this low spatial frequency cutoff [54].

The development of CS across the visual field has also been investigated in monkeys. In the neonatal period there is little difference in CS at 0, 8 and 12 ° from central fixation. However, over the ensuing 4 to 6 months there is differential development of the central and peripheral visual fields. The central field undergoes substantially greater improvements in spatial scale and sensitivity than seen in the peripheral fields, and occur over a longer time period than more peripheral locations. Improvements in the peripheral field do occur, but to a lesser degree both qualitatively and quantitatively. The extent of post-natal development declines with increasing eccentricity, such that there is little change from the neonatal period at 24° [55]. Although these changes are concordant with cone cell density improvement in human infants, the development of CS cannot be accounted for solely at the level of the photoreceptor [56], and thus a post-receptoral origin/locus should be considered.

The earliest post-receptoral level at which the development of neural processing by electrophysiological recording in primates has been studied is the lateral geniculate nucleus (LGN). LGN function shows a simultaneous shift to both higher spatial frequencies and CS, as does that observed behaviourally [57]. The LGN neurones not only increase in overall responsiveness and spatial resolution, but also do so differentially depending on receptive field position. Neurones with receptive fields within the central 10° show a considerably greater post-natal increase than those at more peripheral locations [58]. This suggests that maturation of neural processing at or after the level of the LGN may underlie behavioural CS in infants.

Primate behavioural studies have shown that amblyopic eyes perform similarly to young normal eyes [59]. Amblyopic primates [60] and humans [61] have been shown to have

mid to high CS loss such that the amblyopic eye is relatively more sensitive to lower spatial frequencies.

CS studies in human amblyopes have also shown that strabismic and anisometropic amblyopes differ in the changes from normal that occur across the visual field. In strabismic amblyopia the peripheral region of one or both hemifields may be spared, whilst in non-strabismic anisometropes there is CS loss across the whole of the binocular visual field [62]. In addition, there is evidence that human strabismic amblyopes fall into two categories based on the pattern of loss of CS. In one group of patients CS is depressed only for high spatial frequencies; whilst in others, CS is depressed at all spatial frequencies [63]. As both high and low spatial frequency losses were observed it is likely that the changes are of neural origin. It is unlikely that optical, oculomotor or eccentric fixation changes could account for low spatial frequency changes, although could cause medium/ high losses. This data was based on comparisons between amblyopic and fellow eyes. It was acknowledged that it would not be expected to have significant differences between two normal eyes, but normal eye data was not used comparatively.

McKee et al [64] have argued that the inconsistent abnormalities of psychophysical measurements in amblyopes maybe due to small sample sizes in study populations. In a study of 427 amblyopes categorised by clinical attributes they found that optotype acuity accounts for much of the variance in Vernier and grating acuity and less of the variance in contrast sensitivity. Nevertheless strabismic and anisometropic amblyopes had different patterns of abnormality. These results were similar to those studies with smaller samples. In addition they found that non binocular observers with mild to

moderate visual acuity losses had better monocular contrast sensitivities than binocular observers despite poorer optotype and Vernier acuities at a given level of grating acuity. They suggested that this might be due to cortical pooling of neurons in the striate cortex.

It is generally considered that the synaptic basis of amblyopia lies beyond the LGN, as the spatial resolution of LGN neurones in monocularly deprived monkeys is normal [65] in the presence of profound behavioural loss [66]. Physiological studies of neurons in V1, driven by the amblyopic eye, have found that their response properties are shifted to lower spatial frequencies and it has been argued that this is more in keeping with the behavioural loss found in amblyopia [43]. The changes in LGN cell size are probably secondary to changes in the axons of these cells in the visual cortex. It is becoming increasingly apparent that amblyopia is a disorder also involving higher visual functions [67] and as such it can be anticipated that neural deficits exist beyond the striate cortex.

Anatomical changes resulting from visual deprivation, in the primate LGN, were studied by Sloper et al. [38]. A particular feature of this work is that age of onset was carefully delineated. Monocular deprivation commenced before or after 8 weeks was found to produce significant differences in neuronal morphology in comparison with normal eyes. The relationship between cells in the lower two, M, layers of the LGN and upper four, P, was different depending upon age of onset. The requirement for recovery from deprivation was also different for primates with early compared to late onset amblyopia. It maybe that the two type classification suggested by Hess and Howell [63] is in part, a behavioural correlate of these anatomical differences.

Electrophysiological data

Campbell and Maffei [68] have shown that the pattern visual evoked potential (VEP) amplitude in normal subjects is proportional to the logarithm of the stimulus contrast. Maier et al [69] combined topographical distribution with principal component analysis to locate the sources of pattern-onset VEPs. They considered that the first peak (C1) was generated in area 18 and that the second component (CII) originated in both 17 and18. Both traditional stimulus VEP (i.e. the electrical response of the cortex to visual stimulation, extracted from the higher voltage electroencephalogram using repeated stimulation and computer averaging) and sweep VEP (using Fourier analysis to determine whether a cortical response is present to a series rapidly changing contrast levels) techniques have been used to study the CS function (CSF) in infants. Data from these studies generally agree that the CSF's are similar in shape though not as high in threshold for infants 2 months and older compared to adults [70], [71]. Norcia et al used sweep VEP's in a group of 48 infants to measure CS and compared the results with those found in 10 adults. There appeared to be two phases in the development of CS. Between 4 and 9 weeks overall CS increased by a factor of 4-5 at all spatial frequencies. Beyond 9 weeks, CS at low spatial frequencies remained constant, whilst sensitivity increased systematically at higher spatial frequencies. The development of high spatial sensitivity continued until at least 30weeks of age. The peak of the CSF was nearly adult like at 6 – 8 months [72].

Electrophysiological studies of amblyopia have described both increased latency and reduced amplitude of responses from the amblyopic eye. Sprekreijse et al used pattern onset/offset stimulation with 20' checks and described that both onset and offset VEP`s

were much reduced in amplitude from the amblyopic eye when compared with the fellow eye [73]. Shawkat et al. documented reduced amplitude of the pattern onset VEP and increased latency of the pattern reversal VEP in amblyopic compared to fellow eyes. In addition pattern reversal P100 amplitudes were consistently reduced for a variety of check sizes in fellow eyes compared to normal despite normal vision in the fellow eyes. This would suggest that the fellow eye should not be considered to be normal [74]. Changes in the fellow eye of amblyopes are of particular importance because this is the eye that the patients depend on for visual input and subtle and as yet unrecognised deficits may have a considerable impact on their lives. Campos et al, 1984, compared strabismic and anisometropic amblyopes and found that strabismics only had high contrast losses, whilst anisometropes with or without strabismus had losses at both high and low CS losses. [75].

Magnocellular and parvocellular pathways – normal development and amblyopia

Functional characteristics of M and P pathways

Livingstone and Hubel [76] proposed that M and P cells in the LGN are the first stages of a two way division of function continuing much further through the visual system. From their work on single cell response differences they suggested that M cells are responsible for extracting information about large-scale spatial layout and movement whereas P cells carry out the first steps in extracting information about colour and fine spatial structure. Techniques to create selective, localised lesions in the M and P pathways have shown that there is more functional overlap than might have been predicted from single cell studies. The effect of bilateral ibotenic acid lesions, aimed at the M pathway cause a reduction in sensitivity to stimuli that contain both high temporal and low spatial frequencies. This results in reduced visibility to rapidly moving or flickering stimuli at low, but not high contrast. Lesions of the M pathway do not interfere with discrimination of large-scale spatial patterns. P pathway lesions cause complementary effects to those of M lesions, thus reducing luminance CS for stimuli of higher spatial and lower temporal frequency content. P pathway lesions also result in complete loss of colour vision [77]. Lesions of the P, but not M pathway affect CS.[78]. As there are about eight times as many P cells as M cells per unit area, it maybe that the overall P cell population yields greater sensitivity than the M population despite the lower sensitivity of individual cells. Thus in terms of their frequency characteristics the P pathway can be described as a channel carrying information at all spatial frequencies, but only at low to medium temporal frequencies. The M pathway is a channel, which carries information about high temporal and low spatial frequencies. Nevertheless,

using an interference psychophysical methodology, Kessels et al [79] found that colour flicker interfered with object recognition and luminance flicker interfered with memory for spatial location. These results support the notion of dissociation between what and where processing in the human brain. A colour-sensitive area (V4) had been confirmed in the ventral occipitotemporal cortex [80]. A highly sensitive motion area (V5/MT) has been located at the junction of the temporal, parietal and occipital cortices [25], [26].

Development of visual motion processing

The commonplace observation that newborns readily attend to moving visual stimuli suggests that the motion system is relatively mature at birth. However, this may be due to sensitivity to temporal modulation rather than motion as such; infants readily show a visual preference for dynamic stimuli that do not move such as full-field flicker [81]. Sensitivity to temporal modulation appears to be complete by 12 weeks of age [82]. Full use of motion information requires sensitivity to speed and direction, and these low-level mechanisms provide the foundation for more global perceptual processes. When tested with forced choice preferential looking techniques infants of 3-6 weeks of age did not show any evidence of direction discrimination, but by 7 weeks sensitivity to direction had begun to emerge. Further studies have shown that the development of directional motion is characterised by an expanding velocity range with increasing sensitivity to low and high velocity movement. Sensitivity to intermediate velocity stimuli also continues to improve [83], [84].

Motion onset/ offset VEPs have been shown to consist of two components. Spekreijse at al [85] found that as the interval between motion onset and offset increased to give a real impression of motion at intervals of > 150 msec these two complexes could be distinguished. The latency of the first complex remained constant as would be expected if this complex is due to motion onset and the latency of the second complex changed proportionally to the interval between onset and offset. . The response to motion onset has been shown to have a low threshold , saturating contrast characteristic suggesting the response is generated by the M pathwayl [86]. The authors also studied the contrast dependency of motion onset and pattern reversal VEPs. They observed a negative component at approximately 180 msec to a motion-onset stimulus at low contrast. This component dominated the lateral derivation and was quite separate from the positive component peaking at around 130 msec in the occipital derivation in response to a pattern reversal stimulus. The positive component was enhanced by high contrast. Kubova et al found that the amplitude of the major negativity changed relatively little as contrast was increased, thus agreeing with the concept that the M pathway saturates at low contrast. [87]. One study using a combination of direction reversal and an uncorrelated pattern as a moving stimulus found a response could be measured at 10 weeks. They also found that development of sensitivity to directional motion starts at low velocities and spreads to higher velocities in the maturing infant [88].

Development of colour processing

Colour vision seems to develop relatively early in infants. Behavioural experiments provide evidence for the emergence of colour vision in infants between 1 and 3 months of age[89], [Brown, 1990 #236 Using isoluminant coloured stimuli specific VEP responses have been recorded [90]. In addition VEP components to such stimuli have

linear contrast/amplitude relationships. These observations would suggest a parvocellular neural response underpinning the recorded electrical activity. It has been shown that acuity and CS develop independently for luminance and colour vision [91], [92]. No significant responses could be obtained from equiluminant colour patterns in infants younger than 6-8 weeks. At this age luminance patterns at only 20% contrast give strong and reliable responses. After the onset of response to equiluminant colour patterns, CS and spatial acuity improve rapidly with age, more rapidly than to luminance contrast. As young infants have normal photoreceptors it has been suggested that maturation represents development of the organisation of the receptive fields of neurones responding to chromatic stimuli. Burr et al, [93], studied the development of the temporal characteristics of the pattern visual evoked potentials (P-VEPs) in response to contrast reversal of patterns of low spatial frequency (0.1 c/deg) of either pure luminance contrast (yellow-black plaid patterns) or pure colour contrast (equiluminant red-green plaid patterns) in 15 infants between 6 and 30 weeks of age. Their results suggested that latency to both the colour and luminance of their stimuli increased with age. However, the latency changes were at differing rates suggesting different maturation of the stimulus generators. Data from temporal CS functions (tCSFs) for 3 and 4- month old infants have shown that the chromatic tCSF lacks the sharp high temporal characteristic of the adult function. In addition the luminance tCSF in 3 month olds is elevated above the chromatic tCSF. By 4 months of age, substantial development of chromatic CS has taken place at the lowest temporal frequencies and it begins to develop a more adult shape. By 4 months the luminance and chromatic tCSFs cross at 5 Hz. It has been suggested that this relatively slow development of the chromatic tCSF in infants reflects the relatively immature chromatic responses in the P pathway and/or reliance on chromatic responses in the M pathway [94]. However, an earlier study has

suggested that parvocellular-based systems may become operational slightly earlier than magnocellular systems [95].

Abnormalities of motion and colour processing in amblyopia

Psychophysical assessment has suggested that both motion and pattern detection is affected by amblyopia [96], [97]. Pure strabismic, pure anisometropic and mixed amblyopes have been shown to have a reduction of sensitivity to flickering sine-wave gratings. The reduction is both temporally and spatially dependent and more marked at high spatial and low temporal frequencies. [98]. Work comparing strabismic and anisometropic amblyopes using pattern VEP has found a P anomaly in strabismics and both an M and P anomaly in anisometropes before treatment. These differences improved during treatment [99]. However, other authors argue that motion can be discriminated at detection thresholds in humans with amblyopia [100]. Comparisons are often made between amblyopic and fellow eyes, assuming normality of the fellow eye. However, motion perception in the fellow eye has been shown to be defective in strabismic and anisometropic amblyopic children [101].

Electrophysiological studies of strabismic and anisometropic amblyopic children [102] found that the VEP to pattern reversal stimulation correlated with the level of acuity impairment whereas the response to a motion-onset stimulus was similar for the amblyopic and non-amblyopic eyes. The authors suggested that the motion system might be relatively spared in amblyopia, but their study did not include a normal control group. Other authors found that pattern reversal latencies were significantly prolonged compared to normal for both amblyopic and fellow eyes with small checks (12.5'

checks) in successfully treated amblyopes [103]. VEP responses in strabismic amblyopes and normal children have been compared for M and P pathway involvement using M and P biased stimuli [104]. The M pathway stimulus was an achromatic low spatial frequency sine wave grating; the P pathway stimulus was a chromatic sine wave grating. The strabismic amblyopes showed no difference in either latency or amplitude compared to normal for the achromatic stimulus. The response to the chromatic stimulus was increased in latency and reduced in amplitude to the chromatic stimulus for amblyopic eyes both compared to fellow eyes and normal controls.

Summary

Our understanding of human amblyopia is underpinned by neuroanatomical data from animal models. Nevertheless much of this work relates to amblyopia occurring soon after birth. There is far less reported data on later onset amblyopia, which is the more common human condition.

Human behavioural studies have found different patterns of amblyopic abnormality using a wide variety of clinical paradigms. Equally many of these studies include subjects with a wide range of amblyopic severity and in addition different types of amblyopia. In those studies, which are carefully clinically characterised, it would appear that the presence of strabismus or loss of binocular function is the major determinant of loss of visual function. There are very few studies, which consider age of onset.

Human VEP studies of amblyopes most frequently describe delayed latency and reduced amplitude to the stimulus studied. There are no electrophysiological studies in which age of onset is considered in amblyopia.

Many behavioural and electrophysiological studies of amblyopia consider the preferred or fellow eye of amblyopes to be normal and do not have a normal control group. There has been some recent evidence that this is not the case. I would argue that it is crucial to include a normal data set in any study of amblyopia.

A deficit of the parvocellular pathway has been shown behaviourally and electrophysiologically. However studies have suggested that the magnocellular pathway

is unaffected. This would seem unlikely if the M and P are parallel pathways with some degree of functional overlap.

Age of onset has been suggested, from primate studies, to affect both M and P pathways. This was found to differ according to age of onset. This would translate into age of onset before or after 18 months of age in humans. If human amblyopia could be shown to have different patterns of abnormality related to time of onset, this could explain some of the discrepancies with animal data. More importantly this would provide a basis on which to develop treatment strategies for the future.

