To John & Arabella
Abstract

Pattern stimulation has proved itself to be an effective technique of studying the visual system in health and disease. However, there are differences in the property of VEPs elicited to different forms of pattern stimulation, namely, reversal, onset and offset. Responses to these three stimuli have usually been studied independently and the relationship between the response components is uncertain. This thesis is concerned with assessing properties and interrelationships between reversal, onset and offset pattern VEPs in controls and in a clinical population of amblyopes.

The three stimuli were delivered sequentially in a single recording epoch so that a direct comparison could be made for virtually identical subject and recording conditions. Half-field stimulation was adopted to separate macular and paramacular contributions. The effects of checksize, scotomata and contrast were assessed, and interocular interaction was investigated. The relationship between the three VEP modes was studied by manipulating contrast and spatial phase so that components could be traced from onset/offset modes to the reversal mode. A total of 56 normal and 18 amblyopic subjects were studied.

Ipsilateral reversal (N80, P100 and N145), onset (ipsilateral CII and contralateral P105) and, to a lesser extent ipsilateral offset components were enhanced by using small checksizes. They were also susceptible to central scotomata; degraded when contrast change was low; and showed the greatest extent of interocular interaction. These features indicate that they are predominantly of macular origin. Contralateral reversal and offset potentials, and ipsilateral onset C1, were enhanced by large checks and were relatively unaffected by central scotomata, suggesting predominant contributions from paramacular activity. Onset contralateral P105 waveform was sharply defined with macular stimulation but became broad and bifid with paramacular stimulation. These findings were confirmed in amblyopes, in whom macular vision is compromised. Contrast change studies indicate offset and reversal components are closely related, and suggest similar physiological origins. Onset C1 and CII could be traced through to reversal P100 and N145, respectively. When small checks (12') were used, onset Co could be traced through to reversal N80 component.

The ipsilateral reversal and contralateral onset P105 components have proved to be the most consistent components in distinguishing between macular and paramacular function, and, between normals and amblyopes.
Acknowledgements

My sincere thanks to Dr. Tony Kriss, my supervisor, for his invaluable ideas, guidance and support throughout the years.

I would also like to thank Dr. Martin Halliday for his advice and inspiration, Dr. Steven Jones for loan of equipment, and Mr. David Taylor for his support and for allowing me to carry out my studies in the Ophthalmology Department at Great Ormond Street, and for permission to use his patients as subjects.

I am very grateful to Mr. Jack Pitman for his technical help and maintenance of equipment, to Dr. Mike Hayward for writing the computer software used in data analysis and presentation, and to Mr. Peter West for his assistance with computer software.

In addition I would like to express my gratitude to Ms. Chris Timms for the orthoptic assessment of the amblyopic children and Dr. Dorothy Thompson for her help and for reading the thesis.
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Abbreviations

' : minute of arc

° : degree

c/deg : cycles per degree

dB: decibels

deg: degree

EEG: electroencephalography

Hz: Hertz

ms: millisecond

MRI: magnetic resonance imaging

NPN : negative-positive-negative

PET: positron emission tomography

PNP: positive-negative-positive

sec: second

SE mean : standard error of mean

Std Dev : standard deviation

μV: microvolt

VEMR: visual evoked magnetic response

VEP: visual evoked potential
1. Introduction

1.1 Evoked Potentials: Historical Background

Evoked potentials are voltage fluctuations generated by the brain and spinal cord in response to stimulation of a sensory modality. They are elicited following auditory, visual, somatosensory and gustatory stimulation.

Dawson (1947) first demonstrated that stimulus-specific, cortical evoked potentials could be recorded at the scalp. He showed by using photographic superimposition that the evoked potential could be discerned from the background EEG. In subsequent years, Dawson described signal averaging using an electro-mechanical machine which sampled and added individual responses (Dawson 1951, 1954). Barlow (1957) devised an electronic averager, based on an analog cross-correlator system. Subsequently, Clark et al (1961) described a digital computer (the Average Response Computer) called the "Laboratory Instrument Computer", which is acknowledged as the forerunner of modern laboratory digital computers for signal averaging.

1.2 The Visual Evoked Potential

The visual evoked potential (VEP) may be subclassified into two types: first, the transient VEP in response to slow repetitive presentation of a visual stimulus (<5/sec) so that each individual response is fully discriminable and secondly, the VEP to more rapid repetitive stimulation (usually greater than 10/sec) giving a steady state sinusoidal response. The VEP can also be classified according to the type of visual stimulus used: plain flash, patterned flash, pattern onset or offset, and pattern reversal.
1.2.1 The Flash and Patterned Flash VEP

Early work employed unstructured flash stimulus presented at slow rates (less than 4/sec). The flash VEP is a polyphasic complex of components. Cobb and Dawson (1960) first studied the flash VEP and reported four early components starting with an initial positive peak at 20-25 ms after the stimulus. Different authors have used a variety of ways to classify the VEP components (letters, numbers, etc.). Cigànek (1961), identified components by Roman numerals and divided them into three time groups: the earliest group was labelled 'primary' (components I, II and III), the next group was called 'secondary' (IV, V, Va, VI and VI) and these were followed by the 'after discharge'. The 'primary' components were postulated to be generated by Brodmann's area 17. Gastaut and Regis (1965) compared VEP waveforms described by different laboratories (e.g. Cobb and Dawson, 1960; Cigànek, 1961; Schwartz and Shagass, 1964). Although there was some variation in waveform and in the number of components, there did appear to be a consistent positive component with onset around 80ms and culminating in a peak around 100-150ms. Inter-laboratory variation can be partly accounted for by different stimulus conditions, different electrode montages and stimulus parameters (e.g., intensity, colour and frequency). Intra-individual variability, poor sensitivity to pathological conditions and poor correlation with visual acuity, rendered it unreliable in clinical testing.

Spatially-structured (patterned) stimuli, such as patterned grids placed in front of the strobe tube were found to alter the VEP. Spehlman (1965) found that whereas the response to diffuse light had a maximum positivity around 80 to 120ms post stimulus, the main positivity to patterned light (checkerboards) occurred at approximately 180 to 250ms. and there was a positive correlation between amplitude and checksize. Rietveld et al (1967) found the response to unstructured flash was characterised by a positivity at around 100ms which inverted its polarity into a negativity on patterned flash presentations. This negativity was
followed by another negativity at 170-210ms and a positivity around 200 to 235ms. Amplitude reached a maximum for checksizes 10'-20', independent of the intensity (check sizes tested ranged from 4deg. to 3'). It was also found that if field size was increased beyond 4 degrees, there was no further increase in amplitudes to either structured or unstructured flash stimulation. Harter and White (1968, 1969) studied the effects of defocus on contour sharpness and check size, and found that two components were particularly sensitive: a) a negativity at around 90-200ms and, b) a positivity around 180-200ms. VEP amplitude was greatest for sharply focused patterns using small 10' to 20' checks, however, if the pattern was defocused (by ±1 to ±5 dioptres) progressively larger checks were needed to elicit the largest VEP.

An important problem in interpreting response changes to patterned flash stimulation is that they represent a combination of both luminance and pattern contributions. Jeffreys (1968) sought to overcome this problem by subtracting VEP luminance changes from those to flashed pattern.. Luminance and pattern responses show different waveforms, and have different topographic distributions thus suggesting different origins. Duffy et al (1967) and Lambroso et al (1969) showed that the pattern specific responses were reduced or absent in ambyopic patients, thus strongly suggesting that these responses were associated with stimulation of the central region of the retina.

Pattern VEPs without a luminance change can be obtained by using a neutral grey background of equal mean luminance, that is replaced by the black and white patterns (checks or gratings) of the same overall luminance. This is known as pattern onset or pattern appearance and a distinctive VEP is observed provided the pattern remains for longer than about 150ms. A response differing in waveform and distributions is found when the pattern is replaced by the blank grey field - this is the pattern offset or disappearance response. These two responses (onset/offset) may be separated and distinguished if the 'on' and 'off'
stimuli are sufficiently far apart in time (more than 200ms.).

1.2.2 Pattern-Onset VEP

Jeffreys and Axford (1972 a,b) identified components of the pattern-onset response: The first positive component CI (with a latency range of 65-80ms), the negative component CII (latency range 90-110 ms) and a later positivity CIII (latency range 150-200ms). CI and CII were both greatly influenced by the retinal location stimulated. Their studies showed that corresponding peaks, CI and CII, inverted in polarity following stimulation of the upper and lower half-fields. For the left and right half-field responses, the transverse distribution of CI, but not CII, showed a lateral polarity reversal across the occipital midline. They concluded that CI and CII have spatially separate generator sources. The longitudinal distribution of CII appears to conform with that of a simple dipole model related to the retinotopic representation at the extrastriate cortex (areas 18 and 19). Extrastriate areas also probably generate CIII. They hypothesised that CI reflected dipole-like activity in the striate cortex (area 17) within and bordering the calcarine fissure. The pattern-onset VEP recorded from a midline electrode following lower half-field stimulation, had a similar waveform in most subjects (12 out of 15). The main differences when comparing the subjects were in the relative amplitude of the three components, and in the peak latencies.

Spekreijse et al (1973) studied effects of contrast on onset VEP amplitude. They found that onset VEPs saturate at a relatively low contrast level, but at pre-saturation levels there was an almost linear relationship between VEP amplitude and stimulus contrast.

Vassilev and Strashimirov (1979) recorded pattern VEPs to vertical sinusoidal gratings presented at rates of 0.5 and 1 Hz. Stimulus spatial frequency was varied between 0.5 and 16 c/deg and contrast levels varied from near-threshold to a value of 0.3. At low spatial frequencies, 0.5 and 1 c/deg, the onset and offset VEPs were of similar shape, magnitude and
contrast dependence. However, at spatial frequencies higher than 2c/deg, onset VEPs tended to be larger and persisted at lower contrast levels than did the offset VEPs. At low stimulus contrast and high spatial frequency (above 8 c/deg), onset responses only were detected. Vassilev and Strashimirov ascribed their findings to preferred sensitivity of transient channels to low spatial frequency, and to predominance of sustained channels at higher spatial frequencies. However, there is a wide spatial frequency range (at least from 2 to 8 c/deg.) where suprathreshold gratings effectively stimulate both types of channels.

The latencies and the saturation amplitudes of the onset components are closely related to changes in stimulus parameters. For example Jeffreys (1977) showed that stimulus outlines have higher thresholds, but similar saturation amplitudes, to those obtained to a corresponding pattern of solid squares. In addition, VEPs obtained to different pattern shapes had similar thresholds and latencies, but different saturation amplitudes. The component amplitude thresholds and latencies were found to be dependent on contrast, duration, overall luminance and on the spatial density and dimensions of the pattern elements, but were not greatly influenced by the actual structural details of the pattern elements. By contrast, the saturation amplitudes, especially of CII and CIII were less dependent on contrast and overall luminance but were dependent on the structural details of the pattern.

There are several indications that the observed contrast dependent properties of CIII, and to a lesser extent CII, parallel the subjective impression of the clarity of the contours of the stimulus pattern. The level of contrast at which CIII approaches saturation seems to correspond approximately with the threshold for the detection of the pattern outlines. This could be related to Spekreijse et al's observations (1973), that the onset response to checks exceeding 20' saturates at a lower contrast compared with smaller checks. This behaviour of CIII is opposite to that of CI, whose amplitude can increase substantially when contrast or duration is increased beyond the level at which the pattern contours become clearly detected.
Jeffreys (1977) reported a more rapid rate of attenuation of CII and CIII compared with CI, as the resting contrast level is increased from zero (- i.e. blank field). Thus, this data also indicates an association between the behaviour of CII and CIII and the subjective definition of the outlines of the pattern elements. It seems that once the resting contrast level is raised just above threshold, CII and CIII are relatively more attenuated but the CI amplitude remains fairly large, and the stimulus appears as a contrast change only.

The amplitudes of these pattern-related components, especially CII and CIII are dependent on the structural details of the stimulus pattern (contour), but seem independent of the polarity of the contrast of the pattern - that is, similar onset VEPs are obtained to corresponding patterns whose elements are either lighter or darker than the background. The most striking feature of this structure dependency is the consistent increase in amplitude obtained when patterns of continuous contours are broken up into discontinuous elements.

The effects of pattern pre-exposure on these pattern onset VEPs show further differences in the properties of the individual components (James and Jeffreys, 1975; Jeffreys, 1977). Comparatively short pre-exposure periods have been found to produce a marked attenuation of CII and CIII, but not of CI. This selective attenuation to adaptation of CII and CIII can occur even for the successive presentations of the stimulus pattern in a conventional averaging run if relatively long pattern durations are used. Experiments by Jeffreys, using independently controlled adapting and test patterns, have shown that, following pre-exposure, there is an increase of the component thresholds and latencies in addition to the eventual attenuation of CII and CIII. These adaptation effects appear to be both orientation and dimension specific.

Hudnell, Boyes and Otto (1990) elicited pattern onset VEPs using sinusoidal gratings of 0.5, 1, 2 and 4 c/deg, following adaptation to a blank field, or to one of the gratings. The waveforms recorded after blank field adaptation showed an early positive component around
77-93 ms, (probably analogous to CI) which decreased in amplitude with spatial frequency, whereas the following negative peak at 97-114 ms, (CII) increased in amplitude with spatial frequency. Stationary pattern adaptation to a grating of the same spatial frequency as the test grating significantly reduced the CII amplitude at 4, 2 and 1 c/deg. CI, however, was unaffected by stationary pattern adaptation at all combinations of test and adapting spatial frequencies. Since CII, and not CI, was significantly attenuated following adaptation and testing at 1 c/deg, Hudnell et al concluded that the neurones generating these components are functionally distinct. The use of a common adaptation grating discounted the possibility that CII, but not CI, was affected due to a difference in the rates of retinal image modulation caused by eye movements made while viewing adaptation gratings of different spatial frequencies. It was postulated that the neurones generating CII were adapted at a lower rate of retinal image modulation than that apparently required for adaptation of the neurones generating CI, which suggests a difference between these neurones in the rate of stimulus modulation necessary to activation.

The pattern onset components have been found to be selectively influenced by gradual de-focusing of the stimulus pattern, CI being more resistant to de-focusing than either CII or CIII, especially for large size pattern elements (Jeffreys, 1977). Whereas lenses of 3 dioptres produce a substantial attenuation of CII and CIII to different checksize pattern onset, progressively stronger lenses are needed to produce a comparable attenuation of CI for increases in checksize. Subjectively when there is sufficient defocusing to make the outlines of the pattern elements indistinct, CII and CIII are invariably attenuated. By contrast, CI can remain relatively unattenuated in conditions where the large square pattern appears as an array of very blurred contrast patches.

The presence of steady outlines of the checks in the stimulus pattern produces a substantial attenuation of CII and CIII, as previously mentioned. Jeffreys (1977) also showed
that the alignment of the steady contours and the test pattern is not necessary for this attenuation, but that their relative linear dimensions seem important, especially for CII. For example, CII and CIII are attenuated by the steady pattern of short bars or squares even when displaced, but CII seems little affected by an aligned steady grid pattern. In all cases there is a significant attenuation of CIII. This effect seems to differ from those described by Spekreijse et al., (1973), who found that the pattern reversal VEPs to low contrast checkerboard patterns were almost completely suppressed by the presence of high contrast, fine grid patterns, and this effect required the accurate alignment of the grids with the edges of the squares. Such interaction effects between the steady and test pattern stimuli also exist when these elements are not overlapping and are in fact spatially separated.

The marked differences in the properties of CI compared with CII and CIII, indicate that there are two types of cortical processes. One is a contrast-specific mechanism, that contributes to CI and the other is a contour specific mechanism that contributes to CII and CIII mainly, but also to a minor extent to CI. The contrast-specific process responds predominantly to the onset and/or offset of the stimulus pattern and is relatively unadaptive. The contour-specific process responds to the onset of the stimulus and is highly adaptive, ceasing to respond to prolonged static pattern stimulation and is both orientation and dimension specific.

CI is thought to originate in the striate cortex, and contrast specific mechanisms appear to be confined there too, whereas CII and CIII are both thought to originate in the extrastriate cortex where the contour specific mechanisms predominate, though they are also present to a lesser degree in the striate cortex. Jeffreys and Axford (1972 a,b) found that the onset VEP components from separate sectors within individual quadrants of the stimulus field are independent. This suggests that like the contour-sensitive single neurones found in the visual cortex of the cat and monkey (Hubel and Wiesel 1962, 1965, 1968) these contrast and contour
specific VEP sources are sensitive to stimulation within localized areas of the visual field.

In one of the animal studies designed to investigate the contrast dependency of single units, Maffei and Fiorentini (1973), found that simple cells in the cat consistently showed a linear relationship between the magnitude of their response and the logarithm of the contrast of a sinusoidal grating pattern. Only a small proportion of complex cells in the striate cortex showed such a linear relationship and the response of these cells tended to saturate at relatively low contrast levels. As simple cells are exclusively present in the striate cortex, and as they show partial summation within the 'on' and 'off' regions of their location-specific receptive fields, this suggests their possible involvement in the contrast-specific contributions to CI. Maffei and Fiorentini also found that the subjective estimate of pattern contrast was closely correlated with the logarithm of stimulus contrast, and that a similar relationship was found for simple cells, and for the amplitude of VEPs obtained to the high frequency alternation of a sinusoidal grating pattern (Campbell and Maffei, 1970). The properties of the contour-specific mechanisms however, make it unlikely that they are closely associated with simple cells, and, their sensitivity to discontinuous contours suggests a possible involvement of hypercomplex-type neurones (Hubel and Wiesel 1965).

Ossenblok and Spekreijse (1991) used a dipole localization approach and principal component analysis to investigate the origins of the pattern-onset components. They used high contrast 24' and 48' checks, presented in a 8x8 deg. field. Two components, each corresponding to a single dipole originating in the extrastriate cortex, appeared to account for the variance in the response. The equivalent dipole sources of the two components behaved differently with respect to area of visual field stimulated. The authors concluded that based on topography and response characteristics, the dipole source underlying the initial pattern-onset positivity suggested an origin in area 18, and the later positivity, appeared to be generated "beyond area 18".
Kulikowski (1977) reported an earlier negative component of the onset response at 70-120ms. This component was also studied by Drasdo (1980), who referred to it as 'Co'. He found that the maximum response occurred with checks 3' to 4.5', or with bars 2' to 3' for equal square-wave gratings, when using a foveal field of 2.5deg diameter. He estimated that the optimal bar width at the fovea is about 2', which corresponds to the receptive field characteristics that have been reported by Poggio et al. (1977) for the striate area of the monkey. Drasdo (1980) found that with a small 2.5deg field, CI was reduced in the midline electrode, but was often present in lateral electrodes, whereas conversely, Co was maximal in the midline but decreased rapidly away from the midline. Interestingly, depending on spatial frequency, the peak of CI varied in latency: Drasdo suggested that there were relative latency differences in the behaviour of CI and CII, and that Co emerged when the leading edges of CI and CII changed their relationship with different spatial frequencies. This provided further evidence on the different origins of CI and CII. These findings for the foveally elicited Co, and the fact that it is small and not readily identifiable in all subjects, may explain the wide range of latencies reported for Co.

1.2.3 Pattern-Reversal VEP

Reversal of a black and white checkerboard pattern has become the most commonly used stimulus in clinical investigations. VEPs to pattern reversal are considered to show less inter-individual variability compared with flash stimulation. The requirements for careful luminance matching between stimulus and blank fields needed for pattern onset/offset are eliminated as the same pattern is reversed at the overall luminance level. Both grating patterns (Campbell and Maffei, 1970) or checkerboard pattern (Halliday and Michael, 1970) have been used.
Campbell and Maffei (1970) found that when the grating stimulus is reversed in contrast at a rate of 8Hz, and when the recording band-width is restricted from 8Hz to 25Hz, the VEP waveform is almost sinusoidal. When grating contrast is reduced, the VEP amplitude decreases proportionately and a linear relation was found between amplitude and the logarithm of the grating contrast, which held for a wide range of spatial frequencies from 3.5 to 18c/deg.

Plant, Zimmerman and Durden (1983) found that the first major negative wave (N1) and the first major positivity (P1) were easily identifiable. This N1-P1 complex to gratings below 1c/deg was similar for pattern reversal and onset modes of stimulation. The N1 became more prominent with spatial frequency above 1c/deg. This effect was more pronounced in the case of pattern onset and the response to pattern reversal diminished in amplitude at a lower spatial frequency than did the response to pattern onset. The late positivity (P2) was most prominent in pattern onset recordings and at intermediate spatial frequencies.

Plant et al (1983) have confirmed previous findings with respect to increasing latency as a function of spatial frequency above 2c/deg. However, they suggest that the departure from a monotonic relationship below 2c/deg is the result of an interaction between two components and this supports previous reported observations by Jones and Keck (1978) that the P1 component of the VEP to low spatial frequency gratings have two early waves. This double peak (called P1_A and P1_B respectively) was not found in all subjects and for some subjects larger field sizes and a lower spatial frequency than usual were needed to demonstrate the contribution of the two components. Jones and Keck suggested that the earlier P1_A wave might reflect the activity of a transient system and that it may be related to peripheral retinal stimulation. Plant et al suggest that it is P1_A which should be identified with the major positive wave P1 at the higher spatial frequencies on the basis of latency
Checkerboard reversal stimuli are preferred and more commonly used for clinical testing and experimental studies as they tend to produce more clearly defined components than grating stimuli (Halliday, 1982). The response to checkerboard reversal stimulation has a positive component with a latency of about 100ms, which can be seen in virtually all healthy subjects. The amplitude of this component in healthy subjects is little affected by changes in the mean intensity (about 15% reduction in amplitude per log unit decrease in luminance). For latency, a decrease in luminance of one log unit produces about 15 millisecond increase (Halliday 1982).

The full-field pattern reversal stimulation elicits a characteristic triphasic complex, with a negativity at 75ms, a positivity at 100ms and a negativity at 145ms. (N80, P100, N145). Left and right half-field stimulation (extending beyond the macular area, 0-2°) will elicit triphasic complexes that appear to have opposite polarities over the left and right sides of the occipital cortex. An N80-P100-N145 complex is recorded over the occipital scalp, ipsilateral to the stimulated half-field, whereas on the contralateral side, a complex with a P75, N105, P135 is commonly recorded (Blumhardt et al, 1978). These ipsilateral and contralateral complexes depend to a considerable extent on stimulation of different parts of the visual field. Studies by Blumhardt et al. (1978, 1989) and Halliday et al (1979), in which the macular area of the stimulus field are masked off, and studies involving the progressive construction of the visual fields, have shown that the ipsilateral NPN complex is mainly associated with the macular area of the visual field and that the contralateral PNP complex is mainly associated with the stimulation of the paramacular areas.

Michael and Halliday (1971) compared checkerboard reversal responses to quadrantic upper and lower field stimulation. Upper field stimulation was found to elicit a negative component at around 100ms which was largest 5 to 7.7cm above the inion. This negative
component appeared to be generated by cortical areas subserving the paramacular area (4-8 degree radius). Lower field stimulation gave a positivity, also at around 100ms, but this was maximal at 2.5 to 5cm above the inion, and, predominantly associated with the macular field.

When the distribution of the P100 latency is plotted for a healthy population using a standardized stimulus, high contrast, large field, the range is relatively narrow, the majority (>95%) of values falling within a 20ms interval. Amplitude, on the other hand, is more variable. This makes it more difficult to use amplitude information clinically. However, the difference in the amplitude of the responses when comparing each eye is very small in healthy individuals and a ratio of the amplitude of one eye to the other should approximate 1. Halliday et al. (1977) reported that the mean ratio of left/right eye amplitudes for 30 individuals was 1.03 (std dev: 0.15).

In about 5% of normals, the main positivity at around 100ms (P100) consists of two sub-peaks approximately of equal amplitude and separated by 10-20 ms. This has been termed the W – waveform (Picton, 1979; Shahrokhi et al, 1978). Picton estimated the latency of the P100 as a mid-point between the two peaks. Stockard et al (1979) indicated that the W waveforms are seen mainly in the occipital-vertex derivations, and that the initial positivity is the real P100, whereas the second deflection which appears as a 'positivity' is in fact a negativity picked up by the reference electrode and thus appears inverted.

Stockard et al (1979) have reported a small but statistically significant sex difference in the mean latency of the pattern reversal VEP. A TV stimulator was used in 50 age matched men and women. The mean latencies for the major positivity was around 98.8 ±6 ms for women and 101.5 ±6 ms for men. Halliday (1982), used a projector and mirror stimulator, also found similar significant latency differences between the sexes (mean difference of 3.5 to 3.9ms. for left and right eyes respectively, p<0.001), and in addition reported that women had, on average significantly larger VEPs (mean difference of 4.6 to 4.8uV, for left and right
eyes, respectively, p<0.0001). It has been suggested that smaller head size, thinner skull thickness and the higher body temperatures in women may account for their shorter latencies and larger amplitudes (Stockard, 1979; Christie and McCrearty, 1977).

The latency of the P100 component is not much affected by habituation, fatigue or level of attention. Meienberg et al (1979) measured 20 pattern reversal VEPs obtained consecutively in one recording session. They found that the P100 latency varied less than ±5%, whereas the P100 amplitude varied upto ±15%. The variations appeared to be random. However, Shahrokhi et al (1978) noted that P100 amplitude tended to decrease with a decreased level of attention. It is probable that changes in attention affect fixation accuracy, level of accommodation and alterations of EEG alpha activity.

The checks that produce the clearest patterns subjectively, also produce the largest amplitude VEPs and decrease or increase of that ideal checksize produces progressively smaller VEPs (Harter and White, 1970). The check size that produces the highest amplitude VEP is progressively greater as more peripheral retina is stimulated. This is in good agreement with the fact that visual acuity deteriorates progressively in the peripheral retina (20/50 at 1.5-4.5 deg; 20/100 at 4.0-11.5 deg and 20/200 at more than 9deg - Harter, 1970). Behrman, Nissim and Arden (1972) observed that for 9' checks, the maximum response was obtained with 1.49 degree diameter fields, for 16' checks, the maximum was obtained with 6 degree fields, whereas for 35' checks, the maximum was obtained with 18 degree fields. The relative insensitivity of the periphery to small checks is likely to be due to the decreased density of cones and greater convergence of peripheral cones into ganglion cells at the periphery (and progressive increase in receptive field size) (Hirsch and Hylton, 1984). Peripheral retina is accordingly also represented by relatively smaller cortical areas.

Visual acuity can be estimated by plotting a checksize versus amplitude graph and calculating by regression from checksize giving the largest amplitude VEPs to the smallest
checksize that can just produce a detectable response (Harter et al., 1968).

Latency of the P100 component is not as significantly affected by checksize as amplitude. Van Lith et al. (1978) studied the variance of P100 latency with checksize and noticed no significant differences between 20', 40' and 80' checks, with 26 degree field size. Spekreijse et al., (1972) reported that steady state VEPs to pattern reversal has a maximal amplitude for 11' checks. In an independent study of a group of five subjects, checksizes ranging from 11' to 18' elicited the largest amplitude VEPs. There is, of course an ambiguity in the interpretation of such studies of the effect of checksize, since when checksize is increased, the number of squares/edges falling on the fovea is decreased, thus decreased stimulation which may account for part of the observed attenuation of the VEP amplitude.

From the point of routine clinical testing, moderate sized checks are preferable, (50'), as moderate defocusing or poor refraction tends to have relatively little effect on the P100 latency. However, amplitude tends to decrease in parallel with the reduction in the acuity level.

Collins et al. (1979) showed that a foveal checkerboard stimulus of 12' checks is very sensitive to refractive errors. Harding and Wright (1986) compared the effects of optical blurring on pattern-onset, reversal and flash VEPs. No effect was found for the flash VEP, however, pattern-onset and reversal showed increases in latency with optical blurring which were more pronounced for 14' checks compared with 56' checks. Interestingly pattern-onset CII component showed a much greater shift in latency than the reversal P100, even with relatively low amounts of defocusing (e.g. +1D).

All pattern evoked potentials have a contribution from luminance mechanisms, and as checksize increases, one would expect the amount of the response which is luminance dependent to increase. Bartl et al. (1978) found that stimulation of 1.25 and 2.5 degree fields produced responses of similar size independent of checksize (10'-80'), but with fields of more
than 2.5 degrees, the response became clearly checksize dependent. The VEPs which were not affected by checksize (1.25 and 2.5 degrees) were thought to be predominantly luminance dependent.

1.2.4 Pattern-Offset VEP

The visual evoked potentials to pattern onset and reversal give a relatively stereotype waveform. However, a variety of waveforms have been reported for pattern-offset. Harter (1971), used the cessation of a train of perceptually fused stroboscope flashes, back illuminating a pattern as stimulus. The main component was a negative peak at around 100ms, in one case followed by a positive peak at about 200ms. Spekreijse et al (1973), who recorded pattern-offset responses, reported a single sharp positive deflection around 120 ms. followed in some subjects by a negative peak at 180 ms.

Jeffreys (1977) compared pattern onset, offset and reversal responses elicited using a tachistoscope. Both the offset response and the reversal response had a positive peak around 120ms, though preceded in some cases by a weak negative deflection. Jeffreys used a short duration (25ms) presentation and the offset pattern VEPs contained pattern-onset contributions due to overlap with the onset related components, particularly CII and CIII. By increasing stimulus duration it was found that the most consistent features of these offset related potentials are positive and negative peaks at about 110 and 150 ms, respectively.

Offset related components have different properties from those of CII and CIII. Jeffreys distinguished between the onset and offset related contributions to the short presentation VEPs by changing stimulus parameters which selectively influence these components. For instance, in contrast to CII and CIII, the main offset components vary less with retinal location and are less influenced by the presence of steady outlines of the test-pattern elements.
1.2.5 Comparison of Pattern-Onset, -Reversal and -Offset VEPs

Kriss and Halliday, (1980), and Ochs and Aminoff, (1980) used similar slide projector stimulus systems and large stimulus fields of 50' checks to elicit pattern-reversal and onset/offset VEPs. Kriss and Halliday concluded that the pattern reversal and pattern offset components were essentially of similar morphology, whereas Ochs and Aminoff put forward the view that the pattern reversal waveform could be produced by the effect of pattern adaptation on the pattern onset response: When pattern onset VEPs were recorded immediately after adaptation to the stimulating pattern, the main negativity (CII) was lost and the latency of the main positivity (CI) was increased by 14ms, causing it to appear similar to the pattern-reversal P100 component both in terms of morphology and latency. Ochs and Aminoff thus suggested that adaptation to the pattern, as found in the conventional reversal VEP paradigm, causes the waveform differences between pattern reversal and pattern-onset.

Barber (1984) compared pattern onset, offset and reversal VEPs and found that the pattern offset VEP exhibited much more intersubject variability; in some subjects the response was almost negligible, but in others significant features were distinguishable. Generally, the waveform became better-defined as checksize decreased and as field size increased. In the worst cases only a single peak was seen, in the best, all peaks of the offset response were identified. Barber reported peaks of the offset response were delayed with respect to their counterpart in the onset response. The amplitude of offset components were also much smaller than those to onset, with the exception of Co, which was more pronounced, particularly with small checks or large fields. Co was sometimes seen in some offset responses, when it was not visible in the onset response. Barber explained this in terms of a greater latency increase (for offset as opposed to onset) for CI (positivity) than for the other components. Thus, there was a shift of CI relative to CII and Co, which is the beginning of CII which was uncovered and made visible. This greater latency change for CI was presumed
to be due to its resistance to adaptation compared to CII, and, the presence of residual contrast. It was also reported that the latency of the offset CI was very close to the value to which the onset CI and reversal P100 converged.

Plant et al's (1983) finding that the amplitude of pattern onset is larger at a higher spatial frequency than pattern reversal, has not been reported before, though it may be related to Kulikowski's suggestion that at lower spatial frequencies (< 3c/deg), the reversal VEP is dominated by movement components and the onset VEP by pattern related components. Hence, these may correspond to the activation of Y and X systems respectively, as it is known that the receptive fields of X cells are smaller than those of Y cells.

There are but a few topographical studies of the offset response. Kriss and Halliday (1980) compared the scalp distributions of onset, offset and reversal responses elicited by a half-field (0-16 degrees radius) with 50 minute checks and found that pattern offset responses had a distribution that was almost identical to that evoked by reversal, for lateral and altitudinal half-field stimulation. However, the onset response distribution, was found to be different. Left and right half-field stimulation gave an ipsilateral offset and reversal distribution of the first prominent positive component (P100), relative to the half-field stimulated, and a triphasic (positive-negative-positive -PNP) complex with a conspicuous negative peak (N100) from the contralateral channels. Pattern onset however, produced ipsilaterally, a positivity that was significantly earlier (P90) and on the contralateral side and a broad positivity (P105) which was maximal at about 5 cms lateral from the midline. Kriss and Halliday found that for upper field stimulation, both pattern offset and reversal gave a prominent negativity at 80 ms with little or no polarity reversal below the inion and a positivity at 100 ms for lower field stimulation.

Kriss et al (1984) compared pattern onset/reversal/offset with respect to age, gender and checksize. They found that reversal and offset tended to be smaller in the elderly,
especially in males, but that onset Cl (referred to as P75) and CII (N100) were larger in the elderly. It was found that all offset and reversal potentials, and onset CII (N100) and CIII (P170) showed an inverted 'U' relationship between checksize and amplitude, with 18 minute checks giving the largest responses, whereas onset CI (P75) showed a progressive increase in size from smallest to largest checks. In half of the subjects, stimulation with the smallest checks (4.5 minutes), caused the onset CII to become bifid but offset and reversal were unchanged. There was also a significant tendency, for all reversal and offset components and onset CIII, to give shorter latencies with larger checksizes, whereas CI and CII showed no such relationship between latency and checksize.

Estévez and Spekreijse (1974) examined the relationship between reversal and on/off responses by modulating two sets of checks in counterphase and appropriate luminance levels were chosen so as to go by steps from a pure on/off to a symmetric pattern reversal. In the pattern reversal modulation, a component with a latency of 107-134 ms was identified and this corresponded to the previously reported P100 component. This potential could be identified all the way to the pure offset condition. However, the peak of the negative component of onset responses with a latency of 120 ms, identified by Jeffreys and Axford as CII, became very attenuated and delayed whenever a small amount of asymmetry was present. Estévez and Spekreijse's results showed that this attenuation was mainly due to the presence of an offset component that interacts with the onset response, and not to the actual asymmetry of the stimulus itself. Their data showed that for low contrast stimuli, the main contribution to the 107-134 ms positivity (P100) of the reversal response seems to come from a 'contrast decrease' component which is followed by a smaller negative 'contrast increase' response. This contrast increase component, however, has different properties from the mainly foveal negative CII component of the onset response. It was found that the checkerboard reversal response from foveal stimulation was not an algebraic sum of the corresponding increase and
decrease responses of the pattern onset/offset stimuli. For extrafoveal stimulation, however, the algebraic sum of the increase and decrease responses compares quite well with the pattern reversal response. This evidence suggests that contrast increase and decrease responses differ widely in dynamic behaviour and the dependence on stimulus parameters is thought to portray a different cortical representation for the two responses.