Research Questions

This thesis has used psychophysical and electrophysiological in parallel to examine two questions:

1. Are there different abnormalities in the response to contrast in early and late onset strabismic amblyopes?

2. Do the magnocellular and parvocellular pathways show different changes in strabismic amblyopes, and do the changes differ with age of onset?

Chapter 2. Subjects and Methods

Subjects

Subjects were only included in the study when it could be established that they had a clear history of age of onset before or after 18 months of age. This gave 2 patient groups defined as early (pre 18 months) and late (post 18 months) onset amblyopes. Potential subjects were closely questioned in the clinic. Those with equivocal histories were not recruited. Information was supplemented by details from parents where available. All subjects underwent full orthoptic and ophthalmic assessment prior to testing. Table 1 indicates the experiments completed by each subject. Full clinical details are given in Appendix 1.

Most of the strabismic amblyopes studied had initially been esotropic as children, although a number had now become consecutively exotropic. All patients presented as adults with a manifest squint. Most had no, or only occasional, diplopia indicating suppression of the deviating eye. Suppression was confirmed on testing with Bagolini glasses in 12 subjects in the early group and 6 in the late group. One patient (subject number 36) had a manifest squint with troublesome diplopia. One patient (subject number 33) had an accommodative squint and amblyopia treated as a child and had presented with an increase of her squint angle several years previously. She recovered to have a TNO stereoacuity of 120 seconds of arc following treatment with Botulinum toxin.

Table 1. Experiments completed by each amblyopic subject.

	Subject Number	Psychophysics Contrast	VEP Contrast	Psychophysics M+P	VEP Motion	VEP Colour	fMRI
Early Onset	1	*	*	*	*	*	
	2	*	*	*	*		
	3			*	*	*	
	4	*	*	*		*	*
	5			*	*		
	6	*		*			
	7			*	*	*	
	8	*	*	*	*	*	
	9			*	*	*	*
	10	*	*	*	*	*	
	11			*	*	*	
	12			data excluded	*	*	*
	13			*	*	*	
	14	*	*				
	15	*	*				
	16	*	*	*	*	*	
	17			*	*	*	
	18			*			
	19	*	*				
	20	*	*		*	*	
	21		*	*	*	*	*
Late Onset	22	*	*	*	*	*	*
	23			*			
	24	*	*	*			
	25			*	*	*	*
	26	*		*	*	*	
	27	*	*	*	*	*	*
	28	*	*	*	*	*	*
	29			*		*	
	30		*	*	*	*	
	31	*	*	*	*	*	
	32	*	*				
	33			*	*	*	*
	34	*	*	*	*	*	*
	35	*					
	36	*	*				
	37	*	*	*	*	*	

* = Completed experiments

Control subjects

Normal subjects were recruited from clinical staff at Moorfields Eye Hospital. The median age of early onset amblyopes was 32 years, for late onset amblyopes 32.5 years and for normal subjects 31 years. There was no significant difference in the age distribution of normal subjects and amblyopes. All control subjects had at least 6/6 vision in both eyes. There was no significant difference between the acuities of the normal subjects and those of the fellow eyes of the amblyopes ($t > -0.2$; $p > 0.2$). Normal subjects were naive observers for all the psychophysical measures used. Some of the observers were experienced electrophysiological observers. However, there was no difference in latency nor amplitude of any of the measures used between the experienced and inexperienced observers.

Contrast experiments

Subjects

A total of 12 early and 12 late onset subjects were recruited and completed either psychophysical or electrophysiological testing or both (Table 1). The majority of subjects in the early and late onset groups had low hypermetropic refractive errors in both amblyopic and fellow eyes. There were no significant differences between early and late groups (mean spherical equivalents: early amblyopic = 0.85D; early fellow = 0.18D; late amblyopic = 1.84D; late fellow = 0.77D). Four subjects in each group had anisometropia of more than 1.5 dioptres of spherical equivalent. No control subject had significant anisometropia. Patients in both early and late onset groups showed a similar spread of acuities in their amblyopic eyes (logMAR equivalent means: early onset 0.63, Snellen equivalent means ≈ 6/26, 20/85; late onset 0.83, Snellen equivalent ≈ 6/40, 20/135; P= 0.25). There was no significant difference in fellow eye acuities between the groups (logMAR equivalent means: early onset = -0.04 Snellen equivalent means ≈ 6/5, 20/18; late onset -0.08, Snellen equivalent ≈ 6/5, 20/17; P= 0.34) nor any differences from the normal group. All normal subjects had a visual acuity of at least 6/6 in each eye with binocular single vision.

Psychophysical assessment of contrast sensitivity

Subjects

Eleven early and 11 late onset strabismic amblyopes completed psychophysical testing. Summaries of their clinical details are shown in table 2. Full clinical details are shown in appendix 1. Twelve normal adults formed the control group for the CS measurements.

Table 2. Summary of amblyopic subjects who completed contrast experiments.

	Subject number (see Appendix 1)	Age (Years)	Diagnosis	Anisometropia	Snellen Acuity Amblyopic Eye	Snellen Acuity Fellow Eye	VEPs	Contrast Sensitivity
Early onset	1	18	Residual ET	N	6/36	6/9	+	+
	2	33	Residual ET	N	6/24	6/6	+	+
	4	24	Residual XT	Y	6/18	6/5	+	+
	6	17	Residual XT	Y	1/60	6/5	-	+
	8	40	Residual ET	Y	6/9	6/6	+	+
	10	50	Consecutive XT	N	6/24	6/5	+	+
	14	35	Consecutive XT	N	6/60	6/5	+	+
	15	26	Consecutive XT	N	6/12	6/4	+	+
	19	24	Consecutive XT	N	6/9	6/5	+	+
	20	24	Residual ET	N	6/24	6/5	+	+
	21	23	Residual ET	Y	6/24	6/9	+	-
	16	21	Consecutive XT	N	6/18	6/4	+	+
Mean		**28.9**						
Late onset	22	29	Consecutive XT	Y	6/24	6/5	+	+
	24	31	Consecutive XT	Y	2/60	6/4	+	+
	26	49	Consecutive XT	N	6/60	6/5	-	+
	27	30	Consecutive XT	N	6/24	6/5	+	+
	28	27	Residual ET	N	6/24	6/5	+	+
	30	36	Primary ET	N	6/9	6/5	+	-
	31	30	Consecutive XT	Y	1/60	6/4	+	+
	32	21	Primary ET	N	6/24	6/6	+	+
	34	36	Consecutive XT	N	6/60	6/5	+	+
	35	37	Residual ET	N	6/60	6/6	-	+
	36	28	Primary ET	Y	6/18	6/5	+	+
	37	34	Consecutive XT	N	6/36	6/6	+	+
Mean		**32.3**						

Abbreviations: ET = esotropia; XT = exotropia

Experimental design

A horizontally orientated 3.2 cpd sinusoidal grating pattern with spatial ($\sigma_x = \sigma_y = 0.5$ deg) and temporal ($\sigma_t = 125$ msec) Gaussian windows was PC generated using a 14 bit VSG card and MATLAB. This gave a stimulus subtending approximately 2 degrees of visual angle at the viewing distance of 0.93 m. Monocular detection thresholds were measured for the stimuli using a temporal, two alternative forced choice (2AFC) technique. A staircase procedure driven by the subject's responses and controlled by computer determined the detection threshold. Each trial consisted of two presentations (cued by sounds) one of which contained the stimulus whilst the other was a blank field of the same space averaged luminance. Thresholds were determined by a one-up, one-down staircase procedure in which the contrast was divided by 1.15 after a correct response and multiplied by 1.15 after an incorrect response. Until the first error the divisor was 1.25. Every time an incorrect response was followed by a correct response, a reversal was noted. The session ended after 10 reversals. Threshold was defined as the average contrast over the last 5 reversals. Stimuli were presented in the centre of an NEC multisync monitor at a mean luminance of 90cd/m^2. The stimuli were surrounded by a luminance-matched field (10° x 8°). The room was darkened. The display screen's contrast linearity was measured and found to hold up to 98% contrast. For central measurements a fixation target was computer generated within the luminance-matched field. For eccentric measurements a small fixation spot was fixed to the monitor screen at 5 and 10 degrees along the horizontal meridian and the subject was observed to ensure that fixation was maintained. The patients' normal refractive correction was used during testing.

Statistical Analysis

CS measurements were log transformed. Comparisons of central CS between amblyopic and fellow eyes were made using paired t-tests and between groups using unpaired t–tests. CS across the central field was analysed by ANOVA.

Comparison of electrophysiological responses at different contrast levels

Subjects

Eleven early and 10 late onset strabismic amblyopes were tested. Summaries of their clinical details are shown in table 2. Full clinical details are shown in appendix 1. Fifteen normal young adults formed the control group for the VEPs.

Experimental design

Monocular VEPs were recorded to a chequerboard pattern onset stimulus at 100%, 80%, 40%, 20%, 10%, 5% contrast levels, with the other eye being patched. Space-averaged mean luminance was 90 cd/m^2 for both the stimulus chequerboard and interstimulus display. Field size was 20° x 16°, with a check size of 30 minutes and a viewing distance of 1 metre. The stimulus presentation was pattern on for 40 msec and pattern off for 500 msec. Fixation was maintained using a central red spot. Responses were recorded from an electrode at Oz (midline) referenced to Fz (mid-frontal). A ground electrode was placed on the forehead. The surface electrode impedance was $< 5K\Omega$. The number of responses averaged for each trial was at least 64, with a minimum of two replications being recorded for each contrast level for each eye. The analysis time was 300msec. The filter bandwidth was from 1 to 100Hz. The patient's normal refractive correction was used. Peak latency and peak-to-peak amplitudes of the CII component were measured for each contrast level.

Statistical Analysis

Visual acuities between groups were compared using unpaired t–tests after conversion to logMAR equivalents. Latencies and amplitudes of the CII VEP response were compared using analysis of variance (ANOVA).

Experiments using magnocellular and parvocellular biased stimuli

Subjects

A total of 18 early and 13 late onset amblyopes were tested (table 3). Nineteen normal subjects were recruited in total as controls.

Early and late onset amblyopes had similar refractive errors. The mean spherical equivalent for the early amblyopic eyes was 1.77D and early fellow eyes 1.45D. For the late amblyopic eyes, the mean spherical equivalent was 2.75D and for the late fellow eyes 1.24D. There was no significant difference between the early and late amblyopic eyes (P=0.35). Eight early and 4 late onset subjects had anisometropia of more than 1.5 dioptres of spherical equivalent. No control subject had significant anisometropia.

Patients in both early and late onset groups showed a similar spread of acuities in their amblyopic eyes (logMAR equivalent means: early onset 0.62, Snellen equivalent means ≈ 6/25, 20/83; late onset 0.68, Snellen equivalent ≈ 6/29, 20/96; P= 0.78). There was no difference in fellow eye acuities between the groups (logMAR equivalent means: early onset = -0.07 Snellen equivalent means ≈ 6/5, 20/17; late onset -0.07, Snellen equivalent ≈ 6/5, 20/17) nor any differences from the normal group. All normal subjects had a visual acuity of at least 6/6 in each eye with binocular single vision.

Psychophysical assessment of magnocellular and parvocellular pathways

Subjects

Seventeen early and 13 late onset strabismic amblyopes were recruited. Summaries of their clinical details are shown in table 3. Full clinical details are shown in appendix 1. Fifteen normal adults formed the control group for the measurements. The data from one early onset amblyope was excluded, as the data was significantly different from all other normal and amblyopic subjects tested.

Table 3. Summary of amblyopic subjects who completed magnocellular and parvocellular experiments.

	Subject (see Appendix 1)	Age (Years)	Diagnosis	Anisometropia	Snellen Acuity		VEPs	Psychophysics
					Amblyopic Eye	Fellow Eye		
Early onset	1	18	Residual ET	N	6/36	6/9	+	+
	2	33	Residual ET	N	6/24	6/6	+	+
	3	22	Consecutive XT	Y	6/9	6/4	+	+
	4	24	Residual XT	Y	6/18	6/5	+	+
	5	33	Primary XT	Y	6/60	6/5	+	+
	6	17	Residual XT	Y	1/60	6/5		+
	7	41	Consecutive XT	Y	HM	6/4	+	+
	8	40	Residual ET	Y	6/9	6/6	+	+
	9	33	Residual ET	N	6/9	6/4	+	+
	10	50	Consecutive XT	N	6/24	6/5	+	+
	11	39	Consecutive XT	NK	6/9	6/6	+	+
	12	35	Consecutive XT	N	6/9	6/5	+	data excluded
	13	43	Consecutive XT	NK	6/24	6/5	+	+
	16	21	Consecutive XT	N	6/18	6/4	+	+
	17	19	Consecutive XT	Y	6/24	6/6	+	+
	18	32	Residual ET	N	6/9	6/4	-	+
	20	24	Residual ET	N	6/24	6/5	+	-
	21	23	Residual ET	Y	6/24	6/9	+	+
Mean		**30.8**						
Late onset	22	29	Consecutive XT	Y	6/24	6/5	+	+
	23	20	Primary XT	N	6/6	6/4		+
	24	31	Consecutive XT	Y	2/60	6/4		+
	25	39	Fully Accommodative ET	N	6/12	6/6	+	+
	26	49	Consecutive XT	N	6/60	6/5	+	+
	27	30	Consecutive XT	N	6/24	6/5	+	+
	28	27	Residual ET	N	6/24	6/5	+	+
	29	60	Residual XT	Y	6/12	6/6	+	+
	30	36	Primary ET	N	6/9	6/5	+	+
	31	30	Consecutive XT	Y	1/60	6/4	+	+
	33	49	Primary XT	N	6/9	6/4	+	+
	34	36	Consecutive XT	N	6/60	6/5	+	+
	37	34	Consecutive XT	N	6/36	6/6	+	+
Mean		**37.1**						

Experimental design

CS for stimuli designed to stimulate Magnocellular (M) and Parvocellular (P) pathways respectively were used. These stimuli cannot be guaranteed to be "pure" M- and P-isolating stimuli [105], but at detection threshold the assumption is that they will be detected by the more sensitive mechanism [106]. Luminance CS for M stimuli and chromatic CS for P stimuli were recorded respectively.

Magnocellular stimulus

The M stimulus was an achromatic horizontally orientated 0.8 cpd sinusoidal grating pattern with spatial (σ_x = σ_y = 1.0 deg) Gaussian windows. This gave a stimulus subtending approximately 1° of visual angle at the viewing distance of 0.92 m.

A windowed Gaussian of constant duration was created, $f(t) = e\ (-\ (-t^2/\sigma^2))$. Thus only the centre of the Gaussian is presented which for a large value of σ is essentially a square wave. The value of σ_t = 10,000 msec therefore produced a transient stimulus.

Parvocellular stimulus

The P-stimulus was a red-green horizontally orientated 3.2 cycle/degree isoluminant sinusoidal grating patch presented with a spatial ($\sigma_x = \sigma_y = 0.5$ deg) This gave a stimulus subtending approximately 2° of visual angle at the viewing distance of 0.92m.

A windowed Gaussian of constant duration was created, $f(t) = e\ (-\ (-t^2/\sigma^2))$. Thus only the centre of the Gaussian is presented which for a small value of σ produced a gradual onset/ offset, sustained stimulus. A value of $\sigma_t = 125$ msec was used.

Both M and P stimuli were PC generated using a 14-bit VSG card and MATLAB (Cambridge Control Ltd., Cambridge, UK).