There are marked differences between pattern onset, reversal and offset responses in the rate at which latency is increased with logarithmic decreases in stimulus luminance. Van der Tweel et al (1979) reported that the CII component of the onset response, elicited by either 40' or 60' checks, increases in latency by some 30ms for each log-unit luminance decrease. They also reported that if stimulus contrast at each step of luminance decrease was adjusted so that it was a fixed multiple of its threshold value, the response waveforms and amplitudes remained very similar despite the change in overall luminance. The pattern reversal response has a rate of latency increase associated with luminance decrease that is about half that of the onset response. Halliday (1982) showed that the reversal P100 potential showed a latency increase of some 15ms and a 15% decrease in amplitude for each log unit of luminance decrease.

Kriss et al (1984) looked at the effect of stimulus luminance decrease on onset, reversal and offset, elicited by 18' checks in a 10deg diameter field, in healthy subjects. The three responses were averaged in a single sweep lasting one second, using a TV monitor screen. The results showed that the latency increase for later components was largest for each mode of pattern stimulus. This effect tended to broaden and degrade the responses at low luminance levels. There was a tendency for the offset responses to show the greatest rate of latency and amplitude change with decreasing luminance, followed by onset and then reversal responses.

Checkerboard contrast is usually expressed as the ratio of luminance difference
between black and white checks to the sum of their individual luminances. Spekreijse and his team have extensively studied the effects of contrast using 20 minute or smaller checks in a field less than 4 degrees in diameter (1972, 1973). The amplitude of all pattern responses saturate at relatively low contrast of 10-20% (i.e. amplitude reaches a maximum despite further increases in contrast). For onset responses, checks of 20' or more tend to saturate at lower contrasts than smaller checks. Spekreijse and Estévez (1972) looked at two contrast levels: 3.8% and 15%, and found that onset responses were largest for 15'-20' checks at both contrast levels; offset responses were largest for 15'-20' checks at 3.8% contrast and for 5'-7.5' checks at 15% contrast. Spekreijse et al (1973) showed that the onset responses were more resilient than offset responses to decreases in stimulus luminance at low contrast levels (10%, 5% and 3%).

1.2.6 Pattern, Motion and Contrast Contributions to the VEP

Estévez and Spekreijse (1974) and Kriss and Halliday (1980), showed a close resemblance between pattern reversal and pattern offset. However, several workers have suggested that the pattern reversal VEP is likely to contain contributions from motion-specific and pattern-specific mechanisms and thus pattern reversal itself cannot fully be attributed to pattern onset/offset alone.

Kulikowski (1977, 1978) showed that for coarse patterns, the reversal VEP can be attributed to movement processing, while for stimuli with spatial frequencies higher than 3c/deg, both pattern and motion processing systems contribute to the reversal VEP. Van der Tweel and Spekreijse (1968) have also suggested that large checkerboard reversal VEPs have two constituents related to the sharply focused edges of the pattern, and secondly, those related to changes in local luminance inside individual checks. This local flicker contribution is more evident in large check VEPs than in small check VEPs. The experimental evidence
for this suggestion stems from the fact that the reversal VEPs were abolished when the sharply focused edges of the checks were occluded by fine lines, but this occurred only when the checks were small. Kulikowski (1977) based his work on the psychophysical evidence that a reversing pattern has two distinguishable thresholds: one for detection of motion or flicker, the other for the detection of the pattern. He considered VEPs to a grating of low spatial frequency to be related to a transient (i.e. ac-coupled) neural mechanism as such a VEP depends only on the change in contrast and not on the initial or final value. Kulikowski argued that the criterion for transient related VEPs is that the onset and offset waveforms are similar and that the two constituents sum linearly so that the pattern related constituents of the VEP could be obtained by subtracting the offset responses from the onset responses. These dichotomies are similar to those proposed by Jeffrey's (1977) where a contrast specific mechanism contributes predominantly to onset C1, and a contour specific mechanism contributes to CII and CIII, and to a lesser extent to C1.

Spekreijse et al (1985) argued that the reversal response is better described in terms of motion-onset and motion-offset. Their evidence for this included the fact that responses to abrupt displacement of less than one square width are qualitatively similar to the reversal VEP produced by abrupt displacement of exactly one square's width, also the displacement VEP can be seen as the limiting case of a motion onset response followed by a motion offset response, and the topographical distributions of motion onset and offset responses are similar to the distributions of the two corresponding components of the displacement VEP. The displacement VEP is adapted by steady motion to an extent that depends on the speed of motion. This study, and further studies from the same Amsterdam group (Dagnelie et al, 1986, De Vries et al, 1989), consider responses to motion onset as dominated by a single positive peak (P1) at a latency around 120ms. Other groups such as Yokoyama et al, (1979), Gallicchio and Andreassi (1982), Manning et al, (1988) Kubova et al, (1990) and Kuba and
Kubova (1992) described motion-onset VEPs in terms of a positive-negative-positive (PNP) complex with the negative N\textsubscript{2} peak at a latency of about 160-200ms as the most prominent component. Kuba and Kubova (1992) believe that the major positive peak for motion onset VEPs reported by the Amsterdam groups is primarily pattern generated and is a variant of pattern offset VEPs, caused by pattern disappearance effect at the onset of motion with a high temporal frequency (>6Hz). Such P\textsubscript{1} dominated VEPs also occur mainly when the stimulus is limited to the macular area (central 6 degrees). Kuba and Kubova (1992), however, have shown that the N\textsubscript{2} dominated motion-onset VEPs are recordable as far out as 50 degrees of eccentricity with amplitudes being significantly larger for extramacular compared with macular stimulation. They state that their observed lateralization of this major negativity supports their hypothesis of its motion specificity and the extrastriate origin of the visual perception of motion.

In a subsequent study, Kubova et al (1995) compared checkerboard reversal VEPs with motion-offset VEPs taking into account the relative effects of contrast. Both reversal and motion onset VEPs consist of an NPN complex with the positivity P\textsubscript{1} (peak latency ~ 120ms) being followed by the negativity - N\textsubscript{2}, (peak latency ~ 160-200ms). However, whereas the P\textsubscript{1}, dominates the reversal VEP, particularly at the mid-line occipital electrode, the N\textsubscript{2} dominated the motion-onset VEP especially when recorded from unipolar lateral occipital electrodes. It was found that whereas the P\textsubscript{1}, component decreases in amplitude with decreasing contrast (becoming undetectable at a contrast of about 2% for motion-onset VEPs), N\textsubscript{2} amplitudes for both reversal and motion-onset VEPs did not vary significantly with contrast, above a contrast of 1.3%. It was also found that peak latencies increased with decreasing contrast and again this was more pronounced for P\textsubscript{1} than for N\textsubscript{2} for both reversal and motion-onset VEPs. The motion related studies described above used motion-onset stimuli which may not invoke pure motion mechanisms. Random-dot patterns (kinematograms), where coherent versus incoherent
motion are used, are proposed as being more appropriate in eliciting motion specific responses. These stimuli are perceived as motion only, and they appear to evoke motion responses without other confounding factors such as change in position of contrast (Manning and Mazzucchelli, 1992; Probst et al, 1993; Snowden et al., 1995).

In summary, studies have shown that components related to pattern-onset, -reversal and -offset VEPs behave differently depending on the stimulus characteristics. Pattern-reversal and -offset VEPs appear to be of similar morphology: a triphasic negative-positive-negative complex. If the stimulus is confined to half-the visual field then this complex becomes lateralised over the ipsilateral side of the scalp, and a VEP complex of opposite polarity (positive-negative-positive) is often recorded on the contralateral side of the scalp. The main ipsilateral positivity has been well studied and behaves as if macular in origin. The contralateral main negativity however, appears to be of paramacular origin. Pattern-onset components have been labelled consecutively as CI (positive polarity), CII and CIII. These components also appear to lateralised ipsilaterally on half-field stimulation, with a positive component (P105), often present on the contralateral side of the scalp. Experiments have shown that contrast-specific mechanisms contribute to CI and contour-specific mechanisms contribute to CII and CIII.

1.3 Basic Physiological Aspects and the VEP

The primate visual system is thought to consist of a composite of parallel systems often referred to as channels (Regan, 1982). Subsystems of the direct retino-cortical pathways are stimulus specific, a certain stimulus characteristic may engage the activity of some pathways more than others. Studies have shown that selection of spatial frequency is important in influencing the VEP. This is thought to reflect the fact that VEPs are predominantly the outcome of the activation of those specific retinal and cortical neurones
that respond to the particular stimulus used. Even though a VEP is generated by cortical cells, some of its properties may be pre-determined at the retinal level. As described above, pattern VEP amplitude and latency are influenced by a number of factors such as contrast, spatial frequency, orientation and luminance. The following sections will outline the relevant neurophysiology and neuroanatomy of the visual pathway as related to mechanisms thought to generate the VEP. These will predominantly involve the retino-geniculo-cortical pathway, though the subcortical visual pathway will be briefly described.

1.3.1 The Retina

Signals from a large number of photoreceptors (rods and cones) converge on bipolar neurones which in turn synapse with retinal ganglion cells. The ganglion cell axons, which form the optic nerve, synapse at the lateral geniculate nucleus (LGN) and these in turn synapse at the visual cortex reaching it via the optic tract. A few axons are involved in the control of pupillary diameter and connect to the Edinger-Westphal nucleus and accessory optic tract and some other axons connect to the superior colliculus.

Bipolar cells can be classified into two types: ON bipolars, which depolarise to light, and OFF bipolars which hyperpolarise to light. Pathways for these two types of cells are anatomically distinct and segregated: the dendrites of ON-centre ganglion cells (excited by light) branch in the inner two thirds of the inner plexiform layer, whereas OFF-centre ganglion cells (inhibited by light) branch in the outer one third of the inner layer (Nelson et al., 1978). On and Off ganglion cells receive their inputs from the corresponding types of bipolar cells. These pathways remain distinct throughout the LGN where there is a predominance of ON-centre cells in layers 6 and 5 and a predominance of OFF-centre cells in layers 4 and 3. This ON/OFF distinction appears to carry on to the striate cortex (Schiller and Colby, 1983). It is thought the distinction between ON and OFF gives rise to orientation
and direction selectivities of cortical cells, however, Schiller et al (1986) suggested that it may be thus organised so as to provide fast transfer of information regarding light increments and decrements with equal sensitivity.

The concept of the 'receptive field' of individual neurones (Fischer, 1973) is important for understanding why spatial contrast influences VEP studies. Neurophysiologists have thought of the visual system in terms of neurons that are driven by stimulation within a small discrete area of the visual field. These receptive fields have been mapped by flashing or moving stimuli such as spots or bars. Signals from photoreceptors of a portion of stimulated (illuminated) retina converge onto two separate pools of the receptive field, these pools are known as centre and surround (Enroth-Cugell and Robson, 1966; Rodieck and Stone, 1965). The ganglion cell response recorded from its axon is a fluctuation (increase or decrease) from the mean background firing rate. When both centre and surround organisation of the ganglion cell receptive fields are illuminated, the modulated response decreases compared to if the illumination is of the centre alone. As a result of this antagonistic organization, the size of centre relative to the surround establishes the spatial selectivity of individual neurones. Because of the antagonistic organisation of separate pools for centre and surround portions of the receptive fields of bipolar neurones and ganglion cells, the retinal output does not simply represent the illumination of a patch of retina. Instead the response of bipolar and ganglion cells is determined by spatial contrast, that is a difference in illumination between adjacent retinal areas, rather than by the sum of their separate illuminations.

The distinction between centre and surround and hence their interaction, is sharpest for foveal ganglion cells. The more sharply defined is each mechanism, the more selective is each neuron for stimulus size. For example, bipolar cells of the retina are less selective for stimulus size and shape than cortical neurones. Retinal and LGN neurones are radially symmetrical- that is show little orientation selectivity. A vertical or horizontal slit-like
stimulus or grating will have a similar effect in the retinal and LGN but not so in the cortex (Hubel and Wiesel, 1968).

Ganglion cells differ in the size of their receptive fields. Generally, the closer a neurone to the anatomical fovea, the smaller its receptive field. However, each region of the retina is subserved by a range of ganglion cells with different receptive field sizes. The population of parafoveally located ganglion cells have larger receptive field centres than the foveal ones. It is estimated that the human foveal ganglion cell receptive field is smaller than 20' (Jones and Keck, 1978; Plant et al, 1983; Skrandies, 1984). Pattern element size thus relates strongly to the area of retina giving the strongest response.

The organisation described in the previous paragraphs applies to X ganglion cells (based on cat physiology). Another type of retinal ganglion cell is the Y cell. Y cells are somewhat different in that, opposing forces for centre and surround are never completely in balance. Y ganglion cells have a particular 'subunit' organisation that contributes signals that are not cancelled- i.e. centre and surround responses are not in perfect spatial opposition. (Hochstein and Shapley, 1976). Y cells are particularly sensitive to transient changes in illumination, and to spatial variation occurring in their receptive fields. Hochstein and Shapley (1976), and Victor and Shapley (1979), showed that an essential difference between X and Y cells is in the temporal relationship between stimulus and neuronal response. Y cells and target neurones in the LGN are more sensitive to low contrast stimuli than are X cells (Kaplan & Shapley, 1982). Enroth-Cugell and Robson (1966) noted that in cat, Y cell receptive field centres are generally larger than those of X cells and that X cells are more common in the area centralis of cat retina and thus associated with high resolution vision. Y cells, on the other hand, subserve motion detection and possibly low resolution pattern vision. In general, they have larger diameter axons with faster conduction compared with X cells (Stone and Freeman, 1971; Stone, 1983).
In monkeys, X cells are more common than Y and this is thought to reflect the higher spatial and chromatic acuity in the primate (Shapley and Perry, 1986). In monkey, the X ganglion cells synapse onto neurones in the parvocellular layers in the LGN and are thus called P-cells, and Y cells synapse onto the magnocellular layer and are called M-cells (see LGN section below).

1.3.2 Lateral Geniculate Nucleus:

Six stacked layers can be recognised in the LGN (three layers for each eye). The synaptic terminals from the two eyes distribute themselves in the 6 layers so as to produce six topographic maps of the contralateral half-field of vision. There are 4 parvocellular layers (2 for central 20 deg. of vision and two for peripheral vision) and two magnocellular layers.

![Figure 1.1: Diagram of a coronal section through the left LGN of the macaque showing main projections of P and M cells in LGN to striate cortex. P-cells project primarily to 4Cβ and 4A. M-cells project primarily to 4Ca. From Lennie et al., (1990).](image-url)
The magnocellular layer only represents 8-10% of the total volume of the LGN (Kaas et al., 1978). The LGN layers also have different projection sites; the parvocellular cells projecting to cortical layer 4α and the deeper β sublayer of 4C (4Cβ), while the magnocellular cells project to the superficial sublayer of 4C (4Cα) (Valverde, 1985). A diagram showing these projections can be found in Figure 1.1.

Parvocellular LGN cells are mostly served by colour-opponent receptive fields and respond in a sustained fashion to visual stimuli, although a small number (15-20%) lack colour sensitivity. They have small receptive fields, linear summation properties and have axons which conduct at medium velocities to the striate cortex. Magnocellular LGN cells lack colour-opponent receptive fields, and are not very colour sensitive. They respond in a transient fashion, have relatively large receptive fields and tend to have non-linear spatial summation properties. They conduct at faster velocities to the cortex (by about 4ms.-Lennie et al., 1990) and have greater contrast sensitivity than parvocellular cells (Schiller and Colby, 1983). The receptive fields of LGN cells in general are nearly circular with the greatest width being less than that of cortical cells. It has been suggested that the separation of parvocellular layers from magnocellular layers corresponds to the distinct processing for contrast (magnocellular) and colour (parvocellular) at the LGN level (Shapley, 1986). Grating contrast sensitivity is much higher for magnocellular cells than parvocellular cells (by 10 to 20 times) and it is thought that magnocellular cells respond and mediate the perception of patterns at low contrasts and at low to intermediate spatial frequencies, whereas parvocellular cells are involved with perception at high contrasts and high spatial frequencies.

1.3.3 Cortex

A brief anatomical description of cortical visual areas, which are relevant to VEP studies and inferences will be presented in this sub-section. Approximately 30 cortical visual
areas in macaque, and 10 in man have been described and the majority have been found to be homologous. Topographically, however, human areas are about twice as wide as the macaque counterparts, with the exception of human V3 and VP, which are disproportionately enlarged in man, furthermore distance from foveal areas V2, V3 and VP to V5 (MT) is also disproportionately expanded in humans and it has been suggested that this is related in form processing (Tootell et al., 1996). Cortical areas in man have been studied using PET (positron emission tomography), fMRI (functional magnetic resonance imaging) and magnetoencephalography (MEG). In primates, areas V1 to V5 appear to have an important role in determining VEP characteristics with respect to activation by features such as spatial and temporal frequencies, luminance change, the visual field and areas of stimulation, and on/off stimuli versus reversing stimuli which has a motion contribution.

The main projection from the LGN is to the striate cortex (area 17 or V1) and in monkey it is retinotopic. Cortical cytoarchitecture tends to be distinct. Physiological recordings have shown that the area contains a single representation of the visual field which is foveally weighted. The representation of the central 1 degree of the fovea extends over about 15mm of cortex, whereas, at 10 degree eccentricity, 1 degree is only represented by about 1mm (Van Essen et al., 1984) - see figure 1.2.

Parvocellular neurones project mainly to layers 4Cβ (deep layer) and 4A. Magnocellular neurones, on the other hand, project almost exclusively to layer 4Cα (Hubel and Weisel, 1972; Hendrickson et al., 1978). The receptive fields of layer 4 are almost all monocularly driven and inputs from the two eyes are sharply segregated into long strips running parallel to the surface of VI. These ocular dominance columns have been demonstrated anatomically (LeVay, Hubel and Wiesel, 1975) and physiologically (Hubel and Wiesel, 1968). The magno and parvocellular streams do not remain segregated beyond layer 4, although they do project to different places (Hubel and Wiesel, 1977). Magnocellular
neurones in layer $4C\alpha$ sends major projections out of the striate cortex and to visual areas 2, 3 and middle temporal (V2, V3 and MT). Cortical areas above and below 4C receive inputs from both eyes and have elongated receptive fields that are tuned to orientation.

Figure 1.2: The primary visual cortex contains an orderly map of the visual field at the posterior pole of the cerebral hemisphere and lies predominantly on the medial surface. Each half of the visual field is represented in the contralateral hemisphere. Approximately half of the neural mass represents the fovea. Upper fields are mapped below the calcarine fissure and the lower fields above it.


In the striate cortex a common type of orientation tuned neurons are known as 'complex cells' (Hubel and Wiesel, 1968). They respond weakly to stationary targets or give ON and OFF responses when the stimulus goes on and off. Complex cells tend to be both orientation and direction specific. LGN cells do not show direction selectivity, thus this property of cortical cells, like orientation, is created by cortical connections. 'Simple cells' in the striate cortex resemble LGN cells in that they have excitatory and inhibitory regions in their receptive fields. However instead of being concentrically arranged, they are organised in parallel strips and respond well to a moving bar oriented parallel to the parallel strips.
Simple and complex cells differ in the linearity of their spatial summation. Hubel and Wiesel (1962, 1968) originally identified a third subset of striate cells, the hypercomplex cells, however others have classified them as subsets of simple and complex cells (Dreher, 1972). The defining hypercomplex property is the presence of inhibitory zones at one or both ends of the excitatory region of the receptive field, with the consequence that these cells respond to say stimulating bars of preferred orientation only if they are of a particular length.

The striate cortex interacts with three main extrastriate areas: V2, V3 and MT (Figure 1.3). These three regions receive topographically organised projections from V1, (except for V3 which only represents the lower visual field) and is topographically represented (Van Essen et al., 1986). Receptive fields of extrastriate neurones are considerably larger than those for neurones in V1. When V2 is stained with cytochrome oxidase, then tangential sections reveal densely striped areas; these appear alternately thick and thin in the squirrel monkey, although this distinction is less clear in the macaque (Tootell et al, 1983, Livingstone and Hubel, 1984). Livingstone and Hubel (1984) showed that the V1 blobs projected to the stripes in V2 and that the interblob regions of V1 projected to the interstripe (pale areas) of V2. Hubel and Livingstone (1987) observed that the thicker stripes in squirrel monkey received inputs from layer 4 mainly. They postulated that they were associated with the magnocellular pathway and contained a preponderance of complex cells that showed selectivity for binocular disparity. The thinner stripes were presumed to be associated with the parvocellular pathway, and contained a preponderance of cells that lacked orientation selectivity, and in the macaque were chromatically opponent. V1 and V2 contain neurones that respond to a wide range of spatial frequencies, with V1 showing preferences for high and middle frequencies, and V2 showing preferences for middle and low spatial frequencies (Foster et al, 1985).
Figure 1.3: Diagrams of human (A and C) and macaque (B and D) brains showing topography of presumptive cortical visual areas. A and B show one hemisphere of the human and macaque brains in folded normal state, and C and D show the same anatomical (right hemisphere) and functional data in 'flattened' cortical format. Human visual areas are based on data from functional magnetic resonance imaging (fMRI), and macaque visual areas are based on published data. Names for human visual areas have been adapted from corresponding areas in macaque based on topographical and functional evidence for homology, for example; for V1, V2, V3, ventral posterior (VP), V3A and middle temporal (MT, or V5). Other areas are V4v (ventral V4), VIP (ventral intraparietal), SPO (superior parietal occipital), pMSTd (posterior division of dorsal medial superior temporal area), MSTd (dorsal division of MST), LIP (lateral intraparietal), LSPO (lateral superior parietal occipital), LO (lateral occipital).
Adapted from Tootell et al., (1996).
Lower visual field is represented in V3, whereas a corresponding representation of the upper field is found in the ventral posterior area, which is no longer thought of as part of V3 due to different cytoarchitecture. V3 receives inputs from layer 4B of the striate cortex and from the thick stripes of V2 (Felleman et al 1988). Most of the cells in V3 are strongly orientational and directional specific as well as being sensitive to binocular disparity, but not many are chromatically sensitive. There are distinctive cells that are narrowly tuned for several orientations or directions of motion (Felleman and Van Essen, 1987).

V4 is an area that extends from the anterior bank of the lunate sulcus on to the prelunate gyrus and contains a large number of neurones that are chromatically sensitive (Zeki, 1973). It receives direct but small input from V1 with the majority coming from V2 and V3. The projection from V2 arise in one set of cytochrome oxidase stripes (thin ones in squirrel monkey and in the interstripe (DeYoe and Van Essen, 1985; Shipp and Zeki, 1985), the former by implication being the source of the chromatically opponent signals. However not all neurones in V4 are involved in colour analysis and receptive fields, despite being large, have spatial and orientational selectivities just like those in V1. Heywood and Cowey (1987) showed that bilateral damage to V4 in macaque impaired chromatic discrimination and also resulted in gross disturbance of pattern and orientation discriminations. Cells in V4 (as well as those in V3 and V5) are selective for binocular disparity. These binocular disparity V4 cells receive projections from the parvocellular stream via the V1(inter blob) - V2 (inter stripe) stream (see figure 8.2 -De Yoe and Van Essen, 1988).

The middle temporal area (MT - V5) has been implicated in the analysis of motion since Dubner and Zeki (1971) discovered that the majority of neurones here were directionally selective. MT receives direct projections from V1, arising in layer 4B (Maunsell and Van Essen, 1983 a) and layer 6 (Fries, Keiser and Kuypers, 1985) and indirect via the thick stripes in V2 (De Yoe and Van Essen, 1985). MT also receives inputs from V3 (Maunsell and Van
Evidence linking MT to the analysis of movement come from experiments that have shown neurones responding distinctively to complex pattern motion and from experiments involving chemical lesions in MT. Newsome et al., (1985) made small chemical lesions in MT and found monkeys were temporarily less able to capture and pursue targets in the affected area of the visual field. There has also been a clinical report (Zihl et al., 1983) of a patient who had a lesion in area MT and who had impaired motion perception, particularly with tests involving moderate and fast movement. Magneto-encephalography has been used to determine the location, temporal dynamics and functional properties of the human homologue of primate area MT (Anderson et al., 1996). Anderson et al., studied 3 subjects and found human V5 was located in a minor sulcus near the occipito-temporal border and immediately below the superior temporal sulcus, and had response properties consistent with a predominant input from the magnocellular pathway. Macaque area MT has a large proportion of velocity-tuned cells (Maunsell and Van Essen, 1983 b) with optimal velocities ranging from 2 degrees to 256 degrees per second. About half the cells in MT are disparity selective and there are a small percentage of cells that prefer opposite directions of movement in the two eyes in MT (Zeki, 1974), this is also found in V1 and V2 and thought to signal movement away or towards the head.

A property present in MT but not in V1 relates to stimulation with a 'plaid' pattern made up from two superimposed gratings moving in different directions. Approximately 20% of MT neurones respond selectively to the direction of motion of the plaid pattern rather than the direction of motion of either grating component. MT contains two distinct kinds of directionally selective neurones: Component direction selective neurones, (like those in area V1) which provide signals about local motions of individual contours and orientations, and
direction selective neurones that are only found in the MT and carry more fully integrated information about motion from several different contours and orientations (Movshon et al, 1986).

Areas beyond MT are thought to be involved in motion processing, however this is not fully understood. Areas such as MST (medial superior temporal), 7a and VIP (ventral intraparietal) are implicated. Neurones in MST and 7a respond to complex pattern of motion but not to simpler rigid motion of objects across the visual field. Motter and Mountcastle (1981) have shown directional responsiveness in parietal neurones that is related to optic flow produced by locomotion through the environment. Saito et al., (1986) have reported complex patterns of response in MST neurones including preferences for rotations and optic flow.

Figure 1.4 shows an overview of the anatomical and functional segregation of the visual system as described above. The evidence for the segregation of motion processing and pattern processing, through magnocellular and parvocellular pathways is likely to be an important factor relating to different sub-components of the VEP. VEP potentials that appear to be differentially influenced by such variables as contrast, spatial and temporal frequencies and optimal location for maximal VEP component recording (e.g., mid-line or lateral channels) may help in identifying their different striate and extrastriate origins and relationship to magno- and parvocellular activity.
Apart from the cortical visual pathways, there are other projections from the retina to sub-cortical structures, including the superior colliculus, pulvinar and the accessory optic nuclei (see figure 1.5). A third class of primate retinal ganglion cells (γ) project to the superior colliculus and have similar properties to M ganglion cells, although they lack a
distinct centre-surround arrangement. They have been shown to be sensitive to motion (Perry and Cowey, 1984). These \( \gamma \) retinal ganglion cells and their subcortical projections are important as in the primate eye they form about 10\% of retinal ganglion cells, with the M-cells forming 10\% and P-cells 80\% of the ganglion cell population (Perry et al., 1984).

Figure 1.5: Diagram showing known projections from eye to brain (excluding connections from cortical areas to further visual areas). Thicker arrows indicate the most studied projections. The class of retinal ganglion cell to most of the brainstem targets are unknown.

(Adapted from Cowey & Stoerig, 1991).

1.3.4 Generators of the VEP

The different components in the evoked potential are thought to reflect different cellular events (Creutzfeldt and Kuhnt, 1973). Potentials are usually classified as excitatory or inhibitory, depending on whether they depolarize or hyperpolarize a cell's membrane, respectively. Excitatory postsynaptic potentials (EPSP) that occur on apical dendrites near
the cortical surface generate a superficial current sink and a corresponding deep current source and thus producing a surface negativity. Inhibitory postsynaptic potentials (IPSP) occurring at a similar site produce a superficial source and corresponding deep sink and hence produce a surface positivity. However, EPSPs and IPSPs occurring near the soma of pyramidal cells deep in the cortex produce surface potentials of opposite polarity to those produced by EPSPs and IPSPs occurring on apical dendrites in superficial layers. This deep activity needs to be synchronised to be reflected in surface recordings. So following a synchronised afferent volley, a positive wave is initially observed in the surface evoked potential which is thought to reflect depolarization near the soma of pyramidal cells (Eccles, 1951). The subsequent negativity is thought to reflect the initial depolarization up the apical dendrites and EPSP, mediated via intracortical pathways, generated directly on superficial segments of apical dendrites. Initial positivity is associated with afferent activity arriving at the cortex from the LGN and initial depolarization occurring deep in the cortex. The following negativity is correlated with superficial depolarization. It has been found (Creutzfeldt and Kuhnt, 1973) that the post-stimulus time histograms of firing rates of cortical cells in cats, closely reflect the surface potential: enhanced activation associated with the negative wave and diminished activity associated with the positive wave.

Pharmacological studies (Purpura et al., 1957) have shown that application of GABA (γ-aminobutyric acid), an inhibitory neurotransmitter in the cerebral cortex (Krnjevic and Schwartz, 1967) attenuated the negative wave and enhanced the later positive wave. Thus it seems that GABA-mediated intracortical inhibition is important in influencing the evoked potential waveform. Zemon et al. (1980) studied the effects of bicuculline (a GABA blocker) on VEPs in cats and found that the first negativity represents an excitatory process and that the later positivity represents a GABA-mediated inhibitory process in the visual cortex.

Schroeder et al. (1991) investigated striate cortical contributions to the surface
recorded reversal VEPs in awake monkey by studying the laminar profiles of the VEP and the current source density recorded systematically at incremental depths in area 17. The VEP consisted of a small negativity (N50) followed by a positivity (P60), a large negativity (N80) and a broad positivity (P125). N50 was primarily generated by current sinks in lamina 4C and the P60 from current sources in the supragranular laminae. The N80 and 'P125', (which presumably are analogous to human reversal N80 and P100) were found to be composite waveforms reflecting contributions from local activity and from activity arising outside the foveal and parafoveal representations in area 17. Schroeder et al concluded that pattern VEP are consistent with the cellular anatomy of area 17 with the initial activation of the thalamorecipient subdivisions of lamina 4C.

There have been few studies that dealt with the relationship between scalp - recorded VEP and the underlying areas of visual cortex in man. Jeffreys and Axford (1972 a,b) were amongst the first to tackle this aspect theoretically for pattern-onset VEPs. Their analysis was based on the assumption that the potential distribution of the underlying cortical activity at the instant of each peak can be ascribed to a single current dipole. By analysing the responses to stimulation of different parts of the visual field, they concluded that CI and CII originate from areas 17 and 18 respectively. Drasdo (1980) reanalysed Jeffrey's findings taking into account more details of anatomical layouts of areas 17, 18 and 19, and the concept of cortical magnification, but still assuming that a single dipole described one peak. Drasdo concluded that CI originates from area 18 and CII, area 17. A third study by Lesèvre and Joseph (1979) resulted in areas 19 and 18 as sources of CI and CII respectively. This latter study was the first in which it was stated that each peak in the pattern onset response need not be the result of a single dipole, but might reflect simultaneous activity of several visual areas. They showed that the dipole responsible for CII is already active at the time of CI.

Comparable studies have been carried out for pattern reversal stimulus of which the
best known studies are from Dr. Halliday's laboratory. Unlike Jeffreys and colleagues, Halliday and Michael (1970) reported that no peak of the pattern VEP originates in the striate cortex but that all components are generated by areas 18 and 19. Barrett et al. (1976) suggested that the major reversal positivity to half-field stimulation appears largest in the midline and ipsilateral electrodes (paradoxical lateralization), because their orientation in relation to a mid-frontal reference, positions them maximal for a radial source. The electrodes on the contralateral side of the scalp see the source as tangential and therefore do not receive a large signal. The larger the stimulus field size, the VEP responses are represented further away down the calcarine fissure. However, Harding et al (1980) showed that if the stimulus field was small (0-2.5 degrees), then this lateralisation became less. They used 4 different field sizes (0-14, 0-10, 0-5 and 0-2.5 deg) and found that with reduction in field size, the reversal P100 decreased in amplitude and changed in distribution from being ipsilateral to the stimulated half-field with 0-14deg field, to being slightly contralateral with the 0-2.5deg field.

Lehmann et al. (1982) used dipole modelling techniques to investigate the origins of half-field VEP generators. They found that with large stimuli, the dipole was situated in the contralateral hemisphere with the positive end pointing towards the ipsilateral scalp electrode; however when field sizes were reduced the positive end of the dipole moved towards the midline. Flanagan and Harding (1986) used source derivation to investigate the distribution of reversal VEPs to a large hemi-field (0-14 degrees). They showed that for either half-field, the source was maximal at the midline occipital electrode and that the sink was contralateral to the stimulated half-field.

More recently, visual evoked magnetic responses (VEMR) have been used to topographically map pattern responses. Harding et al. studied pattern-reversal responses (1992), and pattern-onset/offset responses (1994). The main positivity of the VEMR to left half-field reversal stimulation showed an outgoing magnetic field over the posterior right
visual cortex, and with right half-field stimulation, the field was in the left anterior visual cortex, thus identifying the source in the contralateral hemisphere. Source localisation techniques suggested the dipole was located in the contralateral side of the midline to the stimulated half-field. Quadrantic pattern-onset stimuli produced dipoles situated in the lingual gyri on the floor of the calcarine fissure for upper quadrantic field stimulation, and on the cuneal gyrus and ceiling of the calcarine fissure, for lower quadrantic stimulation (Harding et al., 1994).

Haimovic and Pedley (1982) studied the reversal positivity and contralateral negativity in patients with cortical lesions. They predicted that patients with defects in the geniculostriate pathway or area 17, the VEP would be totally absent, but those with defects in the extrastriate area would only have the contralateral negativity abolished. Their studies confirmed these predictions and confirmed that P100 was generated in the striate cortex and the contralateral negativity in the extrastriate areas.