Method of threshold detection

Monocular detection thresholds were measured for the stimuli using a temporal, two alternative forced choice (2AFC) technique based on the Quest methodology [107]. A staircase procedure driven by the subject's responses and controlled by computer determined the detection threshold. Each trial consisted of two presentations (cued by sounds) one of which contained the stimulus whilst the other was a blank field of the same space averaged luminance. Assessment of threshold began with an estimate of threshold based on previous testing experience of the investigator. Thresholds were determined from the mean of the probability density function (pdf) [108]. The next trial was placed at the current most probable estimate of threshold using the mean of the posterior pdf. The session ended after 40 trials. The final estimate of threshold was given by the final mean of the posterior pdf.

Stimulus presentation

Stimuli were presented in the centre of an NEC multisync monitor at a mean luminance of 90cd/m^2. The stimuli were surrounded by a luminance-matched field (10° x 8°). The

room was darkened. The display screen's contrast linearity was measured and found to hold up to 98% contrast. The patients' normal refractive correction was used during testing.

Statistical Analysis

Chromatic and luminance CS measurements were compared using paired t-tests to compare amblyopic and fellow eyes. Between groups comparisons were made using unpaired t–tests.

Electrophysiological assessment of magnocellular and parvocellular pathways

Subjects

Sixteen early and 10 late onset strabismic amblyopes were tested. Summaries of their clinical details are shown in table 3. Full clinical details are shown in appendix 1. Seventeen normal adults formed the control group for the colour VEP measurements and 19 for the motion VEP measurements.

Experimental design

Monocular VEPs were recorded to M and P biased stimuli with the other eye being patched. Fixation was maintained using a central red spot.

Magnocellular stimulus

The M biased stimulus was an achromatic motion onset 0.8 cpd, vertical square wave grating. The field size was 20 ° x 16 ° at a viewing distance of 1 m and the grating moved horizontally from left to right at 4.9 deg/sec. Initial recordings were performed on normal subjects to confirm that there was no change in latency with increasing contrast to this stimulus. The M stimulus used was 100% contrast. Responses were recorded from an electrode at the midline (Oz), referenced to Fz (mid-frontal) and at 5 cm to the right and left of the midline, referenced to linked ears. A ground electrode was placed on the forehead. The peak latency and amplitude of the N200 component, recorded from the right and left hemispheres was measured off-line.

Parvocellular stimulus

The P biased stimulus was a 3.2 cpd, red/green, pattern onset (onset 200ms, offset 600ms), sine wave grating. The field size was 16 deg x 10 deg at a viewing distance of 1.5 m. Isoluminance was determined by hetrochromatic flicker photometry for each subject.The peak latency and amplitude of the N130 component, recorded from the midline was measured off-line. Responses were recorded from an electrode at Oz (midline) referenced to Fz (mid-frontal) and two electrodes at O1 and O2, referenced to

temporal electrodes 5cm above the mastoid. A ground electrode was placed on the forehead.

Stimulus presentation

Stimuli were presented in the centre of an NEC multisync monitor at a mean luminance of 90cd/$m^{2.}$ The room was darkened. The display screen's contrast linearity was measured and found to hold up to 98% contrast. The patients' normal refractive correction was used during testing.

For both stimuli the surface electrode impedance was < 5KΩ. The number of responses averaged for each trial was at least 64, with a minimum of two replications being recorded for each contrast level for each eye. The analysis time was 500msec. The filter bandwidth was from 1 to 100Hz. The patient's normal refractive correction was used.

Statistical analysis

Paired t-tests were used to compare colour and motion VEP latencies and amplitudes between amblyopic and fellow eyes. Between groups comparisons were made using unpaired t–tests.

Equipment calibration

Calibration for contrast psychophysical experiments

The stimulus was calibrated using a Minolta CS100 spot photometer (Table 4). The calibration curve determined is shown in fig 1. Contrast levels were measured using a 120-minute check at a viewing distance of 1 m

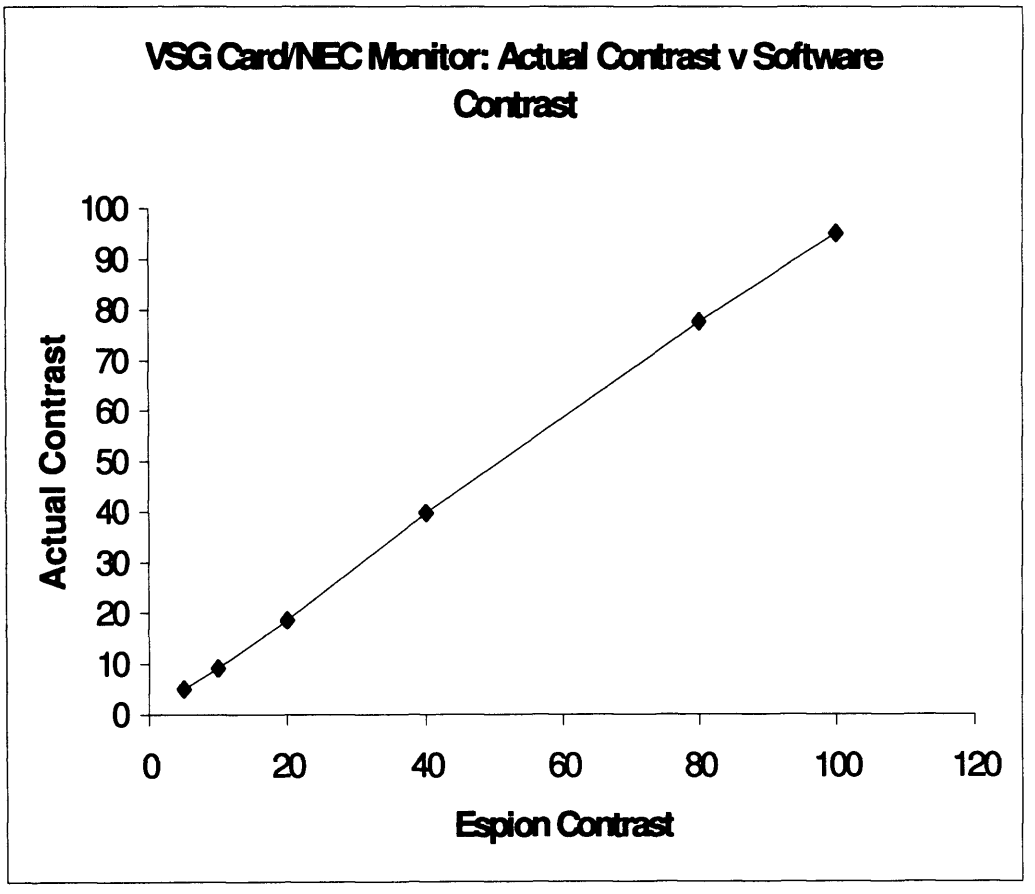
This system was also used to generate stimuli for contrast VEP, magnocellular psychophysical experiments and motion VEP. Thus this calibration curve is also valid for these experiments.

Table 4. Calibration luminance values and calculated contrast % for contrast psychophysical experiments, contrast VEPs, magnocellular psychophysical experiments and motion VEPs.

Espion/VSG generated contrast %	Minimum Luminance (Cd / m^2)	Maximum Luminance (Cd / m^2)	Actual Contrast %
100	4.49	175	**95.0**
80	21.6	172	**77.7**
40	56.5	131	**39.7**
20	75.5	110	**18.6**
10	83.5	100	**9.0**
5	87.3	96.4	**5.0**

Abbreviation: Cd=candelas

Figure 1. Calibration curve for contrast psychophysics, contrast VEPs, magnocellular psychophysical experiments and motion VEPs.



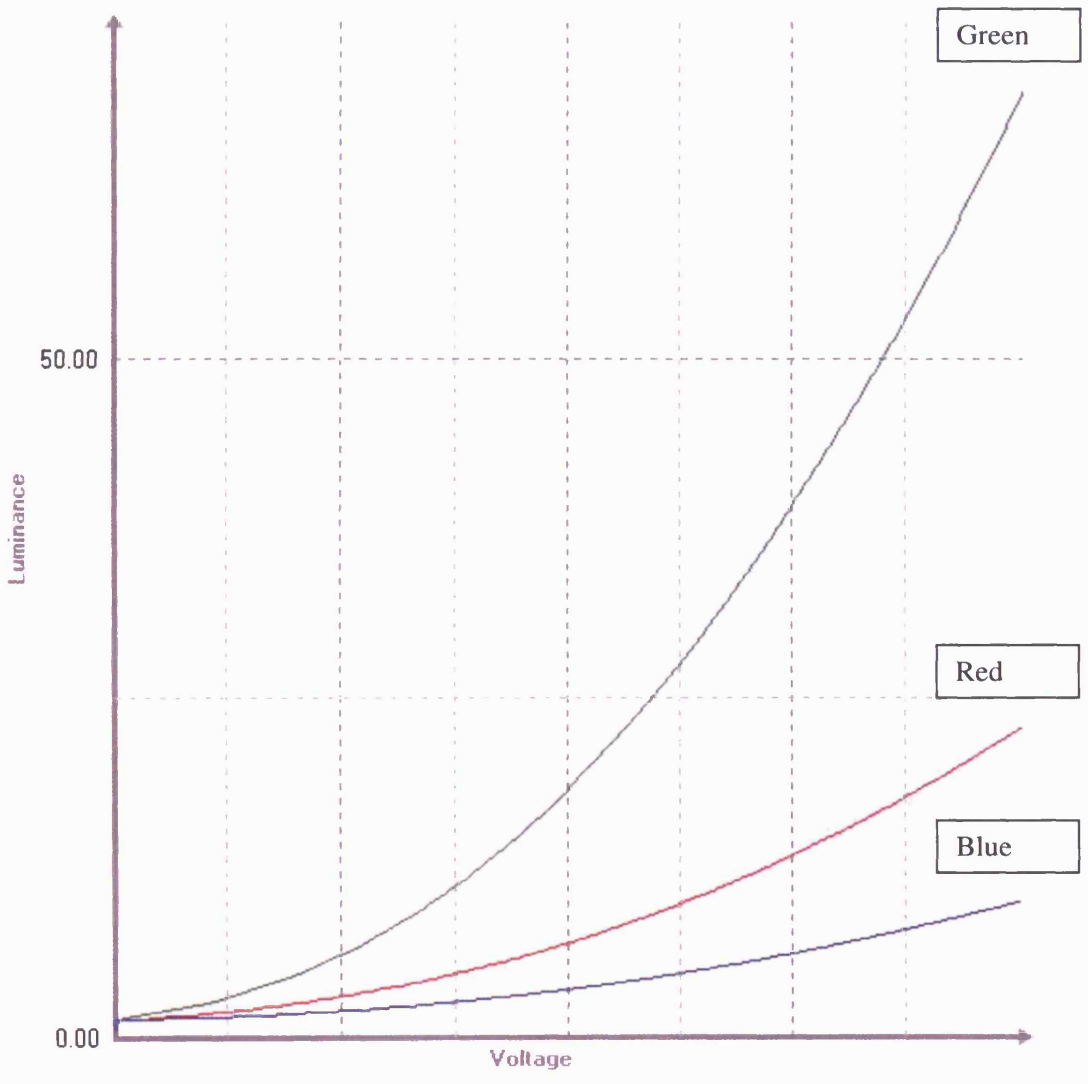
Calibration for parvocellular psychophysical experiments

The stimulus was gamma corrected using Optical (Cambridge Research Systems Ltd, Rochester). The X and Y phosphor co-ordinates for the red, green and blue guns are shown in table 5. The calibration curve measured is shown in fig 2.

Table 5. X and Y phosphor co-ordinates for the red, green and blue guns

	Red	Green	Blue
X	0.610	0.280	0.140
Y	0.040	0.595	0.070

Figure 2. Calibration curve for parvocellular psychophysical experiment.



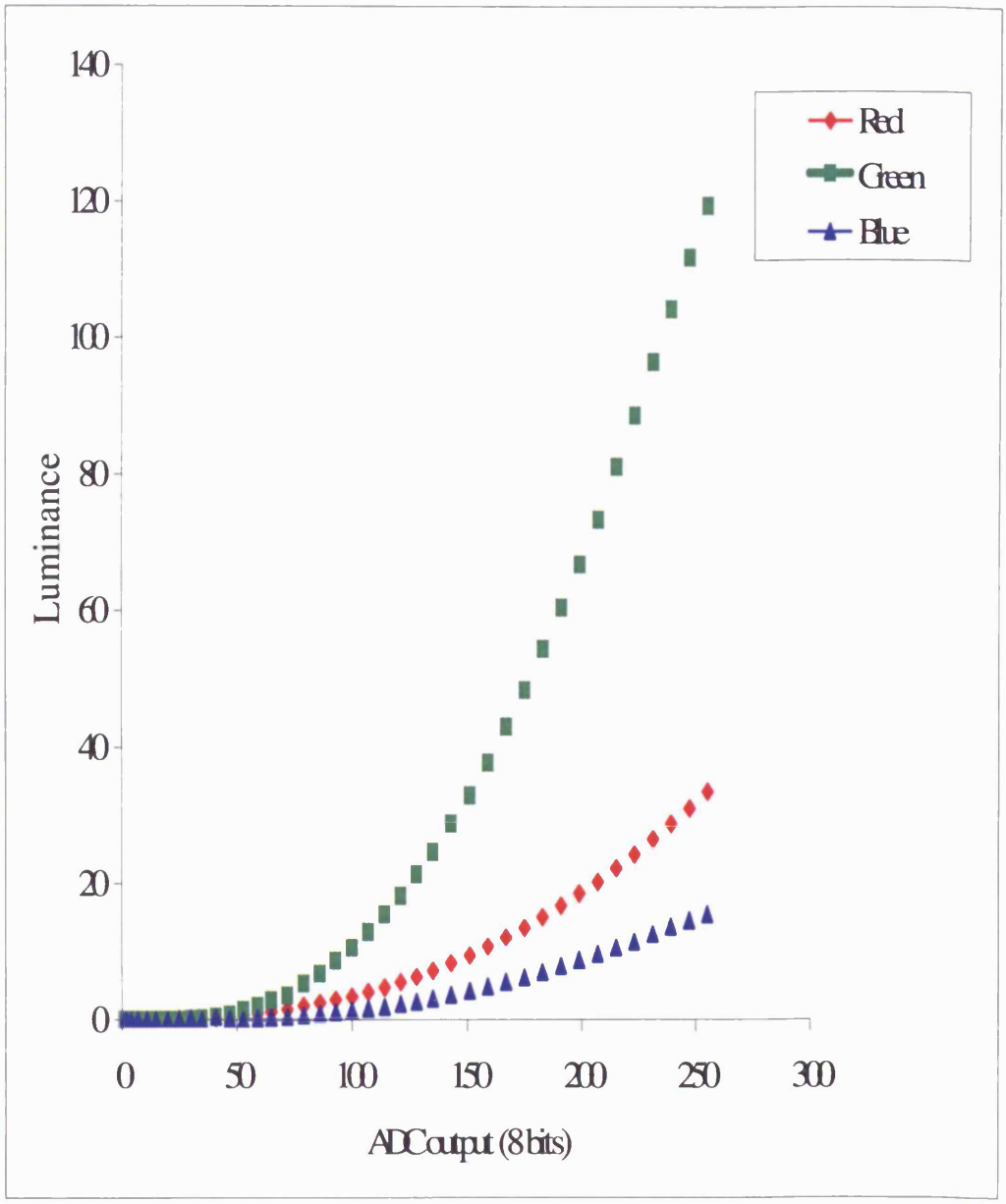
Calibration for Colour VEP

The stimulus was gamma corrected using a purpose built photometery / colourimetry system within the software suite. The X and Y phosphor co-ordinates for the red, green and blue guns are shown in table 6. The calibration curve determined is shown in fig 3.

Table 6. X and Y phosphor co-ordinates for the red, green and blue guns.