Maier et al (1987) reported on their study in which they used a specific physical model to analyze the potential distribution of the VEPs to various stimuli such as pattern onset, offset, reversal, pattern motion and high frequency luminance flicker. Responses were recorded from 24 occipital derivations and these were examined using a three sphere conductance model to represent the head, with the assumption that activity from the underlying cortical is equivalent to a single dipole. Principle component analysis was used to find the dimensionality of the data space. From this analysis, it was concluded that all stimuli, evoked responses in the primary visual cortex, and that only pattern onset and to a lesser degree pattern offset and reversal yielded activity in the higher visual areas. In particular, it was shown that the CI, CII epoch of the pattern onset response has its origins in two different cortical regions. A fast positive (CI) - negative (part of CII) component arises from area 18 (or 19), and a slower negative (initial part of CII) component comes from
area 17. Their model was adequate to distinguish between activity of areas 17 and 18 as long as a restricted area of the visual field is stimulated. They indicated that to optimally study responses from area 17, high frequency luminance flicker, the appearance of a grid pattern or motion onset are needed. To study activity of area 18, one records CI to half-field stimulation, using a differential derivation from two electrodes 4.5 cms left and right of the midline, and both positioned 4.5 cms above the inion. Because of the symmetry of the area 17 component in pattern onset response, this method would yield mainly the area 18 component.

1.4 Aims

Pattern VEP stimulation has been widely utilised in normal subjects and in various clinical conditions, where it has proved useful in studying the visual system both in health and in disease. However, it is apparent from the description in the previous sections that studies have described differences in the property of VEPs elicited to different forms of pattern stimulation, predominantly pattern-reversal, and pattern-onset. Responses to these stimuli have been usually studied independently and it is not clear, if and how, the response components may relate to each other and represent activation of different visual mechanisms. Some VEP stimulus components, such as those to pattern-reversal stimulation have been more extensively studied than others, and have been described as indicating activation of different parts of the visual pathway, namely macular or paramacular. Other studies have attempted to characterise certain VEP responses and their behaviour in terms of reflecting different underlying physiological mechanisms, such as the parvo / magnocellular, and transient / sustained mechanisms.

This thesis is concerned with assessing properties and possible interrelationships between reversal, onset and offset pattern VEPs. Stimulus characteristics, such as pattern
size, stimulation field and contrast, were manipulated to explore the behaviour of major components of pattern-onset, -reversal and -offset VEP modes in healthy control subjects. The three stimuli were delivered sequentially in a single recording epoch so that a direct comparison could be made for virtually identical subject and recording conditions. Half-field stimulation was adopted to better separate and unmask the contributions from macular and paramacular areas of the visual field. A novel approach involving the manipulation of contrast change, so that an onset/offset VEP stimulus can be transformed into a reversal stimulus, through a succession of varying contrast and checkerboard phase steps was utilised to study the transitional changes between pattern-onset, -reversal and -offset components.

The behaviour of the different VEP components, their interrelationship and, possible physiological origins were thus described in control subjects. Pattern-onset, -reversal and -offset VEPs were then studied in a clinical population of amblyopic children. Stereodefective amblyopes were compared with normal children: the effects of pattern size and the extent of interocular interaction were investigated to gauge the effectiveness of the three stimulus modes in elucidating macular/paramacular function and binocular interaction.
2. Sequential Pattern - Onset, -Reversal and -Offset VEPs:

Rationale and Methodology

2.1 Introduction

Experimental and clinical VEP studies tend to use either pattern-reversal or pattern-onset. There are very few publications concerned with direct comparisons of VEPs elicited by these two stimulation techniques and these show that the morphology, distribution and sensitivity of the two VEP modes have important differences. All forms of pattern VEPs are influenced closely by variations in stimulus parameters, recording methodology and equipment settings.

Estévez and Spekreijse (1974) used 20' checks and systematically altered stimulus contrast thereby creating a sequence of stimuli that were intermediate between an onset/offset and complete pattern reversal. Their aim was to understand component relationships between onset/offset VEPs and reversal VEPs when contrast was changed in small steps. They concluded that the reversal VEP response represented an interaction between onset and offset responses, though it appeared to have a predominant contribution from offset. Jeffreys (1977) used a similar protocol and 15' checks, he concluded that the reversal response consisted of a composite of the onset CI potential and the main positivity (P110) of the offset response.

On the other hand, Skrandies et al (1980) concluded that the reversal and offset mechanisms were not closely related, as their topographical distributions were significantly different. They found that VEPs to large 1 degree checks were largest at different locations: responses to onset and reversal stimuli were more anteriorly located with respect to the inion, for upper than for lower hemi-retinal stimulation. For pattern-offset VEPs, an opposite spatial relationship was found, with VEPs being largest more posteriorly for upper than for lower
hemi-retinal stimulation.

Kriss and Halliday (1980) showed polarity reversal across a sagittal row of occipital electrodes. Lower field VEPs were larger than upper-field responses, for all three modes of stimulation, being largest 5cm above the inion. The upper field VEPs originate largely from the areas around the inferior surface of the occipital lobe, and electrodes at the scalp surface above the inion tend to pick up activity from tangentially orientated dipoles, whereas lower field VEP activity tends to be generated by radially orientated dipoles at the cortical surface immediately underlying these electrodes (Michael and Halliday, 1971). Kriss and Halliday (1980) used larger checks (50') and stimulus field (32 deg diameter). They recorded onset, offset and reversal VEPs to upper and lower half-field and to left and right half-fields. Waveform differences between the stimuli were not as varied as those obtained in the studies using small checksizes. It was concluded that, based on morphology, latency and topographical distribution, reversal and offset responses were similar. Kriss and Halliday (1980) described that onset gave an ipsilateral half-field response with a positivity at around 90ms (P90) and a larger contralateral positivity at around 105ms (P105). This onset contralateral P105 appears to be different to that described by reports in which the onset contralateral positivity is earlier (70-125ms) and associated with an ipsilateral negativity of approximately the same latency (Jeffreys, 1977; Lesèvre and Remond, 1972; Shagass, Amadeo and Rohmer, 1976). Kriss and Halliday attributed this difference to their use of larger checks and a wider stimulus field, implying that the P105 may have a paramacular contribution.

In this thesis, the three stimuli were delivered sequentially in a single recording epoch so that a direct comparison could be made for essentially identical subject and recording conditions. Half-field stimulation was adopted to separate contributions from macular and paramacular areas of the visual field. It is known that when the whole full-field is used,
components of opposite polarity, algebraically summate and are not detectable or become very
difficult to discern (Blumhardt and Halliday, 1979). In particular, those components recorded
from the contralateral side of the scalp to the stimulated half-field, namely reversal N105,
offset N115 and onset P105 are often not well seen for full-field stimulation.

Experimental protocols were varied in terms of key stimulus properties (checksize,
stimulus field, contrast change), in terms of subject population (healthy control adults and
children; amblyopes) and in terms of viewing condition (monocular vs binocular to assess
interocular interaction). The method of stimulus delivery was standardized to a sequential
half-field presentation of the three VEP modes in order to maintain near identical
experimental conditions for each subject, and for separating the ipsilateral and contralateral
components.

2.2 Recording Technique and Equipment

Occipital VEPs were recorded using EEG silver/silver chloride electrodes, attached to
the scalp with collodion. The electrode impedance was reduced by gentle skin scarification
to < 10kΩ. A seven-channel montage was used; this consisted of a transverse row of five
electrodes placed 5 cm above the inion and 5 cm apart, so that two electrodes were spaced at
5 and 10 cm to either side of the midline electrode. Electrodes were also placed at the inion,
and 2.5 cm above the inion. All occipital electrodes were referred to a common mid-frontal
reference (Fz). Figure 2.1 is a diagram showing this montage. Hobley and Harding (1988)
reported that for pattern stimulation a negativity around 100 ms can be present at a midfrontal
location. However, using this paradigm such activity was not conspicuous and furthermore
if present, would likely be a constant confounding factor.

Subjects were seated in a darkened room, on a height-adjustable chair. The forehead
was placed on a cushioned rest and the seat was set so that the subject's eyes aligned
symmetrically in the mirror stereoscope. Two oscilloscopes each subtending 24 degrees by 18.5 degrees were clamped together. These were Hewlett Packard displays (1321A, X-YDisplay; P4 phosphor; frame rate 100Hz interlaced; line rate 41.1kHz; 0.72ms flyback time, frame locked triggering). Subjects sat at a distance of 1 metre from the screens. The oscilloscopes were viewed through the stereoscope so that the subject's left eye viewed one oscilloscope and the right eye viewed the other oscilloscope (Figure 2.2).

Figure 2.1: Diagram showing 7 channel montage (all occipital electrodes referred to common mid-frontal reference at Fz), and typical pattern-onset -reversal and - offset responses to 15' checks. Ipsilateral and contralateral VEP components are indicated with arrows and were measured from channels 2 and 4 respectively.

This experimental set up was used for the studies reported in chapters 3 (checksize effect), 6 (comparison of normal and amblyopic children) and 7 (interocular interaction
studies). For experiments described in chapters 4 (effects of scotomata) and 5 (step contrast change), only one oscilloscope was used and the techniques are described in greater detail in the relevant chapters.

For experiments described in chapters 3, 4 and 5, signals were recorded and averaged using a Medelec Sensor ER94A (Medelec Ltd. UK). Input sensitivity was 20μV per cm display (gain). Band pass filtering was 1Hz (-3dB) to 125Hz (-6dB.) and digitally sampled at 500 samples per data epoch.

For part of the experiments in chapter 3 and those in chapters 6 and 7, an averaging system based upon a PDP11 computer was used and each input signal was amplified with a time constant of 1 s and a high frequency response less than 3dB at 80Hz. The input was digitised with a sampling rate of 0.5 points/ms.

Data was stored on a PC-computer. Waveform measurements, plotting and group averaging were carried out using the 'Filer Program' written by Dr. Mike Hayward.

2.3 Stimulus

Black and white checkerboard patterns were presented in the left half-field (0-12 degrees) of each oscilloscope screen; the right half consisted of a uniform grey field of the same average luminance as the checkerboard. A small ring (diameter subtending 1.5 degrees) at the centre of the vertical border of the pattern/blank interface provided a fixation spot. The luminance levels were 11.5cd/m² for white squares, and 0.004cd/m² for black squares and were constant across checksizes.
Figure 2.2: Photographs of the experimental set up showing subject seated in front of the two oscilloscopes and mirror stereoscope (top picture), and the fused image of the oscilloscopes viewed through the mirror stereoscope (bottom picture).
Several studies (e.g. Barrett et al., 1976; Blumhardt and Halliday, 1979) have established that half-field testing is effective in separating macular and paramacular responses, at least for pattern reversal stimulation, and that there are no significant amplitude and latency differences when comparing left and right half-field VEPs for each eye.

VEPs to pattern-onset, -reversal and -offset were acquired in a single epoch, lasting 900ms. A 20ms pre-stimulus interval preceded the stimulus sequence, after which the checkerboard appeared for 300ms, was then replaced by the complementary pattern which stayed on for a further 300ms (i.e. reversal), and then disappeared for 300ms (pattern-offset). A 300ms interval for each stimulus presentation was chosen for recording each of the three VEP complexes, as this avoids the contamination and interaction of VEPs from one stimulus mode with the next. An average of 100 such sequences was recorded for each checksize and viewing condition. When necessary averaging was repeated (e.g. if subject was attending poorly, or responses were corrupted by myogenic artifact). Although 3 responses were generated over a 900ms epoch, previous studies (Kriss et al., 1984), and pilot studies associated with the present work did not indicate that the responses overlapped to approach steady state stimulation.

2.4 VEP Measurements

Left half-field VEP stimulation was used. In general, ipsilateral and contralateral, half-field occipital VEPs were best seen in the 5 cm lateral channels (this agrees with the data of Blumhardt et al., 1979) Therefore, components were measured from the electrodes 5cm to the left and to the right of the midline (Fig. 2.3). The following VEP components were identified and measured: for pattern-onset, ipsilateral CI, CII, CIII and contralateral P105; for reversal, the ipsilateral N80, P100, N145 and contralateral N105; and for offset, the ipsilateral N85, P110, N165 and contralateral N115. Component recognition was based on their polarity.
and latency (see Jeffreys, 1971; Halliday, Barrett, Blumhardt & Kriss, 1979 and Kriss & Halliday, 1980, for details regarding component identification). Peak latency and peak-to-peak amplitude measurements were made for each of these components (figure 2.3). Component amplitudes were measured peak-to-peak instead of from a baseline as there was a significant amount of baseline shift for the recording runs which would have influenced amplitude measures.

Figure 2.3: Left half-field ipsilateral and contralateral pattern-onset, -reversal and -offset components as recorded from channels 2 and 4. Certain ipsilateral components are indicated by arrows to illustrate their relationship to the contralateral components.
3. Effects of Checksize

3.1 Introduction

Several authors have reported that the largest VEPs are produced by checks of 10'-20' when a macular field of around 4-6 degrees diameter is used (Spekreijse et al., 1973; Regan and Richards, 1971; Kriss et al., 1984). The spatial tuning curve of the reversal VEP amplitude tends to show a single peak with amplitude increasing progressively, maximal for checksizes between 10' and 20' (Regan and Richards, 1971). However, the spatial tuning of either pattern-onset or -offset VEPs, appear to be more complex. Spekreijse, van der Tweel and Zuidema (1973) found that checkerboard onset responses had a bandpass characteristic tuned to checks of about 15', whereas the offset response had a more low-pass characteristic, and was minimally influenced by checksize. It was found that spatial tuning results were affected by stimulus contrast. Smaller checks needed to be of greater contrast to produce similar amplitudes to those of larger checksizes. Kriss et al (1984) compared pattern-onset, -offset and -reversal VEP and found that both pattern reversal and offset responses showed shorter latencies with larger checks, whereas pattern-onset responses did not show this trend.

Responses to both the reversal and onset stimulation using sinusoidal gratings have also been described (Kulikowski, 1977; Parker and Salzen, 1977; Plant, Zimmern and Durden, 1983). The peak amplitude to both VEP modalities occurred at a lower spatial frequency with increasing field size, and this was postulated to reflect the increasing size of the receptive fields further away from the fovea. Plant et al (1983) found that the onset response was largest at a higher spatial frequency than the reversal VEPs and that the onset VEP is dominated by an early negativity (70-100 ms) rather than by the subsequent positivity at frequencies greater than 2 c/deg. Peak latencies of both onset and reversal responses were found also to increase for spatial frequencies higher than 2 cycles/degrees (Plant et al 1983).
It was suggested that the departure from a monotonic relationship at spatial frequencies lower than 2 c/deg was the result of an interaction between the two components. This suggestion supports observations by Jones and Keck (1978), that the main positive component of the VEP to low spatial frequency gratings has contributions from two components. The double peak (called P1_A and P1_B respectively) was not found in all subjects, and for some subjects larger field sizes and lower spatial frequencies (< 1c/deg) were needed to demonstrate the contribution of the two components. Jones and Keck suggested that the earlier P1_A wave reflected activity of the transient system and that it was associated predominantly with peripheral retinal stimulation.

The aim of this first study was to compare the effect of changing checksize on the ipsilateral and contralateral VEP components evoked following half-field sequential pattern-onset, -reversal and -offset stimulation. This sequential recording method was used as a direct comparison of the three modes of stimulation can be made for essentially similar subject and recording conditions, thus allowing control for a number of important physiological and psychological variables, including fixation, accommodation, level of background EEG and EMG activity, attention and mental set. It is well established that half-field stimulation gives ipsilateral and contralateral responses and that the two half-fields algebraically summate for full-field stimulation (Blumhardt and Halliday, 1979). Half-fields are not exact mirror images as neuroanatomical representations in each hemisphere are not exactly the same. Half-field stimulation allows separation of macular and paramacular contributions and varying checksizes is expected to enhance these differences, it is thus better to perform half-field VEP studies.
3.2 Subjects and Methodology

Eleven normal subjects were recorded in this study. All had corrected Snellen acuities of 6/6 or better, with no history of clinical visual problems. There were 5 males and 6 females. Age range was 25 to 38 years (mean 31 years). One of the eleven subjects (male, aged 32 years) was recorded for full-field as well as left and right half-field stimulation to illustrate the occipital distribution of the components.

The recording technique, equipment and VEP measurements employed in this study are as described in chapter 2. Ten sizes of black and white checks were presented and these subtended: 6, 9, 12, 15, 18, 21, 35, 50, 80 and 110 minutes of arc at the subject's eye. Binocular, left and right eye responses for each of the ten checksizes were recorded; the test order was randomly varied from subject to subject.

3.3 Results

Half-field, pattern-onset stimulation elicits a triphasic complex which is best seen at the lateral occipital channels ipsilateral to the stimulated half-field (figure 3.1). The contralateral channel is dominated by a single major positive component, around 105ms, which can be labelled the contralateral P105 (Kriss et al 1984). Pattern-reversal and pattern offset responses are characterized by a negative-positive-negative triphasic complex. The components of the pattern-offset VEP (N85, P110 and N165) are generally 5-20ms later than those obtained with pattern reversal stimulation (N80, P100 and N145). For both reversal and offset, the triphasic complex becomes lateralized on half-field stimulation to the side of the scalp ipsilateral to the stimulated half-field. The contralateral channels, also show a triphasic complex but of opposite polarity (i.e. PNP). The negativity is considered to be the major contralateral component and has been labelled N105, for pattern-reversal response, and N115 for pattern-offset response (Halliday et al, 1979).
Figure 3.1: Full and half-field VEP responses to pattern-onset, -reversal and -offset stimulation as recorded from the mid-occipital and two lateral channels 5 cm. to the left and right of the midline (all referred to Fz). Arrow shows the reversal P100 component to illustrate its distribution with full and half-field stimulation.

Recordings were obtained with each eye viewing in turn, hence a multivariate analysis of variance (MANOVA) was conducted to ascertain whether viewing order influenced the VEP parameters with respect to checksize. No significant effect of checksize by viewing condition was present (F=1.4, p=0.88), nor was there a significant effect of viewing condition alone (F=0.23, p=0.15). Checksize, on the other hand, showed a markedly significant effect (Pillais test, F=2.1, p<0.001). Because of the absence of differences between the left and right eyes and the lack of any significant effect of viewing condition on the VEP parameters, further experiments and analyses in this thesis were carried with the left eye viewing for all our normal control subjects, with the exception of studies involving comparison of normals and amblyopes- (chapter 5), and those involving normal and abnormal interocular interaction- (chapter 6).

The group average (11 subjects) ipsilateral and contralateral VEP responses to all ten checksizes are presented in figure 3.2. A clear effect of checksize on amplitude for certain components can be observed.

MANOVA of left eye VEP measures with checksize were essentially in line with the findings reported above: a significant effect of checksize was present (Pillais test, F=2.0,
p<0.008). Univariate analyses showed checksize to significantly influence the amplitudes of onset CI (p<0.02), CIII (P<0.03) and reversal N145 (p<0.03), and, the latencies of onset CII (p<0.04) and contralateral P105 (p<0.03), reversal N80 (p<0.002), P100 (p<0.001) and N145 (p<0.008), and, offset N85 (p<0.003) and P110 (p<0.007).

Figure 3.2: Group average ipsilateral and contralateral VEP responses for the control subjects across all checksizes. Some components have been labelled for responses to 9' checks. Contralateral reversal N105 and offset N115 are labelled for responses to large 110' checks, where they are better defined.
3.3.1 Pattern-Onset VEPs

It can be seen from figure 3.2 that pattern-onset CII is relatively large, whereas CI is small when small checksizes are used. As checksize increases, CI enlarges and CII decreases in amplitude. CII amplitude was greatest at the smallest checksizes, with a maximum mean amplitude occurring for 9 minute checks (2.8 ± 1.9μV.). Thereafter, CII amplitude decreased gradually reaching mean minimal amplitude for the largest 110'' checks (1.9 ± 1.1μV.), however these changes did not reach statistical significance (see figure 3.3).

As mentioned above, univariate analysis showed pattern-onset CI and CIII amplitudes to be significantly influenced by checksize. CI amplitude remained fairly unchanged as checksize increased from 6' to 50' (mean 1.6μV), however after this, it increased fairly rapidly reaching a maximum mean amplitude for the largest 110' checks (3.33± 2.4μV). This component shows the opposite trend to the other onset potentials - its amplitude increased at checksizes greater than 50', whereas the other components all decreased in amplitude with checksizes greater than 50'. To identify more specifically which checksizes caused the most marked changes an analysis of variance, with post hoc Bonferroni adjustment, was carried out. The largest onset CI amplitude was elicited by the largest checksize (110'), and this was significantly different (F=2.73, p<0.008) from amplitudes to 12', 21' and 50' checks.

CIII amplitude shows a bimodal distribution with two amplitude maxima (see figure 3.3): a steep increase in amplitude from 6' checks reaching the first peak at 9' checks (6.3± 3.2μV), amplitude is attenuated for 12' and 15' checks and increases, reaching the second maximal amplitude plateau which spans from 35' to 80' checks (6.8± 2.2μV), after which the amplitude drops again.

The contralateral P105 component, shows a fairly steady increase in mean amplitude with increasing checksize (with smallest 6' checks, mean=5.1± 3.7μV) and reaching a maximum value at 50' checks (8.9± 5.3μV), after which it appears to decline. It is interesting
to note that the P105 waveform appears to alter with the larger checksizes (≥ 35') the
positivity is sharply defined for checksizes 6' to 21', however with checksizes 35' and greater,
the P105 appears broadened and on some occasions it appears bifid (figure 3.2).

\[\text{Figure 3.3: Graph showing mean onset component amplitude changes with checksize}\]

\[\text{Figure 3.4: Graph showing mean onset component latency changes with checksize}\]

Latencies of pattern-onset components showed less conspicuous trends (figure 3.4). Univariate analysis showed onset CII latency (p<0.04) and contralateral P105 latency (p<0.03) to be significantly influenced by checksize. For onset CII, there is a slight decrease in latency between checksizes 6' and 18', but subsequently, latencies gradually increase between 21' and 110' checks.

Analysis of variance with post hoc Bonferroni adjustment showed the contralateral onset P105 latency to 80' checks to be significantly prolonged compared with VEPs to 18' checks (F=2.52, p<0.02). This prolonged P105 latency for large checks is due to the broadening of the positive waveform (figure 3.2), particularly as latency measures are taken from the mid-point of the component. This broadening of the P105, may in fact represent the emergence of a second later positive component (around 130ms) which leads to the appearance of a bifid waveform on some of the recordings (e.g. P105 component to pattern-onset using 35' and 110').
CIII mean latency differs slightly from the other components in that after the initial decline (from 6' to 10' checks) which is seen in all components, there is a second phase (with checksizes 18' and larger) where latency increases reaching a maximum at around 35' checks (see figure 3.4). This bimodality in the distribution of CIII latencies with checksizes is similar to the bimodal amplitude distribution for this component which was described above. This is suggestive of two separate underlying mechanisms which are reflected in the onset CIII, one dominating at small checksizes (macular) and the other at large checksizes (paramacular).

3.3.2.2 Pattern-Reversal VEPs

The univariate analysis showed that only reversal N145 amplitude was statistically significantly influenced by checksize (p<0.03). Both ipsilateral P100 and N145 components showed similar trends of increasing mean amplitude with increasing checksize (figures 3.2 and 3.5): Reversal stimulation using the smallest 6' checks gave the smallest amplitudes (mean P100=2.5± 1.2μV; mean N145=3.5± 1.8μV), however, these increased gradually reaching maximum values at 50' checks (mean P100=4.8± 2.8μV; mean N145=6.5± 2.2μV). P100 and N145 amplitudes showed a relatively marked decrease with checksizes greater than 50'. Analysis of variance with post-hoc Bonferroni adjustment indicated that N145 amplitude was greatest to 50' checks and this differed significantly from those obtained to the smallest 6' checks (F=2.09, p<0.04).

The ipsilateral N80 component did not show significant amplitude trends with checksizes, although figure 3.2 shows N80 to be larger with the smaller checksizes (9' to 15'). The contralateral N105 component was similar to the ipsilateral P100 and N145 potentials, in that the smallest checks (6') produced the smallest components (mean=2.1± 1.1μV) with amplitudes increasing thereafter. However, whereas P100 and N145 reach a maximum at 50'
checks and then decrease in size, the N105 increases in amplitude with increasing checksize and the largest mean response was obtained to the largest 110' checks (4.1±1.5μV).

![Figure 3.5: Graph showing mean reversal component amplitude changes with checksize](image)

![Figure 3.6: Graph showing mean reversal component latency changes with checksize](image)

Latency changes for the reversal components, all showed a similar trend with checksize: as checksize increased from 6' to 35' checks, latency decreased. The P100 latency continued to decrease in latency with increasing checksizes greater than 35', however, the N80, N145 and contralateral N105, all showed increasing latencies for checksizes greater than 35' (figure 3.6). These trends with checksize were statistically significant for all the ipsilateral reversal components. Both the N80 and P100 components had the longest latencies to the smallest checks, with a highly significant difference between the two smallest checksizes (6' and 10') and latencies to 110', 80' and 50' checks (N80, F=5.8; P100, F=5.50; both p<0.0001). The N145 latency, on the other hand, showed significant differences between the smallest 6' checks, which gave the most prolonged latencies and that to 35' checks, which gave the shortest latencies (F=2.41, p<0.02).
3.3.2.3 Pattern Offset VEPs

It can be seen from figure 3.2 that the pattern-offset N85 component is better defined for the smaller checksizes (< 21'). Checksizes of and greater than 35' elicited very poorly-formed and attenuated N85 components. This offset component showed the most conspicuous change with checksize, even though it did not reach statistically significant levels.

The ipsilateral N85, P110 and N165 showed similar trends, that is, tuning effect for the small and moderate sized checks (6' to 50') - see figure 3.7. There was an increase in amplitude with increasing checksize, reaching maximum values at 15' checks (mean N85= 2.6± 1.4µV; mean P110= 3.5± 1.6µV; mean N165=3.8 ± 2.4µV). Further increase in checksize resulted in a decrease in amplitude for all three VEP components. This decrease was essentially continuous for the P110 component reaching minimum values at the largest 110' checks (2.6± 1.8µV). The N85 component also showed a steady decrease in amplitude, however, a minimum was reached at 80' checks (1.7± 1.4µV). Conversely, N165 components showed an increase in amplitude with increasing checksize, with maximum mean values obtained to the largest 110' checks (4.1± 2.4µV). The contralateral N115 component was less
variable with checksize. Mean amplitudes showed a steady increase from 6' checks (2.5±1.7μV) to the largest 110' checks (3.5 ± 2.3μV). All these amplitude changes with checksize did not reach statistically significant levels.

Latency changes with checksize showed an essentially similar general picture for all the components (figure 3.8). After an initial increase in latency from checksizes 6' to 10', there was a gradual decrease in latencies until the minimum was reached. For ipsilateral N85, and contralateral N115, this minimum occurred at 18' checks; for P110, it was at 35' checks and for N165 it was 21' checks. After these minimal mean latency values, increasing checksize resulted in more prolonged latencies. The univariate analysis showed that of the offset components only N85 (p<0.003) and P110 (p<0.007) components were significantly affected by checksize. Analysis of variance with full Bonferroni adjustment showed that the P110 latency differed significantly between 10' checks (most prolonged) and both 50' and 80' checks (least prolonged) (F=3.64, p<0.0007).

In summary, of the 12 components measured across the three modes of stimulation, latencies of 7 components were significantly affected by checksize, whereas only 3 components showed significant amplitude change. An overall comparison of the three modes of pattern stimulation shows that pattern-reversal VEPs are more consistently influenced by checksize than pattern-onset or offset VEPs. All three ipsilateral reversal components, in terms of both amplitude and latency, were affected by checksize, and to a marked degree of significance. Offset components were the least affected with only latencies of N85 and P110 being significantly influenced by checksize; all onset components were influenced by checksize, however the levels of significance were low (p< 0.02 to 0.04) compared with the reversal components (the majority p<0.002).
3.4 Discussion

The marked influence of checksize on VEP component amplitude and latency found in this study is for the most part in keeping with other published reports. However the majority of these reports, confined their analyses to the major component of the VEP complex obtained with each stimulus mode (P100 for reversal, P110 for offset and CI for onset). By using half-field stimulation and analysing the different components of each VEP complex, the inter-component relationships can be investigated and their possible physiological origins can be explored.

The amplitude changes with checksize of the onset components are in agreement with the findings of Jeffreys and Axford (Jeffreys, 1971; Jeffreys & Axford, 1972a,b). These authors have suggested that onset components have different origins: CI and P105 are postulated to be of striate origin, whereas CII and CIII have an extrastriate origin. These conclusions were derived from assessing the relationship of retinal location stimulated to the amplitude distribution of VEP components. Stimulation with a foveal 1 degree field was found to contribute mainly to the CII component, and minimally to the CI and P105 components, which appeared to be primarily derived from the perimacular 2-6 degree region of the field (Jeffreys & Axford 1972a). CII and CIII have been found to be less dependent on contrast and more on pattern detail, as their amplitudes correlated with the subjective impression of the focal clarity of contours (outlines) of the pattern elements. CI on the other hand, has been found to be large for the more marked changes in stimulus contrast.

CI amplitude demonstrated little change when comparing small and moderate sized checks (> 50'). However, for checks greater than 50', a steady increase in amplitude was evident. Larger checks imply less pattern detail (i.e. edges, corners and borders) and a greater effect of luminance change. This trend with checksizes of 50' and greater, is in contrast to the observed decrease in amplitude of the other onset CII, CIII and P105 components. CII
appears to be a primarily macular-derived component, and was largest with 10' checks, deceasing progressively in amplitude with increasing checksize. Kriss et al (1984) found CII and CIII to be largest with 18' checks, whereas CI was maximal when the large, 72' checks were used.

The behaviour of onset CIII component is more complex. For the smaller checksizes (<21'), CIII and CII show similar trends (even though CIII amplitudes are approximately 3 times that of CII) being of maximal amplitudes around 10' checks (figure 3.3). With checks larger than 18', the CIII amplitude again increases in amplitude and reaches a second amplitude maximum around 35-80' check. This bimodal distribution of CIII amplitude with checksize may reflect two different mechanisms: a pattern specific, macular-derived contribution (elicited by smaller checks), which is likely to be of extrastriate origin agreeing with Jeffreys' suggestion (1977), and a predominantly luminance-derived contribution (obtained with larger checks), which may possibly be of striate origin.

The findings for the onset contralateral P105 component were interesting. This component was consistently the largest peak of all the onset responses, whatever the checksize. Previous pattern-onset studies have not emphasized this major component, and mainly confined their analyses to the ipsilateral CII. The largest P105 responses were found when using moderately sized checks (50'), which does not suggest a purely macular-origin. The fact that P105 was largest to the 50' checks may be a reason why studies using smaller checksizes did not describe such a main positivity (Jeffreys, 1977; Shagass et al 1976). The broadening of the P105 waveform with checksizes ≥35' may be due to the emergence and amalgamation of another positivity occurring about 20ms after the original P105 component. Evidence for this suggestion stems from the observation that for some checksizes (e.g 110') a bifid waveform is seen, where the first limb of the waveform appears to correspond to the well-defined P105 positivity obtained with small checks, whereas the second positive limb of
the waveform is a novel component specific for large checks. Thus, this latter part of the waveform to large checks may represent a luminance-specific, paramacular constituent of the P105 response and the first part of the component may represent the macular-derived, pattern-specific constituent which can be traced down to the response recorded to the smallest checksizes (figure 3.2). This finding is reminiscent of the observations of Jones and Keck (1978) who described a double peaked reversal VEP to low spatial frequency gratings, in some of their subjects.

The macular representation is at the posterior occipital pole. Activation of this area in which neurons are mainly radially orientated, is associated with activity which is picked up in the midline and adjacent contralateral cortex (Blumhardt et al., 1978; Brecelj and Cunningham, 1985). The discrete localization of P105 and its sensitivity to small checks, suggest that it results from macular activation. However, there also appears to be another later contribution to P105 which is best seen when stimulating the paramacular area with larger checks. It is not clear at present if this is due to a slower conducting pathway or reflects activation of different cortical areas.

The reversal VEP has been more extensively studied compared with other pattern VEP modes. Spekreijse et al (1973), Regan and Richards (1971) and Kriss et al (1984) reported that the largest responses are evoked by checksizes which ranged from 10' to 20'. Reversal P100 amplitude decreases in parallel with the reduction in the acuity level. A foveal checkerboard stimulus of 12' checks in particular has been shown to be markedly sensitive to induced refractive errors (Collins et al, 1979). Mauguière et al (1982) compared reversal VEPs to 44', 22' and 5.5' checks and found that 5.5' VEPs were very small and variable in normals. They also found that the P100 amplitude did not otherwise vary with checksizes, although latency was longer for the smaller checksizes. However, the data in this study indicate that the largest mean P100 amplitudes were obtained between 12' and 50' checks,
with only a difference of about 1μV in the mean P100 amplitudes between these checksizes. 50' checks gave the largest mean response, but these changes with checksize were not significant.

There is disagreement regarding the properties of the reversal N80. Several studies have found N80 is enhanced by small checksizes (Halliday et al., 1979, Kurita-Tashima et al., 1991, Bodis-Wollner et al., 1992) and that it is selectively attenuated when macular function is poor (Shawkat et al., 1993). However, Yiannikas and Walsh (1983) reported a progressive reduction in N80 amplitude with reduction of the stimulus field; it becomes undetectable in the majority of subjects when the stimulus field was of 4 degrees diameter or less. Furthermore it was found that a central scotoma of 8 degrees did not affect this component. In this study the N80 reversal component was largest with small 9'-15' checks (figure 3.2).

Offset VEPs have been reported to have similar properties to reversal VEPs (Estévez and Spekreijse, 1974; Kriss et al, 1984), although not as strongly influenced by checksize (Spekreijse, van der Tweel and Zuidema, 1973). The offset VEP results from this study showed less amplitude variation with checksize, compared with onset and reversal VEPs and this was not statistically significant. Interestingly, the largest mean amplitudes for the main positivity (P110) occurred with 15' checks. This is similar to reported findings for pattern-reversal, where amplitudes were largest between 10' and 20' checks (Spekreijse et al., 1973; Regan and Richards, 1971; and Kriss et al., 1984).

Offset contralateral N115, on the other hand, did show similar trends to the reversal contralateral N105. Both components increased in amplitude with increasing checksize, and were largest when the largest checksizes were used. This behaviour suggests a paramacular origin for both components. The reversal N105, at least, has been found to be accentuated or 'unmasked' when the central field was progressively occluded and the stimulus becomes essentially paramacular (Blumhardt et al, 1978). It is known that the peripheral parts of the
visual field are represented deeper in the anterior parts of the calcarine sulcus. Electrodes at
the scalp, over the contralateral hemisphere to the field stimulated are advantageously placed
to pick up activity of the negative end of the dipole when peripheral parts of the visual field
are stimulated.