	Red	Green	Blue
X	0.618	0.297	0.143
Y	0.355	0.603	0.06

Figure 3. Calibration curve for colour VEP.



Chapter 3. RESULTS

Psychophysical assessment of contrast

Contrast sensitivity analysis for early and late onset amblyopes

Central CS at 3.2 cpd showed no difference between amblyopic and fellow eyes in the early onset group (Table 7), nor between amblyopic or fellow eyes and normal (Table 8; Fig 4). In the late onset amblyopes the CS of the amblyopic eyes at 3.2 cpd was significantly less than that of the fellow eyes (Table 7). The CS of the amblyopic eyes was also significantly less than normal, whereas the fellow eyes had a significantly greater CS than normal (Table 8; Fig 4).

Contrast sensitivity analysis for combined amblyopic group.

Data for central CS in early and late onset groups were combined and analysed as a single group. The CS of the combined amblyopic eyes was significantly less than that of the combined fellow eyes (Table 7). Neither the CS of the combined amblyopic eyes nor of the combined fellow eyes differed significantly from normal (Table 8).

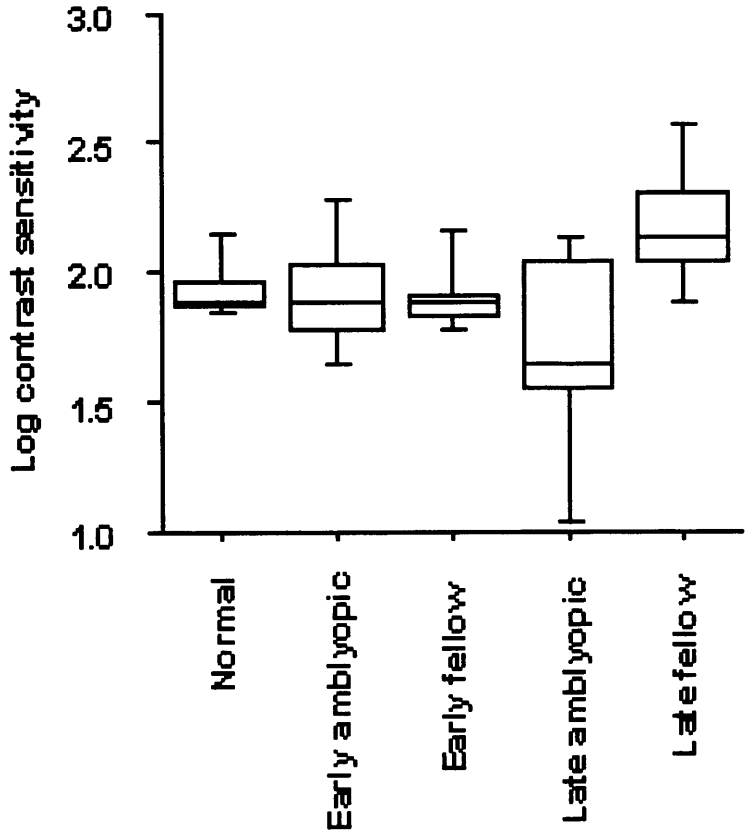
Table 7. Comparisons of log central contrast sensitivity at 3.2 cpd for amblyopic and fellow eyes.

	Mean % difference amblyopic vs. fellow eyes	t value	P
Early onset	+1.9	0.59	0.57
Late onset	-21.6	3.97	0.0026
Combined	-10.3	2.51	0.021

Table 8. Comparisons of log central contrast sensitivity at 3.2 cpd between amblyopic and fellow eyes and normal.

	Mean % difference from normal	t value	P
Early amblyopic	-0.5	0.13	0.9
Early fellow	-2.4	0.85	0.41
Late amblyopic	-16.6	2.93	0.0081
Late fellow	+6.4	2.21	0.039
Combined amblyopic	-8.5	1.64	0.11
Combined fellow	+2.0	0.7	0.49

Figure 4. Box –and –whisker plots for log central CS at 3.2 cpd for early- and late-onset amblyopes and normal control subjects. Amblyopic and fellow eyes of early onset amblyopes do not differ from normal. In late onset the CS of the amblyopic eye was reduced and that of the fellow eye increased compared with normal. Upper and lower box limits represent 75th and 25th percentiles, respectively.



Comparison of CS across the central visual field between late onset amblyopes and normal subjects

In the late onset amblyopes and normal subjects CS measurements were also taken at 5 and 10 degrees along the nasal and temporal horizontal meridians. This showed that the differences in CS extend across the central field (Table 9). Comparison with normal subjects showed both that the CS across the central field of the late onset amblyopic eyes is reduced and the CS of the fellow eyes is increased compared to normal (Table 9).

Table 9. Log contrast sensitivity across the central field for late onset amblyopes and normals.

		ANOVA	
	Mean % difference	**F(1,100)**	**P**
Late onset amblyopic vs. fellow	-21.0	36.2	<0.0001
Late onset amblyopic vs. normal	-16.1	18.61	<0.0001
Late onset fellow vs. normal	+7.0	8.5	0.0046

Contrast sensitivity thresholds for 3.2 cpd horizontal sine wave grating

1. Early onset amblyopes – there was no significant difference between amblyopic and fellow eyes, nor between either eye and normal subjects.
2. Late onset amblyopes – the amblyopic eyes had reduced CS compared to fellow eyes and compared to normal subjects. Fellow eyes had significantly greater CS than normal subjects.

Electrophysiological assessment of contrast sensitivity

Comparison of amblyopic with fellow eyes

There was no significant difference in C II latency or amplitude between amblyopic and fellow eyes for the early onset amblyopes, across all contrast levels. For the late onset amblyopes the latency from the amblyopic eye was longer and the amplitude markedly smaller than that for the fellow eye (Table 10; Figs 5, 6A and B).

Table 10. Comparison of VEP CII parameters for amblyopic and fellow eyes. There was no significant difference in C II latency or amplitude between amblyopic and fellow eyes for the early onset amblyopes, across all contrast levels. For the late onset amblyopes the latency from the amblyopic eye was longer and the amplitude markedly smaller than that for the fellow eyes.

	Latency			Amplitude		
	Mean % difference	ANOVA		Mean % difference	ANOVA	
	amblyopic vs. fellow eye	F *	P	amblyopic vs. fellow eye	F *	P
Early onset	0	<0.01	0.93	-8.8	0.03	0.86
Late onset	+12.1	10.69	0.0014	-38.8	21.70	<0.0001
Combined	+6.1	5.54	0.019	-25.9	13.45	0.0003

* F (1,108) for early and late groups; F (1,228) for combined group

Figure 5. Group mean pattern – onset VEPs for early and late amblyopes and normal control subjects. The CII responses from both amblyopic and fellow eyes of the early-onset amblyopes are of shorter latency and smaller amplitude than normal. The CII response from the amblyopic eye of the late-onset amblyopes is of increased latency and reduced amplitude whereas that from the fellow eye does not differ significantly from normal.

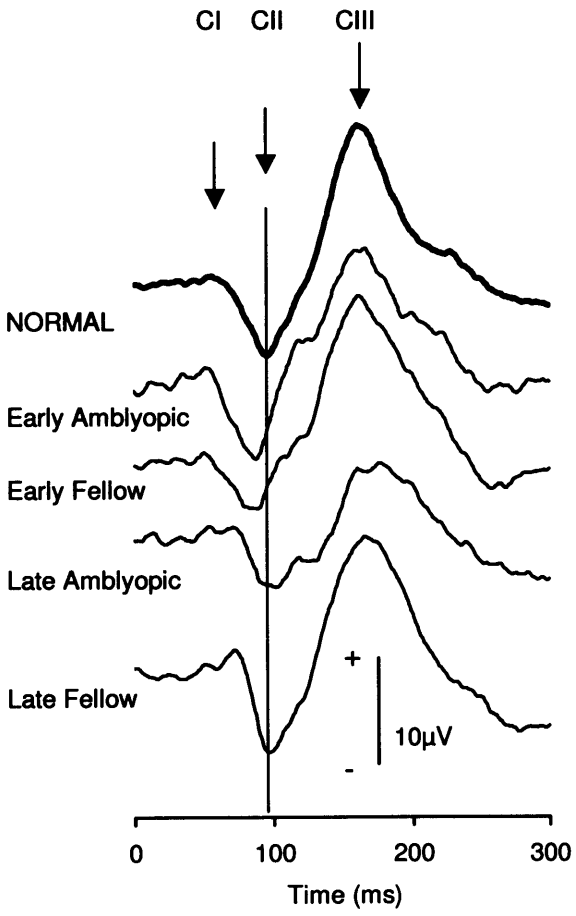
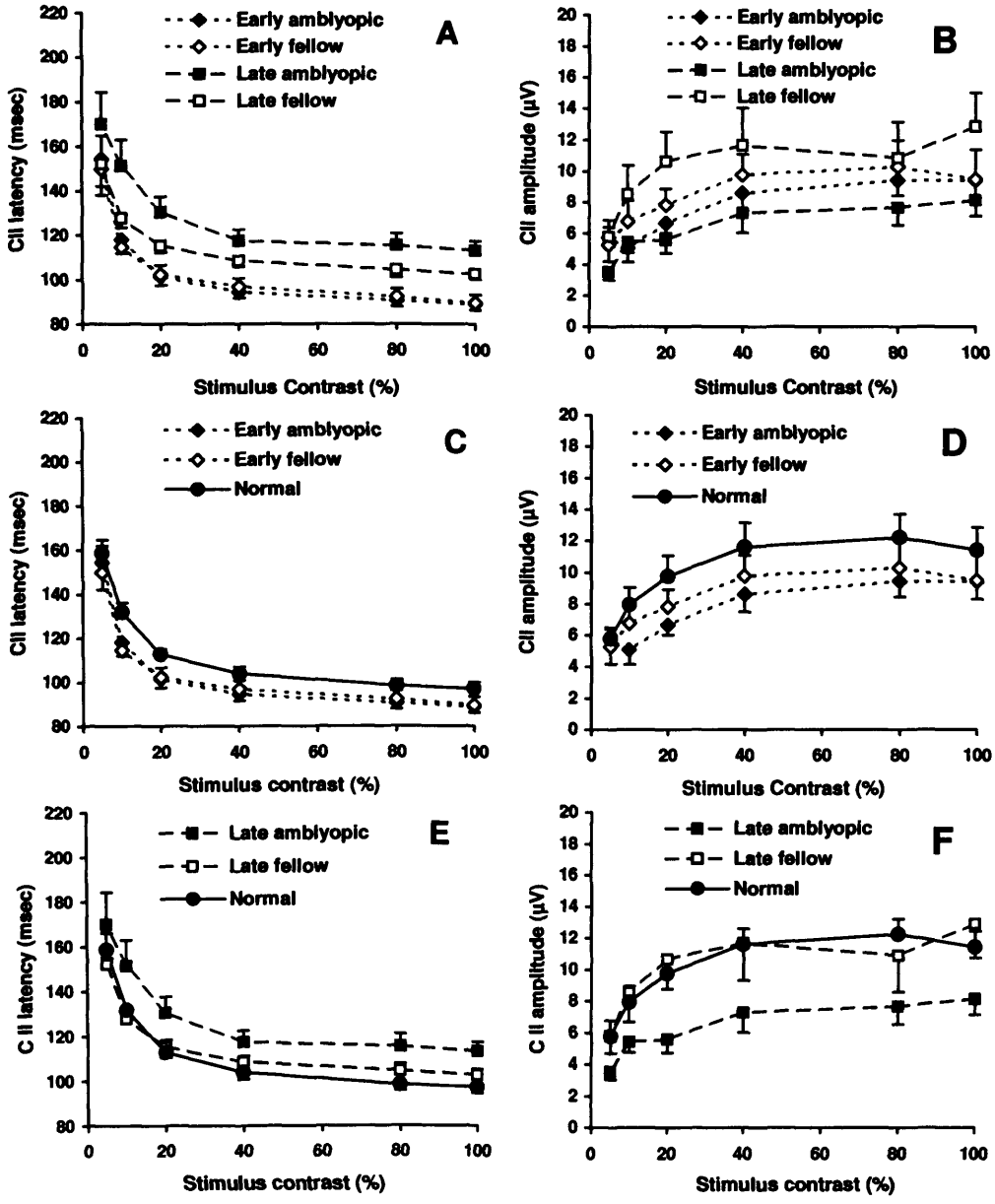


Figure 6. Latencies and amplitudes of the CII pattern onset VEP responses at different contrast levels for early- and late onset-amblyopes and normal subjects. (A) Latencies and (B) amplitudes of early-onset amblyopic and fellow eyes compared. (C) Latencies and (D) amplitudes of early-onset amblyopic and fellow eyes compared with normal eyes. (E) Latencies and (F) amplitudes of late-onset amblyopic and fellow eye compared with normal eyes. Error bars, SE.



Comparison between early and late onset amblyopes

The C II latencies from the amblyopic eyes of the early onset group were markedly shorter and the amplitudes larger than those for the amblyopic eyes of the late onset group across all contrast levels. The latencies for the fellow eyes of the early onset group were similarly shorter than those for the late onset group, but the amplitudes from the late onset fellow eyes were larger than for the early onset group (Table 11; Fig 6A and B).

Table 11. Comparison between early and late onset amblyopes of CII VEP parameters from amblyopic and fellow eyes.

	Latency			Amplitude		
	Mean % difference	ANOVA		Mean % difference	ANOVA	
	late c.f. early group	F (1,108)	P	late c.f. early group	F (1,108)	P
Amblyopic eye	+24.4	31.52	<0.0001	-15.7	4.23	0.042
Fellow eye	+11.0	7.25	0.0082	+22.2	8.56	0.0042

Comparison of early and late onset amblyopes with normal subjects.

The CII latencies from both the amblyopic and fellow eyes of the early onset amblyopes were shorter than in the normal group and the amplitudes smaller (Table 12; Figs 6C and D). For the late onset group the CII latency from the amblyopic eye was longer than normal and the amplitude markedly smaller whereas the latency and amplitude from the fellow eye did not differ significantly from normal (Table 12; Fig 6E and F).

Table 12. Comparison of CII VEP parameters of amblyopic and fellow eyes with normal.

	Latency			Amplitude		
	Mean % difference	ANOVA		Mean % difference	ANOVA	
	from normal	F *	P	from normal	F *	P
Early amblyopic	-8.1	7.15	0.0087	-22.7	6.73	0.0108
Early fellow	-8.1	6.37	0.0131	-15.4	5.40	0.0220
Late amblyopic	+14.1	18.11	<0.0001	-36.1	17.59	<0.0001
Late fellow	+1.9	0.50	0.4808	+3.2	0.59	0.4426
Combined amblyopic	+2.5	1.10	0.2955	-29.2	11.62	0.0008
Combined fellow	-3.3	1.55	0.2150	-4.3	0.29	0.5926

* F (1,108) for early and late groups; F (1,156) for combined groups

Analysis of combined early and late onset VEP data

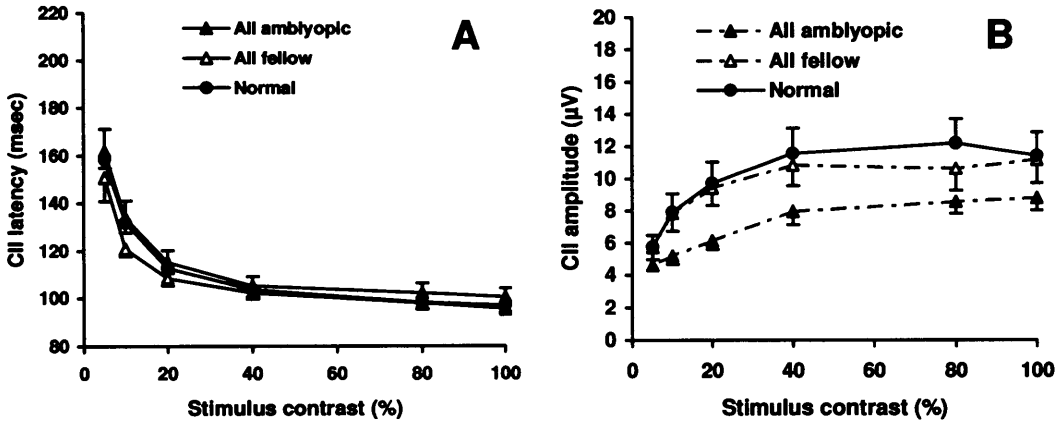
To allow comparison with previous studies, data from the early and late onset amblyopes were combined and analysed together.

The mean CII latency for the amblyopic eye of the combined group was significantly longer than for the fellow eye across all contrast levels and the mean amplitude was smaller (Table 10; Fig 7A and B).

Data from the first seven subjects in the early and late onset groups were combined and analysed as a single group and compared to fourteen normal subjects. The ANOVA method used was with replication as the experiments at different contrast levels represented repeated measures. For this method it is necessary to have the same size groups.

The mean CII amplitude for the amblyopic eyes of the combined group was significantly smaller than normal but the mean latency of the amblyopic eyes and latency and amplitude of the fellow eyes did not differ significantly from normal (Table 12).

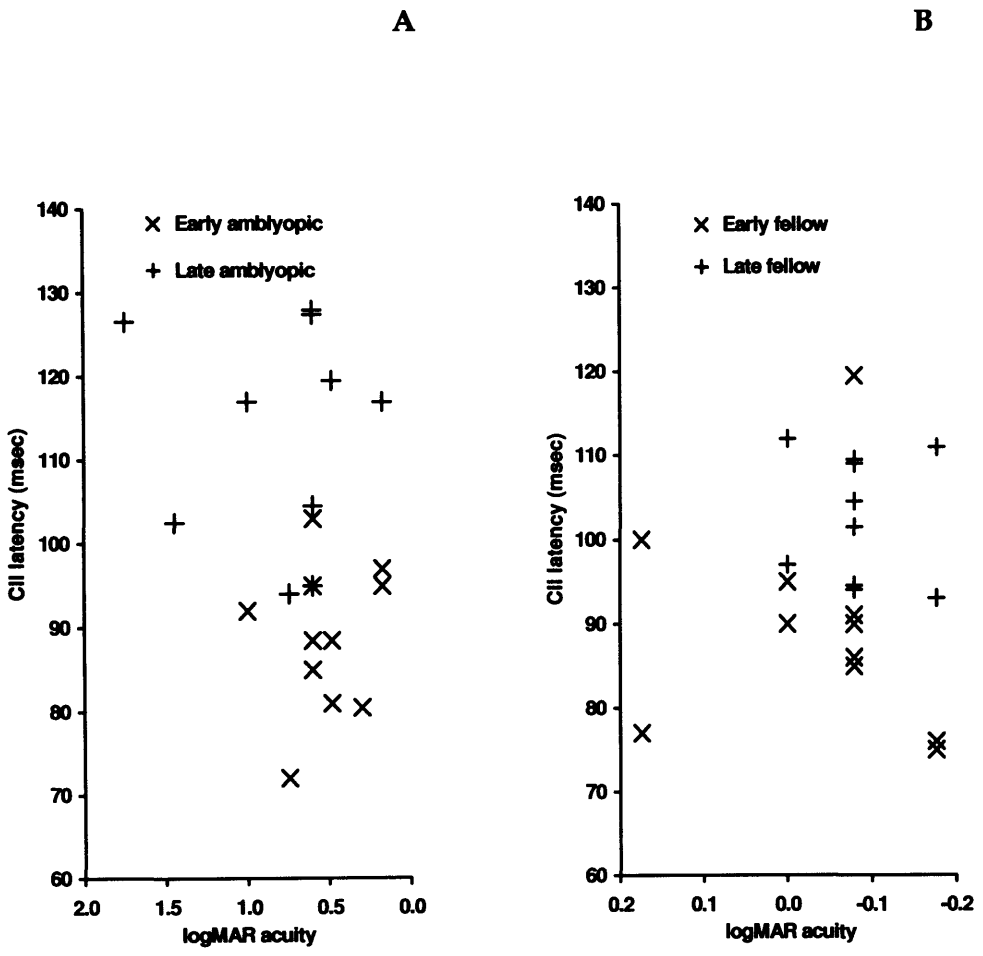
Figure 7 (A) Latencies and (B) amplitudes of the CII pattern onset VEP responses at different contrast levels for combined data from early- and late-onset amblyopes and normal subjects. The amplitude of the combined responses of the amblyopic was reduced, but other differences largely cancelled out. Error bars, SE.



Relationship of pattern onset VEPs to visual acuity

The CII VEP latency to 100% contrast for early and late onset amblyopic eyes is plotted against logMAR equivalent acuity in Fig 8A. There was no relationship between CII latency and acuity, with the latency difference between early and late onset groups being present across the whole acuity range. Similarly there is no relationship between CII latency and acuity for the fellow eyes (Fig 8B).

Figure 8. CII pattern-onset VEP latencies to 100% contrast plotted against logMAR equivalents for (A) amblyopic and (B) fellow eyes. The latency differences between early and late onset amblyopes were present across the whole range of acuities



Experiment 2 - Electrophysiological assessment of contrast sensitivity

Monocular VEPs to a pattern onset (CII component) 30 min check at 5 – 100% contrast

1.Early onset amblyopes – there was no difference between amblyopic and fellow eyes. Both amblyopic and fellow eyes had shorter CII latencies and smaller amplitudes than normal subjects.

2.Late onset amblyopes – the amblyopic eyes had significantly longer CII latencies and smaller amplitudes compared to the fellow eyes. In addition the amblyopic eyes had longer CII latencies and smaller amplitudes compared to normal subjects. Fellow eyes showed no difference in CII latency nor amplitude compared to normal subjects.

3.Combined early vs. late onset amblyopes – the amblyopic eyes had longer CII latencies and smaller amplitudes compared to the fellow eyes.

RESULTS.

Psychophysical assessment of magnocellular and parvocellular pathways

Luminance contrast sensitivity for the magnocellular stimulus

Luminance CS for normal and amblyopic subjects is shown in Table 13 and figure 9. The mean luminance CS to the M stimulus for normal eyes was 36.6.

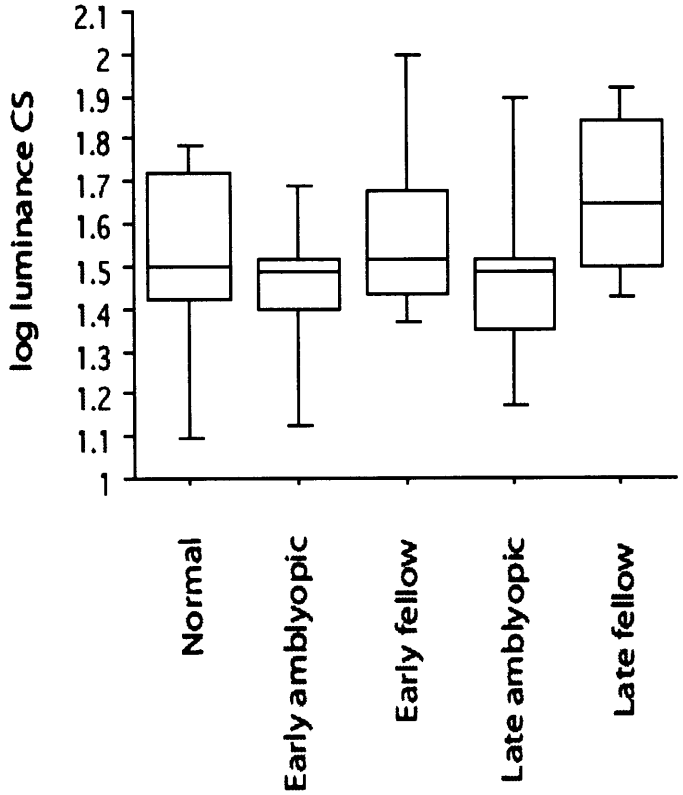
Luminance Contrast Sensitivity of Amblyopes

The CS of the amblyopic eye was worse than that of the fellow eye for combined amblyopes and for late onset amblyopes separately (Table 13). Comparison with normal subjects showed that the difference between amblyopic and fellow eyes was as much due to an increase in the CS of the fellow eyes as to a reduction in the amblyopic eyes, particularly in the late onset group, although the differences from normal did not individually reach statistical significance (Table 13).

Table 13. Luminance contrast sensitivity for the M stimulus.

Subjects	Amblyopic eyes				Fellow eyes				Amblyopic Vs. Fellow		
	Mean luminance contrast sensitivity	% Difference from normal	t value	P cf normal	Mean luminance contrast sensitivity	% Difference from normal	t value	P cf normal	% Difference	t value	P
Normal n=15	36.6				36.6						
All amblyopes n=29	33.3	-9.2	0.82	0.42	47.5	29.7	-1.4	0.17	-30.0	-3.03	0.005
Early Onset n=16	29.8	-18.7	1.10	0.28	43.9	19.9	-0.74	0.46	-32.2	-1.92	0.076
Late Onset n=13	37.3	1.8	0.29	0.77	51.3	40.1	-1.69	0.10	-27.3	-2.40	0.033

Fig 9. Box –and –whisker plots for log luminance contrast sensitivity for early- and late- onset amblyopes and normal control subjects. Late fellow eyes had significantly increased luminance CS compared to the late onset amblyopic eyes.



Chromatic contrast sensitivity (CS) for the parvocellular stimulus

Chromatic CS for normal and amblyopic subjects is shown in Table 14 and figure 10. The mean chromatic CS to the P stimulus for normal eyes was 7.45.

Comparison of amblyopic with fellow eyes

Chromatic CS in the amblyopic eyes was significantly lower than that of the fellow eyes. This was true for the combined amblyopic group and for both early and late onset amblyopes considered separately.

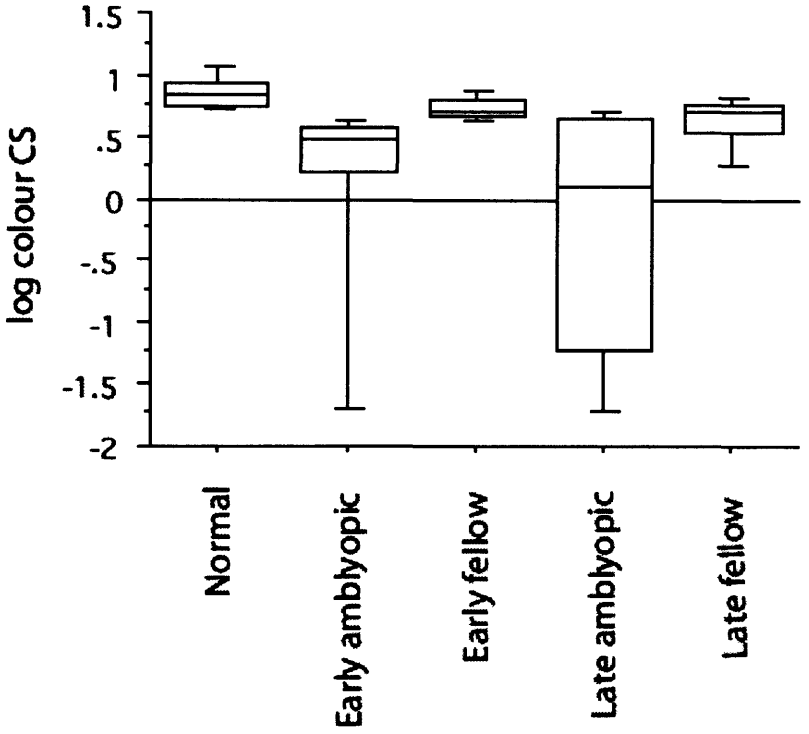
Comparison of amblyopes with normal subjects

There was a marked reduction in chromatic CS for combined, early and late onset amblyopic eyes compared to normal eyes. There was also a significant reduction for combined, early and late onset fellow eyes compared to normal.

Table 14. Chromatic contrast sensitivity for the P stimulus.

	Amblyopic eye				Fellow eye				Amblyopic Vs. Fellow		
	Mean chromatic contrast sensitivity	% Difference from normal	t value	P cf normal	Mean chromatic contrast sensitivity	% Difference from normal	t value	P cf normal	% Difference	t value	P
Normal n=15	7.45	-		-	7.45	-		-	-		-
All amblyopes n=28	2.37	-68.2	3.77	0.0005	5.20	-30.3	3.46	0.0013	-54.4	-4.39	0.0002
Early Onset n=15	2.67	-64.2	3.31	0.0026	5.50	-26.2	2.93	0.0066	-51.4	-2.8	0.0141
Late Onset n=13	2.08	-72.0	4.27	0.0002	4.95	-33.6	3.21	0.0035	-57.9	-3.35	0.006

Fig 10. Box –and –whisker plots for log chromatic contrast sensitivity for early- and late- onset amblyopes and normal control subjects.



Ratio of chromatic to luminance contrast sensitivity (CS)

The ratios of chromatic to luminance contrast sensitivities calculated by (M-P)/M) are shown in Table 15 and figure 11. The mean ratio for normal eyes was 0.78. This ratio was generated in order to determine the relative change of the M stimulus CS compared to the P stimulus CS.

Comparison of amblyopic with fellow eyes

When amblyopic and fellow eyes were compared the (M-P)/M ratio was significantly greater for the amblyopic than fellow eyes in the combined, early and late onset groups. The ratio was significantly greater in the late onset compared to the early onset amblyopic eyes ($P = 0.044$).

Comparison of amblyopes with normal subjects

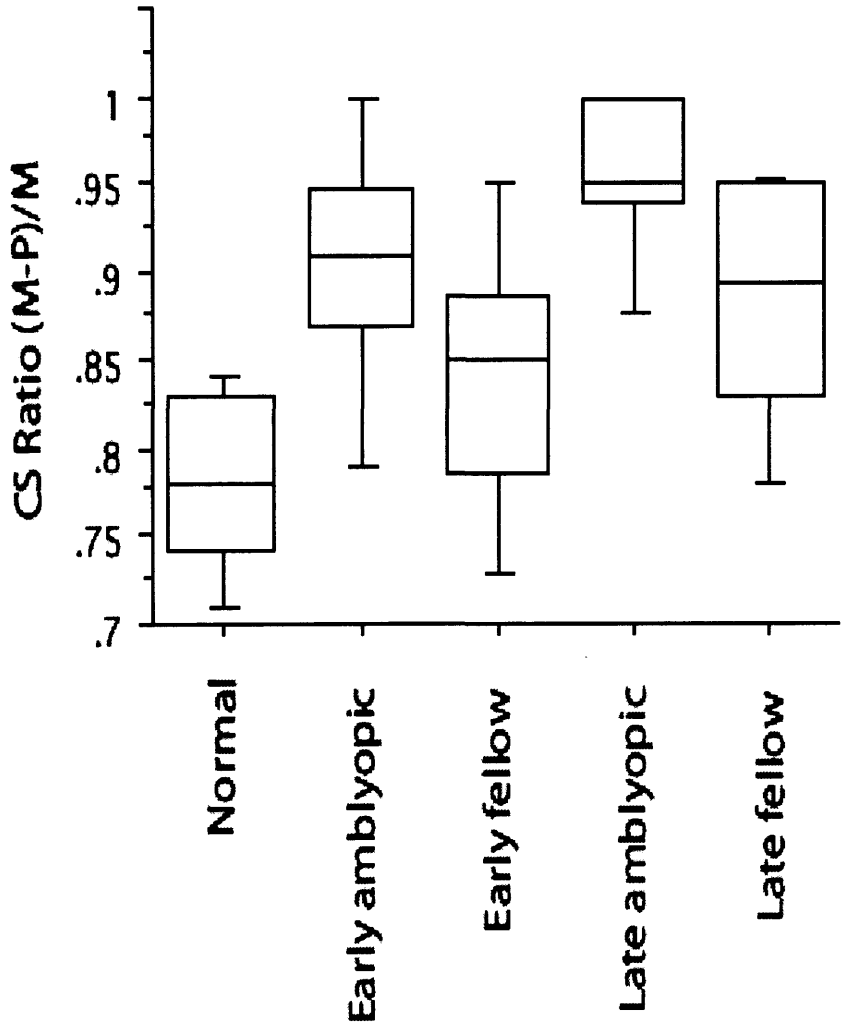
The ratio was significantly greater than normal for the combined, early onset and late onset amblyopic eyes compared to normal. The ratio was also significantly increased in all fellow eye groups compared to normal.