The checksize that produces the highest amplitude VEP is progressively greater as
more peripheral retina is stimulated. Studies have shown that larger checks (>60') produce
largest responses when the peripheral, rather than macular field, is stimulated (Harter, 1971;
Todd Meredith and Celesia, 1982). Behrman et al., (1972) observed that for 9' checks, the
maximum response was obtained with 1.49 degree diameter fields, for 16' checks, the
maximum was obtained with 6 degree fields and for 35' checks , the maximum was obtained
with 18 degree fields. This observation ties in with the evidence that visual acuity
deteriorates progressively away from the fovea (20/50 at 1.5-4.5 degrees; 20/100 at 4.0-11.5
degrees and 20/200 at more than 9.0 degrees) (Harter and White, 1969). The relative
insensitivity of the periphery to small checks is ascribed to the decreased density of cones and
greater convergence of peripheral cones onto ganglion cells away from the fovea (Schein,
1988). Compared with the fovea, peripheral retina is represented by relatively smaller cortical
areas. This has been quantified by the cortical magnification factor, which is defined as
millimetres of cortex per degree of visual angle which decreases with increasing retinal
eccentricity (Daniel & Whitteridge, 1961, Rovamo and Virsu, 1978; Tootell et al., 1982, Van
Essen et al.,1984).

Pattern evoked potentials have a contribution which is luminance dependent. As
checksize increases, the contribution from luminance dependent mechanisms increases
proportionally (Van der Tweel and Spekreijse, 1968; Kulikowski, 1977; Bodis-Wollner and
Hendley, 1977). It has been suggested (Jeffreys, 1977) that two mechanisms are involved in
the generation of pattern VEPs: a mechanism involving the detection of sharp edges, and a
second one which involves the detection of local luminance changes. VEPs to small checks sizes predominantly reflect the edge detection mechanism, whereas VEPs to larger checks (>40') contain predominantly a contribution of the luminance mechanism.

Movement mechanisms also contribute to the pattern reversal VEP. Kulikowski (1977, 1978) suggested that large pattern-reversal VEPs mainly reflect motion processing mechanisms, whereas with stimuli at spatial frequencies higher than 3c/deg, both pattern and motion processing systems contribute to reversal VEPs. He based his assertions on the psychophysical evidence that a reversing pattern has two distinguishable thresholds: one for detection of motion or flicker, the other for the detection of the pattern. Kulikowski considered VEPs to a grating of low spatial frequency to be related to a transient neural mechanism (magnocellular mechanism) as such a VEP depends only on the change in contrast. Van der Tweel and Spekreijse (1968) also suggested that large checkerboard reversal VEPs have two constituents related to the sharply focused edges of the pattern and secondly those related to changes in local luminance inside individual checks. This local flicker contribution is more evident in large check VEPs than in small check VEPs (reflecting magnocellular activity). The experimental evidence for this suggestion stems from the fact that reversal VEPs were abolished when the sharply focused edges of the checks were occluded by fine lines, but this occurred only when the checks were small (reflecting parvocellular activity).

Studies that compare checkerboard reversal with motion-onset stimuli have shown that VEPs from both modes are similar, in that they consist of a negative/positive/negative complex, the latter presumably reflecting primarily motion / magnocellular mechanisms (Kuba and Kubova, 1992; Kubova et al, 1994). However, whereas the positivity (P100) dominates the reversal component, motion-onset VEPs were dominated by the second negativity (N145) with a latency of 160-200ms (labelled N2 by the authors). It was found that whereas the P100
component decreases in amplitude with decreasing contrast, N145 amplitudes for both reversal and motion-onset VEPs did not vary significantly with contrast, above a contrast of 1.3%. It was also found that peak latencies increased with decreasing contrast and again this was more pronounced for P100 than for N145 for both reversal and motion-onset VEPs. These findings are in agreement with those of Müller and Göpfert (1988) that N145 arises from neurones that have high contrast sensitivity, and thought to reflect input from the magnocellular pathway.

The scalp distribution of motion-onset VEPs tend to be largest in the lateral channels and symmetrical about the midline for full-field stimulation (Clarke, 1973; Göpfert et al., 1988; Spekreijse et al., 1985). Although there has been a study of motion-onset VEPs to nasal and temporal half-field stimulation, no comment was made regarding occipital lateralization (Göpfert et al., 1991). Kubova et al (1994) have interpreted the occurrence of a maximal negativity motion-onset VEPs in the lateral occipito-temporal channels as possibly reflecting their origins from the motion-processing area MT (V5, Zeki, 1990). The pattern reversal N145 is also commonly largest at the lateral occipital electrodes and together with its behaviour to contrast changes, makes it remarkably similar to the motion-onset negativity. Thus the reversal N145 may also be related to motion and be of extrastriate origin. However, the positivity of both motion-onset and reversal VEPs are highly contrast dependence and are undetectable at low contrasts. Kubova et al (1994) thus suggest their possible derivation from the pattern-processing system and the striate cortex with inputs from the parvocellular pathways.

Latency has greater sensitivity to checksize than has amplitude as more component latencies were significantly affected by checksize. All ipsilateral reversal components, offset N85 and P110 and onset contralateral P105 were significantly affected by checksize. These results for reversal and offset VEPs support the findings of Kriss et al (1984) who reported that all reversal ipsilateral components, as well as offset N85 and P110 had shorter latencies.
when elicited by large checks (checksizes used were 9', 18', 36' and 72'). Kriss et al also found that onset CI and CII latencies showed no systematic change with checksize. The onset contralateral P105 (not investigated by Kriss et al) was the only onset component to show a checksize effect: it was significantly more prolonged when using large checks (>18') compared with latencies with smaller checks (<18'). However, as mentioned previously, this may be a 'pseudo-latency increase': larger checks evoked broad and sometimes bifid P105 components suggesting the emergence of another, later component, which combines with the pre-existing P105 to give a broad positivity, whose mid-point is taken as the latency measure.

The decrease in latency with increasing checksize for the above VEP components, suggests that the larger checks may be activating mechanisms associated with faster optic nerve fibres. VEPs to larger checks imply a greater contribution of luminance-dependent mechanisms as opposed to pattern-specific, edge detection mechanisms. It is also interesting that latencies appear to be more influenced by checksize than amplitude. It is known that amplitudes show more inter and intra-individual variability than latencies (Halliday, 1982; Stockard et al, 1979) and this could explain the greater statistical significance found for latencies. On the other hand, latencies reflect different physiological origins and mechanisms than amplitudes: latencies are determined by conduction along the post-retinal visual pathway, whereas amplitudes can be influenced more locally (e.g. at the cortical level), and could be a reflection of temporal dispersion, area of cortex activated and depth at which cortex is activated.

The greater effect of checksizes on latencies compared with amplitudes, the decrease of latencies with increasing checksize and the relative insensitivity of components known to be of paramacular origin or to be contrast dependent, such as contralateral reversal N105, offset N115 and ipsilateral onset CI, may be all explained by the differential activation of the magnocellular as opposed to the parvocellular pathways. Larger checksizes would
preferentially activate the larger M-ganglion cells in the retina that have larger receptive fields and conduct faster to the magnocellular LGN cells. Magnocellular LGN cells respond in a transient fashion with fibres that conduct at relatively fast velocities to the cortex (Schiller and Colby, 1983). Parvocellular cells however, respond in a sustained fashion, conduct at relatively slower velocities to the striate cortex (by about 4ms. - Lennie et al., 1990) and are involved with perception at high contrasts and high spatial frequencies. The slower conduction velocities of the sustained, higher spatial frequency, pattern-specific, parvocellular pathways would explain the more prolonged latencies for the smaller checksizes, particularly involving those VEP components known to be of macular origin. Larger checksizes elicit a stronger contribution from luminance mechanisms which lead to the magnocellular pathways being preferentially activated, and this may explain their shorter VEP latencies.

3.5 Conclusions

VEP amplitude and latency changes with varying checksize have highlighted differences and relationships between components and between the three modes of stimulation. These differences, and the behaviour of components may be understood in terms of what is known about mechanisms at the cellular level. Predominantly pattern-dependent, macular-derived components are accentuated by smaller checksizes, whereas luminance-dependent and paramacular-derived components are likely to be enhanced by larger checks. Small checks lead to an increase in pattern-specific stimulation and activate parvocellular pathways, whereas large checks result in reduced numbers of pattern elements for a given retinal area with increased contributions from mechanisms which preferentially activate magnocellular pathways.

The significant decrease in latency with increasing checksize for ipsilateral reversal
and offset components suggest that for the larger checks, they have a predominant contribution from the faster conducting magnocellular pathways. At the smaller checksizes, amplitudes for these components tend to be greater though latencies more prolonged, thus suggesting the predominant contribution of the slower conducting, pattern-specific parvocellular pathways. These reversal and offset components therefore appear to be influenced by both magno- and parvocellular mechanisms. Onset CI is also significantly influenced by checksize, but the opposite is found; amplitudes and latencies are greatest when using large checks. This suggests that CI is mainly a luminance-dependent component with relatively short latencies with smaller checks when compared to other VEP potentials and thus likely to be activating the magnocellular pathways. Although not reaching statistical significance, onset CII appears to be a macular-derived response, being largest with small checks, whereas CIII with its bimodal amplitude and latency distributions for checksize may well represent contributions from both magno- and parvocellular pathways.

The contralateral reversal N105 and offset N115 appear to have similar properties, suggestive of paramacular origin as they were maximal in size with the largest checks. Contralateral onset P105 however, appears to reflect both macular and paramacular components, as it remains a fairly prominent component through all the checksizes with the possible appearance of a second, later peak with larger checksizes, which is likely to reflect paramacular contributions.

Comparing the three modes of stimulation, the effects of checksize in accentuating macular/paramacular origins and magno/parvocellular contributions, appear to be best seen following pattern-reversal stimulation.
4. Effects of Experimental Scotomata

4.1 Introduction

Previous studies have demonstrated that for all three forms of pattern stimulation, VEP components are topographically related to the stimulated areas of the visual field. Pattern-reversal VEPs to half-field stimulation show a marked occipital asymmetry, when recorded using a transverse chain of occipital electrodes and a common reference montage. The asymmetry is characterised by the main positive component (P100), forming part of a triphasic negative-positive-negative (NPN) complex, and is distributed over the ipsilateral scalp to the stimulated half-field (Barrett et al., 1976). This apparently paradoxical distribution is thought to reflect the orientation of the generator neurones in the postero-medial aspect of the visual cortex. A smaller triphasic complex, which appears to be of opposite polarity (PNP), is often seen over the side of the scalp contralateral to the stimulated half-field (Halliday et al., 1979).

Pattern VEPs to foveal half-field stimulation (2 degree radius) appear to have considerable inter- and intra-individual morphological variability. There is a tendency for the NPN complex to be recorded over both the ipsilateral and contralateral sides of the scalp; thus the PNP paramacular complex found on the contralateral scalp with wider half-field stimulation is not usually evident with small field stimulation (Harding et al., 1980; Brecelj and Cunningham, 1985). The occipital symmetry around the midline of the foveal VEPs to half-field stimulation can be explained by the predominant activation of the macular representation area at the occipital pole. When using wide half-field stimulation which involves paramacular areas, the macularly-derived NPN complex associated with activation of the occipital pole and seen contralaterally, becomes masked by the much larger amplitude activity generated by stimulation of paramacular areas, and thus the PNP complex.
predominates and is conspicuous.

Studies manipulating scotoma size and stimulus field size (Blumhardt et al, 1978; Haimovic and Pedley, 1982; Yiannikas and Walsh, 1983) reveal that, the ipsilateral P100 component of the NPN complex is predominantly a macular response, arising primarily from the stimulation of the central field with 8 degree radius, and the contralateral negative component of the PNP complex is mainly of paramacular origin generated predominantly by areas around 6-8 degrees radius. Sakaue et al (1990) found that experimental square, central scotoma greater or equal to 4x4 degrees, caused a significant reduction in the P100 of the transient, monocular VEP when using 60' checks. Katsumi et al (1988), using 24' checks and steady state VEPs (6Hz), found that the minimum scotoma size that caused a significant attenuation in the responses subtended 3.2x3.2 degrees when tested monocularly, and 2x2 degrees binocularly. Geer and Spafford (1994) found no difference in critical scotoma size between monocular and binocular viewing conditions, nor between the horizontal or vertical orientation of a rectangular scotoma. Their study showed that VEP attenuation was dependent on scotoma area, and was evident with a scotoma as small as 1x1 degree, and became marked with a 3x3 degree scotoma.

Increase of the field of stimulation, within limits, produces progressive increase of P100 amplitude. However, to an extent the checksize that produces the largest response also increases progressively. Bartl et al (1978) stimulated with fields of 1.25, 2.5, 5, 7.5 and 10 degrees and found the greatest increase in amplitude when the field was increased from 2.5 to 5 degrees. The maximum amplitude was observed with 7.5 or 10 degree fields, when using checksizes between 20 and 40 minutes. Within the range analysed, they observed that the ratio (amplitude of P100 / stimulated area of retina) becomes progressively smaller as a larger area of the retina is stimulated. This is probably due to the progressively smaller density of cones, and the greater convergence of cones onto ganglion cells in the periphery.
Schein, 1988). Asselman et al (1975) observed that the responses using a 18 degree field were reduced 50% when the central 5-6 degrees were occluded, and by 80% when the central 10 degrees was occluded. Lesevre and Remond (1972) observed that P100 amplitude was maximum for a field size of 10 degrees, which remained the same for 20 degrees, but decreased abruptly when field sizes of 5 degrees or less were used.

Checksize and field size have a marked effect on the scalp distribution of onset components. Kriss and Halliday (1980) used a 0-16 degree half-field stimulus containing 50' checks. This evoked CI and CII components with an ipsilateral distribution, and a broad positivity peaking at around 105ms, over the contralateral scalp. Drasdo (1980) confined stimulation to the foveal area (2.5 degrees, using 4'-6' checks) and showed that CI was recorded over the hemisphere contralateral to the half-field stimulated and was of maximal amplitude over an area presumed to overlie the striate cortex, but was of largest amplitude further laterally (over areas 18 and 19) when 12' checks were used.

The field size producing the highest VEP amplitude response is dependent on the portion of visual field stimulated. The pattern VEP to full-field stimulation has a predominant contribution from the central 5-10 degrees of a stimulated field which activates the macular representation at the occipital pole. This explains why an increase in stimulus field size beyond 10 degrees does not produce usually an increase in P100 amplitude. The ipsilateral distribution of P100 to half-field stimulation is ascribed to activation of occipital neurones which receive their input from the paramacular retina. Stimulus field sizes of more than about 4 degrees will usually elicit an ipsilateral 'paradoxical' P100.

Pattern-onset stimulation using a foveal (1 degree) field is found to contribute mainly to the ipsilateral CII component, but not to the ipsilateral CI, and contralateral P105 components (Jeffreys and Axford, 1972, a,b). The latter components are derived primarily from the 2-6 degree region of the stimulus field. CII and CIII have also been found to be less
dependent on contrast and more on pattern detail. The amplitude of these components parallel the subjective impression of the clarity of the contours of the pattern elements. CI has been found to be large when the stimulus comprises contrast change only, whereas under such conditions, CII and CIII were very reduced in amplitude (Jeffreys, 1970; Jeffreys and Axford, 1972, a,b).

There have been few studies investigating the pattern-offset VEP. It is reported that pattern-offset VEPs have similar morphology and stimulus/response characteristics compared with reversal VEPs (Estévez & Spekreijse, 1974; Kriss & Halliday, 1980). Thus a triphasic NPN complex (representing components N85, P110 and N165) would be expected on the ipsilateral side of the scalp, with half-field stimulation, and a contralateral paramacular negativity (representing N115). On this basis it would be expected that offset components would behave in a similar manner to their reversal counterparts, in relation to field size and effects of scotomata.

Checksize (as well as scotoma size) is known to influence the relative macular and paramacular contributions to all forms of pattern VEP (Harter, 1971; Todd Meredith and Celesia, 1982). Scotoma and checksize interaction and effects, on half-field pattern-onset, -reversal and -offset VEPs have not been previously studied. The purpose of this study was to examine the differential effects of scotomata on ipsilateral and contralateral half-field pattern-onset and offset components and to study their relation to the reversal components with respect to putative contributions from macular and paramacular mechanisms. Sequential pattern-onset, -reversal and -offset VEPs were recorded in a single sweep so as to maintain identical recording and subject conditions for all three stimulus modes. The effects of checksize and its interaction with scotoma size was studied to determine the possible macular and paramacular origins of the components.
4.2 Methodology

Ten healthy subjects were recorded (6 males, 4 females). Their ages ranged between 24 and 46 years (mean= 35.5 years). All had Snellen visual acuities of 6/6 or better, and none had a clinical history of visual problems.

A four-channel montage was used. The electrodes were placed on a transverse row 5cm above the inion and 5 cm apart, with one electrode on the midline and the others 5 cm either side of the midline electrode. A further electrode was placed at the inion. All 4 occipital electrodes were referred to a common mid-frontal reference (Fz).

Subjects sat 1 metre from an oscilloscope which displayed a field subtending 24 degrees horizontally by 18.5 degrees vertically (Hewlett Packard, 1321A, X-Y Display, P4 phosphor). Black and white checks were presented to the left half-field (0-12 degrees); the right half consisted of a uniform grey field of the same average luminance as the checkerboard. A small ring (diameter subtending 1.5 degrees) at the centre of the vertical border of the pattern/blank interface provided a fixation spot for all stimulus conditions. Five sizes of check were presented, subtending 6, 12, 20, 50 and 80 minutes of arc at the subject's eye. The luminance levels were 11.5cd/m² for white squares, and 0.004cd/m² for black squares, and were constant across checksizes.