Table 15. Ratio of chromatic to luminance contrast sensitivity

	Amblyopic eye				Fellow eye				Amblyopic Vs. fellow eye		
	Mean M-P/M	% Difference from normal	t value	P cf normal	Mean M-P/M	% Difference from normal	t value	P cf normal	% Difference	t value	P
Normal n=15	0.78										
All amblyopes n=25	0.93	19.3	-6.03	<<0.0001	0.86	10.6	-3.42	0.002	7.8	4.15	0.0003
Early Onset n=15	0.90	16.4	-4.05	<<0.0001	0.84	8.2	-2.22	0.035	7.6	2.57	0.022
Late Onset* n=13	0.95	22.4	-5.23	<<0.0001	0.88	13.4	-3.24	0.003	8.0	3.59	0.004

*Significantly different from early onset amblyopic (P = 0.044; unpaired t-test)

Fig 11. Box –and –whisker plots for (M-P)/M ratio for early and late onset amblyopes and normal control subjects. The ratio was significantly greater than normal for all groups. The highest ratio was found for the late onset amblyopic eyes.



Experiment 3 - Psychophysical assessment of magnocellular and parvocellular pathways.

M stimulus - achromatic horizontal 0.8 cpd grating with spatial gaussian ($\sigma_x = \sigma_y =$ 1.0 deg) and transient presentation.

1.Combined amblyopes - the fellow eyes had better luminance CS than normal eyes. The amblyopic eyes had reduced luminance CS compared to normal

2.Late onset amblyopes – the amblyopic eyes had reduced luminance CS compared to fellow eyes.

P stimulus - red-green horizontally orientated 3.2 cycle/degree isoluminant sinusoidal grating patch presented with a spatial gausssian ($\sigma_x = \sigma_y = 0.5$ deg) and sustained presentation.

1. The amblyopic eyes had worse chromatic CS compared to fellow eyes for early and late onset amblyopes. Amblyopic and fellow eyes had worse chromatic CS than normal for both early and late onset amblyopes.

Ratio of chromatic to luminance response (M-P/M)

1.The M responses were good and the P responses were poor.

2.The (M-P/M) ratio was higher for late onset amblyopic eyes compared to early onset amblyopic eyes.

RESULTS.

Electrophysiological assessment of magnocellular and parvocellular pathways

Normal values for motion onset (N200) and colour (N130) VEPs are shown in Table 16.

Motion VEPs

Motion onset latency values for amblyopic and fellow eyes are shown in table 17. Motion onset amplitude values for amblyopic and fellow eyes are shown in table 18. Group mean traces are shown for early onset, late onset amblyopes and normal subjects in figure 12.

Comparison of amblyopic with fellow eyes

The combined amblyopic group showed a longer N200 latency and smaller amplitude for amblyopic eyes compared with fellow eyes. There was no significant difference in N200 latency or amplitude between amblyopic and fellow eyes for the early or late onset amblyopes when considered separately (Tables 17 and 18; figure 12).

Comparison of amblyopes with normal subjects

For the combined amblyopic group the N200 latency was significantly shorter for both amblyopic and fellow eyes compared to normal. When considered separately the latency of both the early and late onset fellow, but not amblyopic eyes was significantly shorter than normal (Table 17). The amplitude of the N200 response was significantly reduced in the combined amblyopic eyes. The amplitude was also significantly reduced for the early and late onset amblyopic eyes when considered as separate groups. The amplitude of the N200 response in the fellow eyes of the early and late onset amblyopes was not significantly different from normal (Table 18).

Table 16. Normal values for colour and motion VEPs based on one random eye from each subject.

	Latency	**Amplitude**
	Mean±s.d. msec	**Mean±s.d. μV**
Motion (N200) n=19	178.1±9.5	6.8±3.3
Colour (N130) n=17	125.2±11.3	7.5±3.4

Abbreviation: s.d. = standard deviation

Table 17. Comparison of VEP motion onset latency for amblyopic and fellow eyes.

	Amblyopic eye				Fellow eye				Amblyopic vs. fellow eye		
	Mean ± s.d. msec	% Difference from normal	t value	P cf normal	Mean ± s.d. msec	% Difference from normal	t value	P cf normal	% Difference	t value	P
Normal	178.1±9.5										
All amblyopes n=25	170.7±12.7	-4.2%	-2.13	0.039	167.2±11.0	-6.1%	-3.44	0.001	+2.1%	2.33	0.029
Early Onset n=15	170.1±13.8	-4.5%	-1.99	0.055	166.3±11.3	-6.6%	-3.31	0.002	+2.3%	1.79	0.096
Late Onset n=10	171.6±11.4	-3.7%	-1.65	0.11	168.7±11.0	-5.3%	-2.41	0.023	+1.7%	1.46	0.178

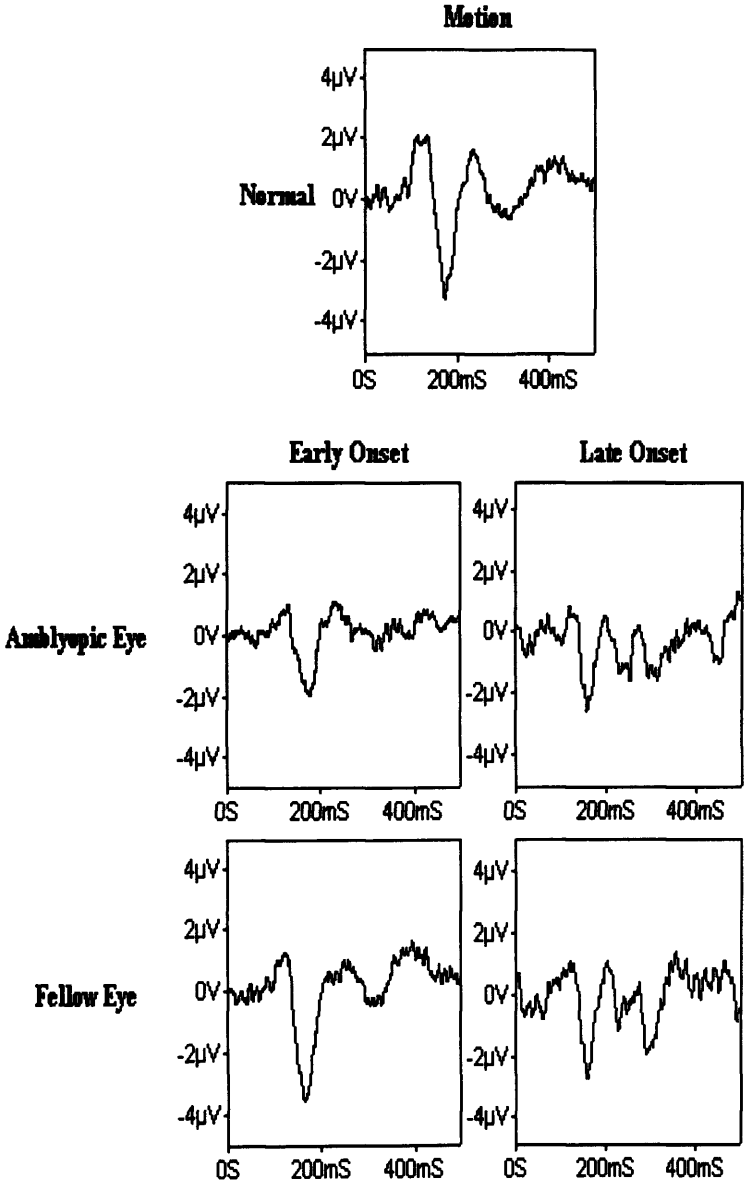
Abbreviation: s.d. = standard deviation

Table 18. Comparison of VEP motion onset amplitude for amblyopic and fellow eyes.

	Amblyopic eye				Fellow eye				Amblyopic vs. fellow eye		
	Mean ± s.d. msec	% Difference from normal	t value	P cf normal	Mean ± s.d. msec	% Difference from normal	t value	P cf normal	% Difference	t value	P
Normal	6.8±3.3										
All amblyopes n=25	4.2±2.1	-37.7%	-3.09	0.004	5.3±2.2	-20.9%	-1.71	0.095	-21.2%	-2.41	0.024
Early Onset n=15	4.3±2.4	-36.3%	-2.39	0.023	5.7±2.5	-15.8%	-1.03	0.311	-24.4%	-1.90	0.078
Late Onset n=10	4.1±1.7	-39.7%	-2.37	0.025	4.8±1.4	-28.6%	-1.72	0.093	-15.6%	-1.66	0.132

Abbreviation: s.d. = standard deviation

Fig 12. Group mean Motion Onset VEP traces for early and late onset amblyopes and normal control subjects. The N200 responses of fellow eyes were of significantly shorter latency than normal in both early and late onset amblyopes. The N200 responses of early and late onset amblyopic eyes were of significantly smaller amplitude than normal.



Relationship of VEPs to visual acuity

The motion onset VEP latency for early and late onset amblyopic eyes is plotted against logMAR equivalent acuity in Fig 13. There was no relationship between motion onset latency and acuity, with the latency difference between early and late onset groups being present across the whole acuity range. Similarly there is no relationship between motion onset amplitude and acuity for the amblyopic eyes (Fig 14).

Figure 13. N200 motion-onset VEP latencies plotted against logMAR equivalents for (A) amblyopic and (B) fellow eyes. The latency differences between early- and late-onset amblyopes were present across the whole range of acuities.

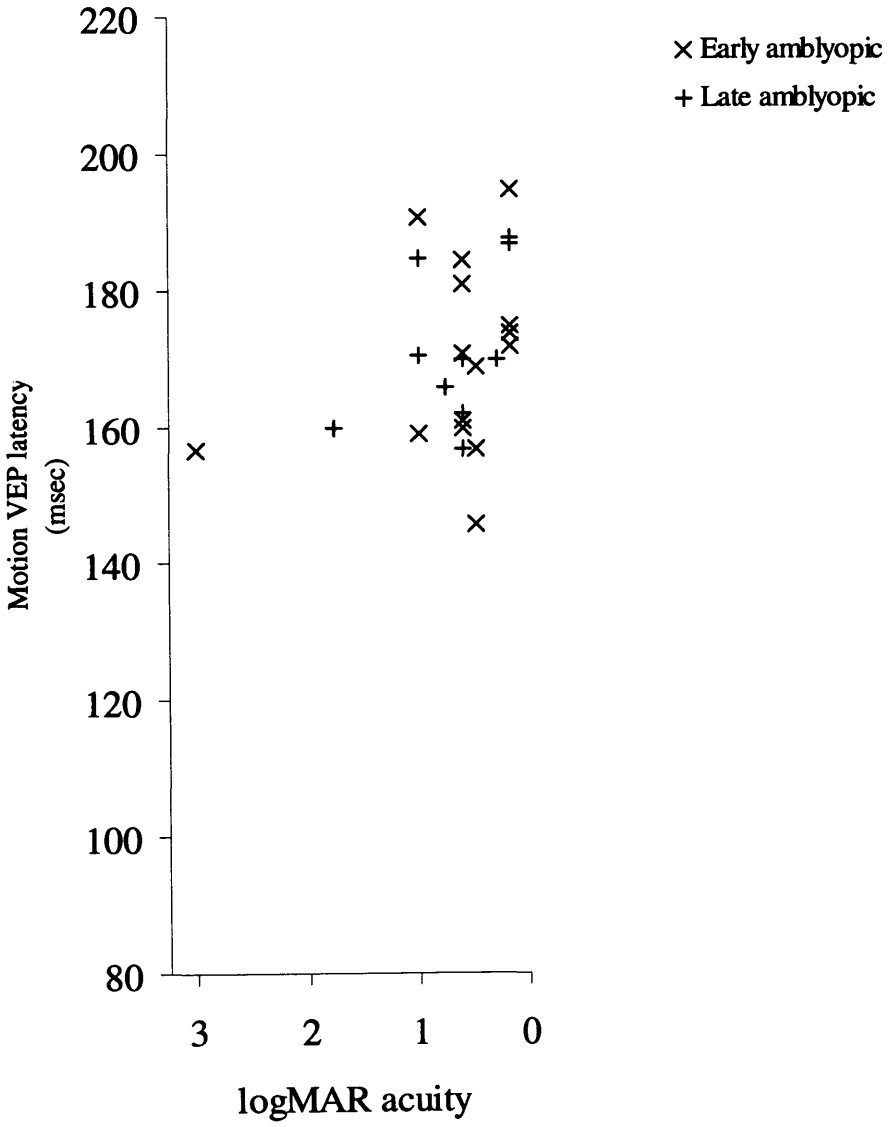
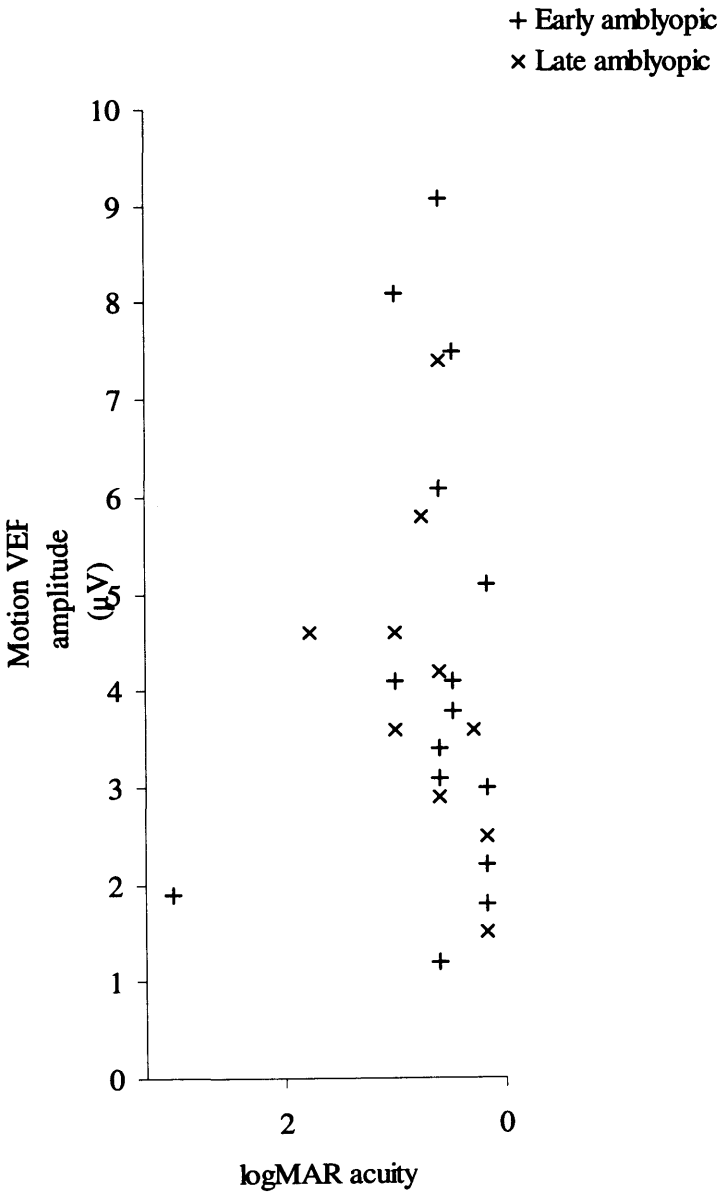


Figure 14. N200 motion-onset VEP amplitudes plotted against logMAR equivalents for (A) amblyopic and (B) fellow eyes. The latency differences between early- and late-onset amblyopes were present across the whole range of acuities.



Colour VEPs

Colour VEP latency values for amblyopic and fellow eyes are shown in Table 19. Colour VEP amplitude values for amblyopic and fellow eyes are shown in Table 20. Group mean traces are shown for early onset, late onset amblyopes and normal subjects in figure 15.

Comparison of amblyopic with fellow eyes

Late onset amblyopic eye N130 latency was significantly longer than fellow eye latency. There was no significant difference in N130 latency between amblyopic and fellow eyes for either the combined or early onset groups. (Table 19; figure 15) The N130 amplitude was significantly smaller for the amblyopic eyes of the combined amblyopic group compared to fellow eyes. Early onset amblyopic eye amplitude was significantly smaller than fellow eye latency. There was no significant difference between the late onset amblyopic and fellow eye amplitudes (Table 20; figure 15)

Comparison of amblyopes with normal subjects

For the amblyopic eyes of the combined group, the N130 latency was significantly longer than normal. When considered separately, only the late onset amblyopic eyes had longer N130 latencies than normal. The increased latency in the late onset amblyopic eyes was significantly longer than that of the early onset amblyopic eyes ($P = 0.036$). There was no significant difference between the N130 latencies from the fellow eyes of the combined, early or late onset amblyopes when compared to normal (Table 19; figure 15).

The amplitude of the N130 was significantly reduced for all amblyopic eyes compared to normal. When considered separately, only the early onset amblyopic eyes had significantly reduced amplitudes. There was no significant difference between the N130 amplitudes from the fellow eyes of the combined, early or late onset amblyopes when compared to normal (Table 20; figure 15).

Table 19. Comparison of VEP colour onset/ offset latency for amblyopic and fellow eyes.

	Amblyopic eye				Fellow eye				Amblyopic Vs. fellow eye		
	Mean ± s.d. msec	% Difference from normal	t value	P cf normal	Mean ± s.d. msec	% Difference from normal	t value	P cf normal	% Difference	t value	P
Normal	125.2±11.3										
All amblyopes n=26	140.5±25.5	+12.2%	2.31	0.026	132.4±14.7	+5.8%	1.71	0.095	+6.1%	1.80	0.085
Early Onset n=14	131.0±22.1	+4.6%	0.93	0.357	132.3±15.6	+5.7%	1.45	0.157	-1.0%	-0.32	0.756
Late Onset n=12	151.7±25.6 *	+21.1%	3.70	<0.001	132.6±14.1	+5.9%	1.54	0.133	+14.4%	2.53	0.028

* Significantly different from early onset amblyopic (P = 0.036; unpaired t-test)

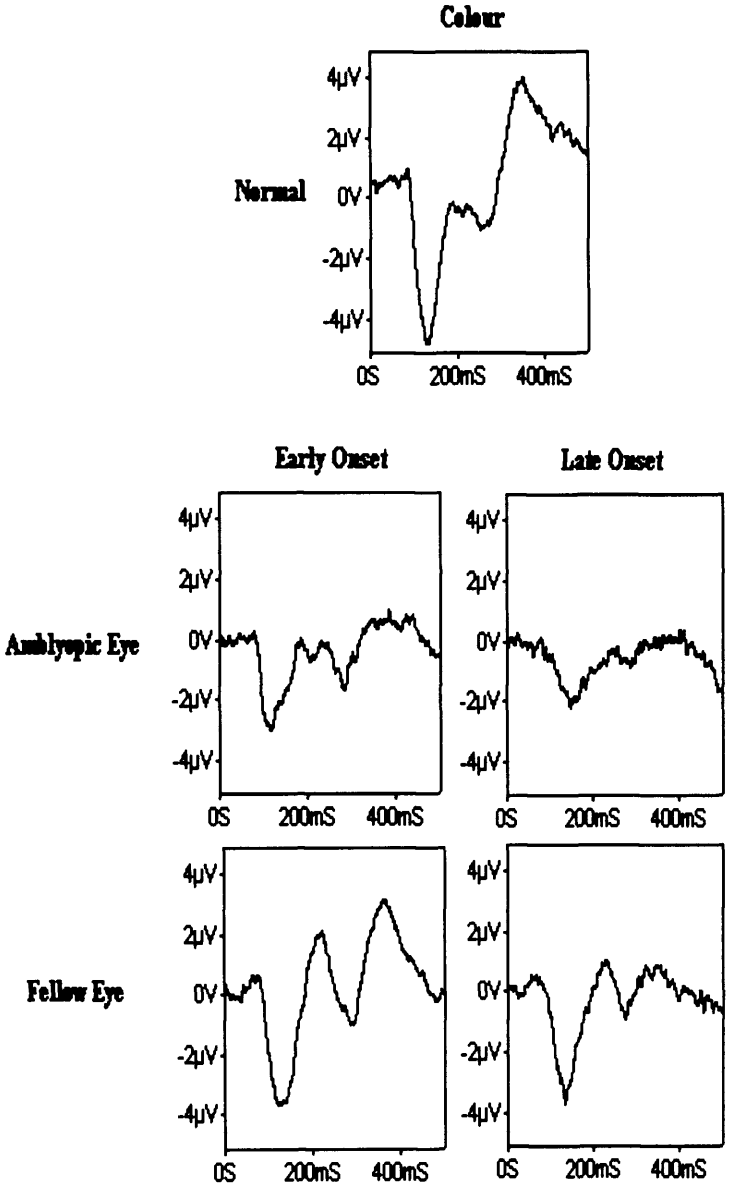
Abbreviation: s.d. = standard deviation

Table 20. Comparison of VEP colour onset/ offset amplitude for amblyopic and fellow eyes.

	Amblyopic eye				Fellow eye				Amblyopic vs. fellow eye		
	Mean ± s.d. μV	% difference from normal	t value	P cf normal	Mean ± s.d. μV	% difference from normal	t value	P cf normal	% difference	t value	P
Normal	7.5±3.4										
All amblyopes n=26	4.9±3.6	-34.0%	-2.33	0.025	7.5±4.0	+1.1%	0.07	0.944	-34.8%	-3.44	0.002
Early Onset n=14	4.5±3.0	-39.1%	-2.49	0.019	7.6±4.4	+1.3%	0.07	0.944	-39.9%	-2.33	0.012
Late Onset n=12	5.4±4.2	-28.2%	-1.49	0.148	7.5±3.7	+0.9%	0.05	0.96	-28.8%	-1.85	0.091

Abbreviation: s.d. = standard deviation

Fig 15. Group mean colour VEP traces for early and late onset amblyopes. The N130 latencies of the combined and late onset amblyopic eyes were significantly longer compared to normal. The N130 latency was also reduced when the late onset amblyopic eyes were compared to the fellow eyes. The N130 amplitude was reduced in the combined and early onset amblyopic eyes compared to both fellow and normal eyes.



Relationship of VEPs to visual acuity

The colour VEP latency for early and late onset amblyopic eyes is plotted against logMAR equivalent acuity in Fig 16. There was no relationship between colour VEP latency and acuity, with the latency difference between early and late onset groups being present across the whole acuity range. Similarly there is no relationship between colour VEP amplitude and acuity for the amblyopic eyes (Fig 17).

Fig 16. Comparison colour VEP latencies to logMAR acuities. There was no relationship between the colour VEP latency and visual acuity.

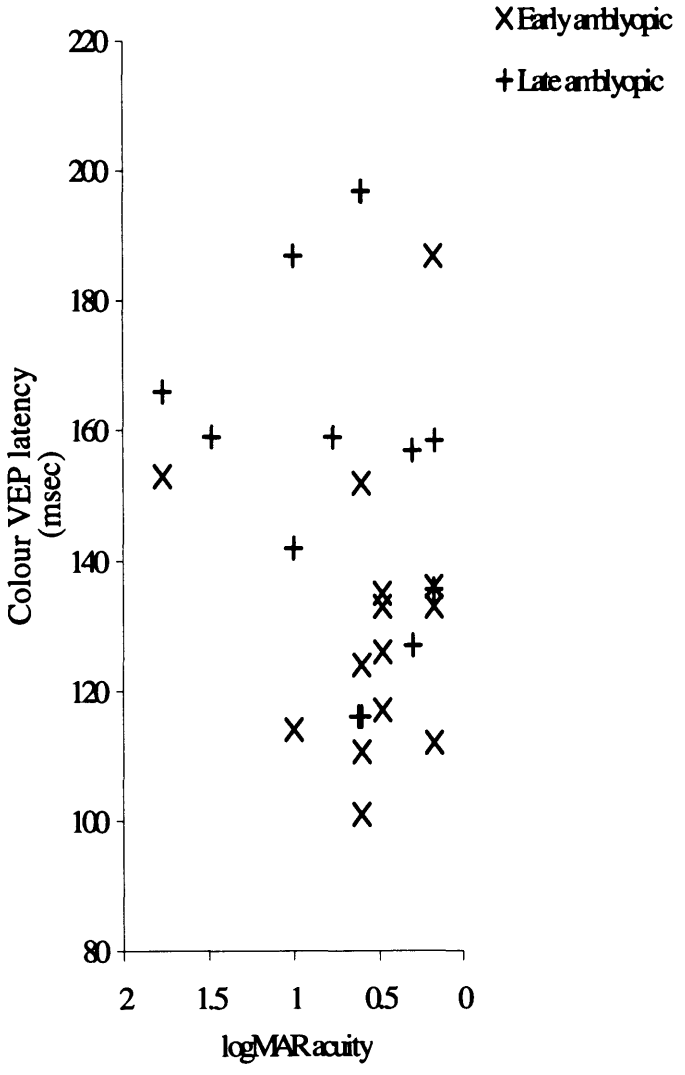
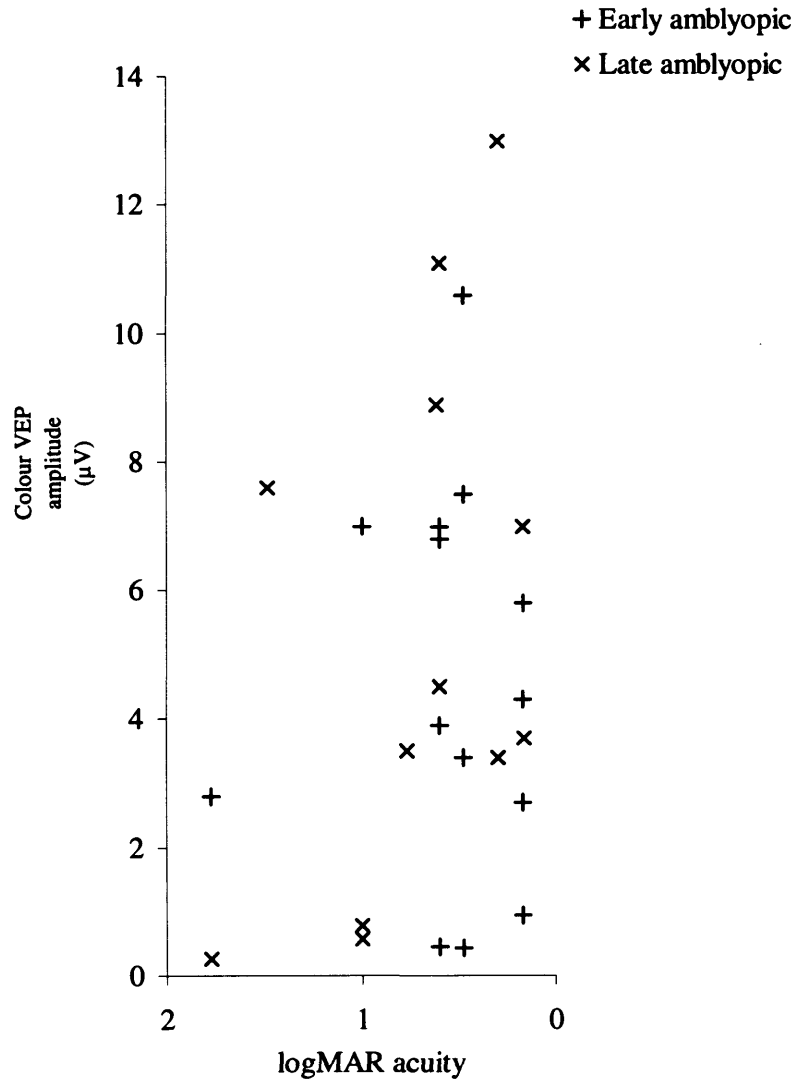


Fig 17. Comparison colour VEP amplitudes to logMAR acuities. There was no relationship between the colour VEP amplitudes and visual acuity.



Experiment 4 - Electrophysiological assessment of magnocellular and parvocellular pathways.

Motion VEP (N200) - The M biased stimulus was an achromatic motion onset 0.8 cpd, vertical square wave grating.

1.Combined amblyopic group - the amblyopic eyes had longer N200 latencies and smaller amplitudes compared to fellow eyes. Amblyopic and fellow eyes had shorter latencies compared to normal.

2.Early and late onset fellow eyes had shorter N200 latencies than normal when considered separately.

Colour VEP (N130) - The P biased stimulus was a 3.2 cpd, red/green, pattern onset (onset 200ms, offset 600ms), sine wave grating.

1. The late onset amblyopic eyes had longer N130 latencies than both fellow and normal eyes.

2. The combined amblyopic group had smaller N130 amplitudes compared to normal.

3. The late onset amblyopes had longer N130 latencies compared to early onset amblyopes. Early and late onset amblyopes had smaller N130 amplitudes compared to normal.

Discussion

The first series of experiments examined the electrophysiological responses to stimuli at different contrast levels and CS as measured by psychophysical methods in strabismic amblyopes. These showed striking differences in the responses obtained from early and late onset amblyopes. These differences were further investigated in the second series of experiments, which demonstrated differences in the relative involvement of M and P pathway involvement with age of onset of amblyopia. An unexpected finding was of the suggestion of enhancement of the M pathway in all amblyopes. An additional finding was the consistent abnormality of fellow eyes.

The present findings of differences between early and late onset amblyopes depend critically on the ability of the patients to identify the time of onset of their amblyopia to before or after 18 months of age. Patients were only recruited to the study when a clear history was available, which was in about half of potential patients. The age of onset of squint was clearly remembered as, in most cases, was the age at which patching was started. Although children with early onset strabismus who cross fixate may not become amblyopic [109], there is evidence that those who become amblyopic do so soon after the onset of their squint [110] [111] [112]. Conversely most of the late onset amblyopes started to squint well after 18 months of age. Although it is possible for anisometropic amblyopia to predate a squint, this is relatively uncommon, and the majority of these patients were not anisometropic.

Subjects were only included in the study when they had a clear history of age of onset either before or after 18 months of age. The majority of early onset subjects were infantile esotropes with histories of cross fixation. An alternative method to detailed history taking would be to develop a structured questionnaire. Nevertheless, any study of adults will be open to confounding based on their knowledge of past events. For future adult studies an additional source of information would be to contact a subject's GP to ascertain further details of childhood presentation and treatment.

It is thus unlikely that amblyopia was present before 18 months of age in this group. It is difficult to explain the striking differences found on any basis other than the age of onset of amblyopia.

The electrophysiological findings in human strabismic amblyopes with an onset before 18 months of age differ significantly from those in amblyopes of later onset. In the early onset amblyopes CII responses from both eyes were of shorter latency and smaller amplitude than normal, with no difference between amblyopic and fellow eyes. In contrast, the CII response from the amblyopic eye of the late onset amblyopes was of increased latency and markedly reduced amplitude compared to normal, whereas both latency and amplitude of the response from the fellow eye showed no such differences. These differences are not attributable to differences in visual acuity, which was similar in the two groups, but rather suggest differences in cortical pathophysiology.

The overall degree of amblyopia, as judged by visual acuity, was similar in the early and late groups. Thus, the greater difference between the CII response from amblyopic and fellow eyes found in the later onset amblyopes does not indicate a greater general

sensitivity to abnormal visual experience at a later age, but rather that a particular aspect of visual processing had been more affected at a later age. It is known that different visual functions develop at different rates [113], [114]; that there is more than one sensitive period in visual development [38], [67], and that the sensitivity of the human visual system to abnormal visual experience reduces with age [115]. However no previous human studies appear to have investigated the possibility that abnormal visual experience starting at different ages within the sensitive period may result in amblyopia with different characteristics. Previous studies presumably combined data from both early and late onset amblyopes, and reported reduced amplitude pattern onset VEPs [74], [73]. In addition, Shawkat et al. demonstrated reduced amplitude from the fellow eye compared to normal [74]. These findings could be replicated in the present study by combining data from early and late onset amblyopes, when the other differences between the groups cancelled out.

This data indicates clear differences in pathophysiology between early and late onset amblyopes; they do not indicate the nature or location of the changes in central visual pathway function. The CII component of the pattern onset VEP was evaluated quantitatively because it is the most consistent component and thus most commonly measured. The exact generators of the different VEP components are not fully ascertained. CII may arise from extra-striate visual areas [116], but nevertheless, changes in CII can occur consequent upon changes at earlier stages of visual processing. Qualitative differences are apparent in the CI component of the group mean waveforms; this component is thought to originate in striate cortex [117].

It is unusual to find a shortening of VEP latency in a pathological condition. One possibility is that the shortened latency is caused by an enhancement of magnocellular relative to parvocellular responses in strabismic amblyopes. Findings in non-human primates show an increase in the ratio of magnocellular to parvocellular cell size in both undeprived and deprived laminae of the lateral geniculate nucleus following monocular deprivation [38]. Also there is evidence of relative sparing of the motion system in human amblyopia as judged by motion VEPs [102].

Despite the use of only one spatial frequency, the CS changes also show clear differences between early and late onset amblyopes in keeping with differences in underlying pathophysiology. They also emphasise the importance of making comparison with normal subjects as well as with the fellow eye [118].

The first aim of this thesis was to answer the question as to whether or not features of amblyopia might differ depending upon age of onset. Thus the first series of contrast experiments, using psychophysical and electrophysiological methodologies, has shown different patterns of abnormality in early and late onset amblyopes. As might have been be expected from previous studies of amblyopia in which age of onset was not considered, the late onset amblyopes had poorer responses to contrast with increased latency and decreased amplitude of the CII component. Although the early onset amblyopes also had reduced CII amplitudes, the CII latency was shorter. The finding of shorter latencies was unexpected [119]. The shorter latency maybe a behavioural reflection of the enlarged magnocellular cells that have been described neuroanatomically in a primate model [41].

Psychophysical and electrophysiological investigations compared responses to stimuli designed to stimulate Magnocellular (M) and Parvocellular (P) pathways respectively. Although, these stimuli cannot be guaranteed to be "pure" M- and P-isolating stimuli [105], at detection thresholds, the assumption is that they will be detected by the more sensitive mechanism [120], [121], [106], [122], [123], [124]. Using motion onset VEP a negative component (N180) dominating the lateral derivation has been shown to have a low threshold saturating contrast characteristic that would suggest it represents motion mechanisms [86]. Using isoluminant coloured pattern appearance VEPs it has been suggested that the P pathway can be selectively stimulated [125]. The choice of stimuli is dictated by the physiological findings that P-neurones are colour-opponent and have relatively 'sustained' temporal frequency responses, while M-neurones are chromatically broad-band and have more transient' temporal tuning [126]. M-neurones also have larger receptive fields and tend to respond better to low spatial frequency gratings.

Studies of the CS function (CSF) in primates and humans have shown mid to high spatial frequency loss [60], [61] with preservation of the response at low spatial frequency. This might suggest involvement of the P pathway and preservation of the M pathway. Although the responses from the amblyopic and fellow eyes did not differ significantly from normal luminance CS the significant difference between amblyopic and fellow eyes was as much due to the increase in fellow eyes as reduction in amblyopic eyes, which would suggest that the M pathway is enhanced. This was particularly the case for late onset fellow eyes.

A previous electrophysiological study of motion onset VEPs in amblyopia showed no significant latency difference between amblyopic and fellow eyes and it was suggested

that the motion system, and by inference the magnocellular pathway, is relatively spared in amblyopia. However fellow eye recordings were used as controls for amblyopic eye recordings [102]. The present study found only a small, but nevertheless significant, difference in motion onset VEP latency between amblyopic and fellow eyes. However the responses from both amblyopic and fellow eyes were strikingly different from normal Rather than a finding of increased latency as might be expected in an abnormal developmental system the findings here are of shorter latencies. The responses from all amblyopic eyes had a significantly shorter N200 latency compared to normal, although this difference did not reach significance when the early and late onset amblyopes were considered separately. Again the fellow eyes were significantly different to normal. N200 latencies were shorter than normal for fellow eyes of both the combined group and early late onset amblyopes separately. For the combined group the N200 latency was shorter in the fellow eye compared with the amblyopic eye. Despite the finding of shorter latencies the amplitude of the N200 was significantly reduced compared to normal for all amblyopic eye groups. This might suggest that whether it is the M pathway processing the information when the amblyopic eye is stimulated the response remains sub optimal. However the fellow eye amplitudes were not significantly different to normal for all groups suggesting an effective and efficient system for processing this information when the fellow eye is stimulated.

It has been suggested that there is a P pathway deficit in amblyopia [97], [99]. The results presented here also indicate a deficient parvocellular system. When the amblyopes are considered as a combined group the chromatic CS was reduced in the amblyopic eyes compared to fellow eyes. This difference was also significant when the amblyopic eyes were compared to normal eyes. When early and late onset amblyopic

eyes are considered separately this reduction was seen in both groups. In addition, fellow eye chromatic CS was also significantly reduced compared to normal for the combined amblyopic group and when early and late amblyopes were considered separately.

In contrast to the findings of the motion VEP, the colour VEP results are perhaps more predictable. Demirci et al [104] recorded responses to a chromatic sine wave grating in strabismic amblyopes. They found an increased latency and decreased amplitude to this stimulus for amblyopic compared to normal eyes. Similarly the results presented here show an increased latency and reduced amplitude for the N130 component for the combined amblyopic group compared to normal. The N130 also has an increased latency and decreased amplitude for the combined amblyopic compared to fellow eyes. When early and late onset amblyopes are considered separately the early onset group shows no significant difference in latency compared to normal for either eye or when the amblyopic and fellow eyes are compared. However, the amplitude of the amblyopic eye response is significantly reduced compared to normal and compared to the fellow eye. The longest latency is seen in the amblyopic eye of the late onset group, which was increased both compared to normal and to the fellow eye. Latency was also significantly increased compared to early onset amblyopic eyes. Despite this marked increase in latency the amplitude of the response was not significantly reduced compared to normal. These findings show that the P pathway is deficient in amblyopic eyes and that late onset amblyopes are more severely affected. This agrees with primate studies of the LGN, which have shown that visual deprivation in the late sensitive period causes a marked selective shrinkage of parvocellular cells [38].

The (M-P)/M ratio was generated to ascertain how the changes between M and P pathways might interrelate. This ratio was significantly increased for amblyopic and fellow eyes for early and late onset amblyopes. However the ratio differences were most marked in the late onset group, and were significantly greater than in the early onset group. It is important to note that both the M pathway responses were better and the P pathway differences worse. No previous studies have discussed a relationship between M and P pathway changes. One possible explanation for the findings in the experiments described here is a potential inverse reciprocal relationship. Findings in non-human primates show an increase in the ratio of magnocellular to parvocellular cell size in both undeprived and deprived laminae of the lateral geniculate nucleus following monocular deprivation [38]. It is possible that the findings of enhancement of the M pathway response and diminishment of the P pathway response are the functional correlates of this neuro-anatomical finding.

The initial findings, from the contrast experiments, of a difference in early and late onset amblyopes, although interesting, did not give any indication as to the site or underlying mechanism of the abnormality. The second research question was generated to determine whether further differences might be found within the M and P pathways. Psychophysical and electrophysiological experiments using M and P biased stimuli have again shown different patterns of abnormality in early and late onset amblyopes. The greater deficit was in the late onset amblyopes, with the late onset amblyopic eyes having the most pronounced P deficiency. Early onset amblyopic eyes also had a P deficit, but less so than the late onset group. The magnocellular results were similar for early and late onset amblyopic eyes, but as was found with response to contrast, the latency of the motion onset N200 was shorter than normal.

Combining the experiments for amblyopic eyes suggests that although M and P changes occur in both early and late onset amblyopes theses changes are disparate. This is confirmed in the finding that when the (M-P)/M ratio for luminance and chromatic contrast sensitivities was compared, the ratio was significantly greater in the late onset amblyopic eyes, even though visual acuities in the amblyopic eyes of the two groups were similar. One hypothesis to explain these changes might be that the amblyopic visual system attempts to compensate for the P deficit, which is the primary abnormality.

There is significant evidence that the M pathway retains developmental plasticity and reaches maturity later than the P pathway. Similarly specific dorsal stream vulnerability occurs in some conditions e.g. Williams syndrome [127]. The findings in this thesis of abnormalities of both the M and P pathways would also fit with a concept of differential vulnerability and development. Thus the M pathway might attempt to compensate for reduced parvocellular function.

In early onset amblyopia, perhaps the M pathway retains more functional plasticity and reduces the P deficit. In late onset amblyopia the reciprocal enhancement of the M pathway has less functional impact. Therefore the P deficit is unameliorated, and is measured as greater than that occurring in early onset amblyopia. Nevertheless it is important to note that the differing patterns of involvement described in early and late onset amblyopia in this thesis are independent of visual acuity. This whilst the potential

enhancement of the M pathway may occur it has no demonstrable effect on visual acuity.

Our finding of enhanced function in both amblyopic and fellow eyes was unexpected. McKee et al [64] were also surprised to find amblyopic subjects who had demonstrably reduced binocular function were somewhat better at detecting low contrast targets using their amblyopic eyes. This stimulus could be considered to be M biased. In addition they found that the monocular contrast sensitivity of non- binocular observers with acuity > 20/100 was better than that of normal observers. They proposed a model in which the sensitivity of each cortical cell is proportional to the number of active inputs, which in the non binocular observer gives monocular cells greater input, compared to binocular observers. They argue that this model is consistent with linear pooling of afferent signal inputs by cortical neurones.[128]. It might be that the enhanced M pathway responses found in fellow eyes in this study are also a result of more cortical input into the M pathway as a result of the P pathway deficit.

Chapter 5

Conclusions and the future

If similar patterns of visual development with two distinct sensitive periods occur in monkey and humans then there are a number of implications. Firstly, the apparent discrepancy between the lengths of the critical period in monkey and man may be explained [129]. Secondly, most of the published data regarding cortical changes following visual deprivation in primates will apply only to early onset amblyopia in humans. In particular changes in the ocular dominance columns in layer IV of the primate visual cortex only occur during the early sensitive period [38] [36] [130]; this would explain the lack of ocular dominance column changes found in a human strabismic amblyope of onset at the age of two [48]. There is very little physiological data with respect to late onset visual deprivation in the monkey.

There was no difference in acuities between early and late onset amblyopes. This is important, as in general the psychophysical and electrophysiological results are worse in the late onset amblyopic eyes. If the experiments from this study were measuring a treatment effect one would anticipate that the late onset amblyopes would have worse visual acuities. The first Cambridge screening study found that partially treating hyperopia reduced the incidence of strabismus whilst the second Cambridge screening study did not confirm this treatment effect. There was a two month delay between starting treatment in the second compared to the first study, which would suggest that early treatment is more successful [6], [15]. As the acuities in early and late onset groups are similar in this study, the results presented in this thesis would suggest that treatment is not the fundamental reason for the differences found.

If studies were conducted on children during their treatment of amblyopia then it would be important to include detailed descriptions of occlusion prescribed and compliance achieved. This would enable the distinction between treatment effect versus a difference in the underlying pathophysiology between early and late onset amblyopes to be determined.

The demonstration of differences between early and late onset strabismic amblyopes has potential clinical implications. In the early sensitive period in monkey reverse suture, equivalent to total occlusion, is required to equalise the sizes of LGN cells and revert the changes in ocular dominance columns [38] [36] [39] [41]. In the late sensitive period substantial recovery in both the deprived and undeprived LGN laminae occurs after simply reopening the deprived eye, although a small difference in cell size between deprived and undeprived cells remains [41]. Simply removing the cause of deprivation in late onset human amblyopes may allow a substantial degree of recovery and could explain the recovery of acuity demonstrated by only correcting a refractive error in amblyopic children [131] [17]. However, greater improvement was obtained by those who also wore a patch either simultaneously [17] or subsequently [131].

Abnormalities of the fellow eye have been described previously [132], [118], [74], [103]. In this series of experiments early onset fellow eyes, shorter CII latencies and smaller CII amplitudes were measured. The fellow eyes of early and late onset amblyopes also showed increased luminance CS and shorter motion onset latencies. The motion onset amplitudes were reduced compared to normal. The chromatic CS was reduced for all fellow eyes compared to normal. The N130 latencies and amplitudes were similar to normal. These results would confirm previous studies that fellow eyes of

amblyopes should not be considered to be normal eyes. It would seem imperative, therefore that studies of amblyopia should include a normal control group.

It is interesting that the findings of abnormities of the fellow eyes, mirror those found in amblyopic eyes. A potential M pathway enhancement is suggested, by the shorter N200 latencies, although again the functionality maybe reduced as suggested by reduced amplitudes. The reduced chromatic CS suggests a P pathway deficit. It has been suggested that the fellow eye abnormalities found may be a result of patching of the fellow when amblyopia is treated [132], [74]. Although this is clearly a possibility, it should be noted that in the primate experiments there are marked anatomical changes in both the deprived and undeprived pathways even though only one eye has ever been deprived [38]. It is clear that once development is disrupted the whole visual system is abnormal.

The conclusions that can be drawn from this thesis are limited by the fact that all the subjects studied are adults. It is not possible to be certain that the differences found are due to the pathogenesis of amblyopia or the treatment these individuals were given as children. A logical extension of this work is therefore to study amblyopic children.

The motivation for this work was to explore the pathogenesis of amblyopia with the long-term aim of developing new and novel treatment strategies. It is clear that although amblyopia improves with occlusion [14], some children improve with spectacles alone [49]. It maybe that spectacles treat a different component of their amblyopia. Methods have been tested previously to try and improve the effect of occlusion, although without

success [133]. Maybe it will be possible to harness the changes seen here, in the M and P pathways, to improve the effectiveness of treatment strategies in the future?

If the ultimate aim of this work has been to further elucidate the underlying mechanisms of amblyopia, then the next step is to look for these changes in children at the onset and during treatment for amblyopia. It will be crucial to study both amblyopic and fellow eyes in comparison with normal controls to discern how treatment affects both eyes of amblyopes. It is hoped that by gaining a greater insight into the pathogenesis of amblyopia that more efficient and effective treatment regimes maybe developed for children in the future.

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