Four sizes of experimental scotomata were used (subtending 0-1.5, 0-2, 0-3 and 0-4.5 degrees). The stimulus field on the left half of the screen was masked with one of the four scotomata which occluded the central zone of the field with a notch for a fixation ring at the vertical edge which always remained visible (Figure 4.1). The left eye only was stimulated, and the right eye was occluded with a pad. Responses for each of the five checksizes were recorded. Checksize and scotoma size were varied randomly between subjects.
The VEP stimulus is identical to that described in the previous chapter with pattern-onset, -reversal and -offset averaged in a single epoch lasting 900ms. The checkerboard appeared for 300ms, was replaced by the complementary pattern which stayed on for a further 300ms (i.e. reversal), and then disappeared for 300ms (pattern-offset). The average of 64 such sequences was recorded for each stimulus condition. Ipsilateral and contralateral occipital responses were recorded from the electrodes 5cm to the left and to the right of the midline, respectively. Component identification was based on their polarity and latency as
describes in chapter 2. Peak latency and peak-to-peak amplitude measurements were made for each component.

4.3 Results

A clear experimental effect was evident. Increasing the size of the experimental scotoma resulted in attenuation of the majority of the VEP components. Figures 4.2 and 4.3 show the group average ipsilateral and contralateral responses, for the 5 checksizes, with increasing scotoma size. Component attenuation was especially conspicuous with occlusion using the two largest scotomata (0-3° and 0-4.5°) (figure 4.3). It was also evident that the VEP changes were most marked when checksizes of 12' and 20' were used, with ipsilateral reversal components and the contralateral P105 components attenuating to the greatest extent with even the smallest scotoma (0-1.5°) (figure 4.2).

When larger checks (50' and 80') were used, the VEP degradation was not as marked with the smaller scotomata (0-1.5° and 0-2°). This is likely to reflect the fact that the primarily macular-derived components, which are enhanced by smaller checks (onset CII, reversal N80, N145 and offset N85), have already attenuated with the use of large checks.
Figure 4.2: Group average ipsilateral and contralateral pattern-onset, -reversal and -offset VEPs without scotoma and with scotomata of 0-1.5° and 0-2°
Figure 4.3: Group average ipsilateral and contralateral pattern-onset, -reversal and -offset VEPs without scotoma and with scotomata of 3° and 4.5°
MANOVA gave highly significant individual effects of scotoma size (p<0.0001) and checksize (p<0.0001) on VEP amplitudes. Surprisingly, there were no significant effects of checksize by scotoma size. The effects of checksize alone will not be discussed further as the trends were in line with those described in the previous chapter.

Examination of the univariate results showed that scotoma size effect was marked for all the ipsilateral (CI: p<0.001; CII: p<0.002; CIII: p<0.0001), and contralateral (P105: p<0.0001) onset components, and the ipsilateral components only of pattern-reversal and offset (i.e., N80, P100 and N145 for reversal, and N85, P100 and N165 for offset - all at p<0.0001). Interestingly, the contralateral reversal N105 component and the offset N115 component did not show a significant change. There was a strong negative correlation between amplitudes of the majority of VEP components and scotoma size - as scotoma size increased, amplitudes decreased (Spearman's r, ranged between -0.22 and -0.48; p<0.001). However, neither the contralateral offset N115 (r= -0.14, p<0.03), nor the contralateral reversal N105 demonstrated a significant change (r= -0.12, p=0.118). These associations were evident for all checksizes (partial correlation). There were no significant correlations between VEP latencies and scotoma size for any onset or reversal components. Conversely, all offset components: N85 (r= -0.144, p<0.03), P110 (r= -0.186, p<0.004), N165 (r= -0.256, p< 0.0001) and contralateral N115 (r= -0.211; p<0.002), showed significant negative correlations with increasing scotoma size.

MANOVA of latency measures showed a significant effect of scotoma size (Pillais' test: p<0.03), but like the amplitude findings, it did not show a significant effect of checksize by scotoma size. The ipsilateral N145 of the reversal VEP (p<0.03) and all of the ipsilateral offset components: N85, P110 and N165 (p<0.0001) showed significant latency changes; however none of the onset latencies were significantly altered by scotoma.

The sensitivity of the VEP to increasing scotoma size was assessed in more detail.
using paired t-tests (corrected using a full Bonferroni adjustment). Attenuation of the VEP with scotoma was greatest for onset contralateral P105 and reversal ipsilateral P100 and N145 (all p<0.0001). These highly significant differences were found across all scotoma sizes (Figure 4.4). Ipsilateral onset CIII, reversal N80, offset N85 and P110 showed significant attenuation (p<0.0001) for the two larger scotoma sizes of 0-3 deg. and 0-4.5 deg. only. CII amplitude on the other hand, showed attenuation only when the largest (0-4.5 deg.) scotoma was introduced. Onset CI, reversal contralateral N105 and offset ipsilateral N165 and contralateral N115 failed to show statistically significant changes for the different scotoma sizes.

The onset contralateral P105 showed the same broadening of the waveform for larger checks (50' and 80') as was found in the checksize experiments described in chapter 3. Interestingly, the sharply defined P105 obtained with small checks (6' and 12') were very conspicuously degraded with the introduction of even the smallest scotoma (0-1.5'). However, the broadened P105 obtained with larger checks appeared to be minimally influenced by small scotomata (0-1.5' and 0-2'), and even with the larger scotomata (> 0-3'), the waveform attenuation was much less marked than that obtained with small checks. Furthermore, the P105 component was one of the very few components still discernible when large scotomata were introduced. These observations lend further support to our suggestion that the altered morphology of the P105 obtained to large check sizes, reflects its different origins and mechanisms than the P105 obtained with small check sizes (the former reflecting paramacular origins and the latter macular origins).
Figure 4.4: Stacked histograms of mean component amplitudes (uV) for scotomata size and checksize.
Figure 4.5: Stacked histograms of mean component latencies (msec) for scotomata size and checksize.
Similar analysis was conducted with respect to latency changes (Figure 4.5). There were no significant findings when comparing VEPs recorded without a scotoma and those recorded using the three smaller scotomata (0-1.5, 0-2 and 0-3 deg.). However, responses recorded using the largest 0-4.5 degrees scotoma, were significantly prolonged for reversal contralateral N105 and offset ipsilateral N165 and contralateral N115 (p<0.0001).

4.4 Discussion

This study has shown that there were significant negative correlations between most VEP component amplitudes and increasing scotoma size. In particular, ipsilateral reversal P100 and N145 and, contralateral onset P105 (to small checksizes only), were significantly attenuated by the smallest 0-1.5 degrees scotoma, implying that these components have major foveal contributions. This assessment of the reversal P100 is in good agreement with conclusions of previous studies (Barrett et al., 1976; Blumhardt et al., 1978; Yiannikas and Walsh, 1983). The ipsilateral reversal N145 component has not been well studied previously, except by Blumhardt et al. (1978) who reported it was somewhat variable, and unlike the findings of this study, it was not consistently affected by experimental central scotomata. Blumhardt et al (1978) found that by occluding the central 1.5 and 2.5 degrees of the stimulus half-field, the ipsilateral NPN complex was attenuated and a contralateral PNP complex emerged. The contralateral N105, the negativity of the PNP complex, was found to be accentuated or 'unmasked' when the central field was progressively occluded and the ipsilateral P100 attenuated. It is likely that the N105 is 'enhanced' because the influence of the P100 is reduced by the scotomata. N105 was largest with a central 5 degree scotoma, with larger scotomata resulting in its attenuation (Blumhardt et al, 1978).

In the present study, scotoma size did not have a significant affect on the amplitude of the contralateral reversal N105 or offset N115. Furthermore, the correlation between these
two components and scotoma size was poor compared with all the other components. The relative insensitivity of these components to central-field occlusion support the reports emphasising their paramacular origin. Neither N105 or N115 showed a significant increase in amplitude with increasing scotoma size as reported by Blumhardt's study (1978). Several factors may have contributed to this: First, the contralateral N105 and N115 components are small and show marked inter-individual variability when compared with the P100 and this would be reflected in the statistical analyses. Secondly, it is known that the N105 is significantly influenced by size of stimulus field and our half-field stimulus of 0-12 degrees, may be sub-optimal for eliciting good contralateral negativities. The results of experiments indicate that for a half-field stimulus size of 0-5 degrees, the contralateral N105 is not discernible, with a 0-10 degrees field, it is only just evident, but with a 0-16 degree hemi-field, it became prominent (Blumhardt et al., 1989)

In this study, the ipsilateral reversal N80 was largest when using checksizes of 12' and 20' and as figure 4.2 shows, it clearly attenuated with the introduction of scotomata. N80 was significantly attenuated by scotoma of more than 0-3, degrees, indicating an important contribution to its generation. In the literature, there is disagreement between studies regarding the possible origins and behaviour of the reversal N80 component. Several studies observed that it is best seen when using small checksizes (Halliday et al., 1979, Kurita-Tashima et al., 1991, Bodis-Wollner et al., 1992), and that it is selectively attenuated in clinical conditions in which there is poor macular function (Shawkat et al., 1993). However, another study (Yiannikas and Walsh, 1983), found a progressive reduction in its amplitude with reduction of the stimulus field, becoming non-detectable in the majority of subjects when the stimulus field was of 4 degrees diameter. Furthermore it was reported that a central scotoma of 8 degrees did not affect the reversal N80. Pattern-offset N85 and P110 were also significantly attenuated by scotomata of 0-3 degrees and 0-4.5 degrees. The resemblance in
behaviour of these offset components with the N80 and P100 reversal components, support the suggestion that they have similar origins (Estévez & Spekreijse, 1974).

The contralateral onset P105 was the only 'non-reversal' component to show significant attenuation to occlusion of the central 0-1.5 degrees hemi-field. Jeffreys and Axford (1972, a,b) reported that the foveal 1 degree field, contributed to onset CII only and was not involved in the production of the contralateral P105 response. Onset P105, together with CI, were shown to be derived from the central 2-6 degree area of the field. In our study, CII was significantly attenuated when the largest 0-4.5 degree scotoma was used; and we did not find any significant findings for CI. Both these results were unexpected in the light of the above mentioned previous studies. The discrepancies between our results and those of Jeffreys and Axford may be due to methodological differences, including stimulus size, duration (they used a 25ms pattern onset presentation), recording derivation and sample size (they used only 4 to 6 subjects in their experiments). CIII was significantly attenuated with scotomata of 0-3 degrees, suggesting the component's probable macular influence, though not to the same extent as for the P105 component, which was significantly reduced with smaller 0-1.5 degree scotomata. As mentioned in the results section, and in support of our presumptions in chapter 3 regarding the derivations of onset P105, this component appears to have macular and paramacular constituents, which differ in morphology and in relation to checksize variation and experimental scotomata. The macular component occurs earlier in latency, is sharply defined and is prominent for small checksizes and is dramatically attenuated with even the smallest of scotomata. The paramacular component, on the other hand, appears later, is broad or may even be bifid, is elicited by larger checksizes (>21') and is relatively unaffected by central scotomata.

Studies have shown that larger checks (>60') produce largest responses when the extramacular rather than macular field is stimulated (Harter, 1971; Todd Meredith and Celesia,
Behrman et al. (1972) observed that for 9' checks, the maximum response was obtained with 1.49 degree diameter fields; for 16' checks, the maximum was obtained with 6 degree fields, and for 35' checks, the maximum was obtained with 18 degree fields. Visual acuity deteriorates progressively in the peripheral retina (20/50 at 1.5-4.5 degrees; 20/100 at 4.0-11.5 degrees and 20/200 at more than 9.0 degrees) (Harter, 1970). Todd Meredith and Celesia (1982) determined the smallest field size and the optimal checksize to evoke a recognizable reversal VEP from different areas of the visual field: For foveal stimulation, the minimum field size was 13'17", but at 8 degree nasal eccentricity, a 3deg 18' field was needed. These field sizes produced similar sized VEPs and they were estimated to activate similar extents of cortex. At 8 degree eccentricity, the largest responses were produced by checksize 2deg33', whereas at 14 degree eccentricity, a checksize of 3deg28' was required to produce maximal VEPs.

The past reports led to an expectation of an interaction between checksize and scotoma, such that VEPs to the smaller checks would be more severely affected by increasing scotoma size, compared with VEPs to the larger checks. This is certainly true of our results as can be seen in figure 4.2: the introduction of small central hemi-scotomata had a marked effect on the VEPs to smaller 12' and 20' checks whereas there appeared to be minimal changes for VEPs to larger 50' and 80' checks. Stimulation with large checks would preferentially provoke paramacular over macular activity, and thus, central scotomata would be less influential in attenuating macular components. It is interesting that even with the second largest scotoma (0-3") a reversal positivity (P100) and onset positivities (C1 and CIII) were recordable with the larger checksizes - offset components and ipsilateral reversal and onset components, on the other hand, were barely discernible to any checksize. Our analysis showed significant independent effects of scotoma size and checksize, however the interaction effect between these two factors did not reach significant levels. This may be due to
insufficient sampling of different small checksizes and scotoma sizes.

4.5 Conclusion:

This study has shown that of the three modes of pattern stimulation, reversal ipsilateral components are the most sensitive to the effects of small central scotomata, and indicate the predominant macular contribution in their generation. Only the contralateral pattern-onset P105 response showed similar sensitivity although this was very much checksize specific. Hence if this mode of stimulation is used, half-field presentation and lateral occipital electrodes are needed to isolate this component, which can only be detected on the contralateral side of the scalp to the stimulated half-field. Offset VEPs showed similar trends to those obtained on pattern-reversal, however the changes were not so marked. Paramacular-derived potentials: contralateral reversal N105 and offset N115, VEPs (in particular the broadened P105 component) to large checks and, contrast dependent onset CI were the least influenced by the experimental scotomata and are likely to reflect paramacular pathway activity.
5. Effects of Contrast Change: A Means of Studying the Transition Between Onset, Reversal and Offset Components

5.1 Introduction

Checkerboard contrast is usually expressed as the ratio of luminance difference between black and white checks divided by the sum of their individual luminances. Spekreijse and his team have extensively studied the effects of contrast change on VEPs using checks of 20' or less (1972, 1973). The amplitude of all forms of pattern VEPs tend to saturate at relatively low contrast of 10-20% (i.e. amplitude reaches a maximum despite further increases in contrast). For pattern-onset stimulation, VEPs to checks of 20' or more tend to saturate at lower contrasts than smaller checks. Spekreijse and Estévez (1972) assessed two contrast levels (3.8% and 15%), and found that onset responses were largest for 15'-20' checks at both contrast levels; but offset responses were largest for 15'-20' checks at 3.8% contrast, and for 5'-7.5' checks at 15% contrast. Spekreijse et al (1973) have found that the onset VEP amplitude saturated with increasing pattern contrast, and saturation was found to occur at a relatively low contrast level, after an initial steady increase from threshold. However, Campbell and Maffei (1970) used steady state pattern reversal stimulation (8Hz) and reported at sub-saturation levels, that the VEP amplitude varied linearly with the logarithm of the contrast, and that the extrapolated zero amplitude correlated with the psychophysical threshold. Their stimulus conditions were likely to preferentially activate magnocellular mechanisms. However, other studies using transient stimuli which invoke both magno and parvocellular mechanisms (e.g. Jeffreys, 1977; Spekreijse et al., 1973), have shown that pattern-onset VEP components tend to have more complicated contrast-dependent characteristics than those described by Campbell and Maffei for steady state VEPs. Regan (1972) reported that stimulus frequency influences amplitude saturation characteristics.
Contrast change appears to produce alterations in component latency, duration and amplitude.

It is reported that VEPs obtained to different forms of stimulus pattern can have similar thresholds and latencies, but different saturation amplitudes (Jeffreys, 1977). The component thresholds and latencies are dependent on contrast, duration, overall luminance, and on the spatial density of the pattern elements. However, they are not greatly influenced by the actual structural details of the pattern elements. In distinction, the saturation amplitudes, (especially for components CII and CIII) are less dependent on contrast and overall luminance, but are very dependent on the structural details of the pattern. The saturation threshold of the individual components can differ for a particular type of pattern VEP. For certain patterns, such as solid squares (as opposed to square outlines), CIII builds up more rapidly and saturates at a lower contrast or duration level compared with CI. Contrast dependent properties of CIII, and to a lesser extent CII, parallel the subjective impression of the clarity of stimulus contours. The level of contrast at which CIII approaches saturation seems to correspond approximately with the level at which the outlines of the pattern are clearly seen throughout the stimulus field. Also, CIII reaches saturation levels at lower values of contrast or duration for those stimulus patterns whose outlines are better defined at the lower values. This is in line with Spekreijse et al's findings (1973), that the onset response to a checkerboard with checks exceeding 20' saturates at a lower contrast compared with that to smaller checks. This behaviour of CIII is opposite to that of CI, whose amplitude can increase substantially when contrast or duration is increased beyond the level at which the pattern contours become clearly defined.

Jeffreys (1977) also reported a marked difference in the behaviour of pattern-onset components obtained to a step-change in pattern contrast - that is, a brief increase from a low resting contrast level which was systematically varied to a control high contrast level and back again. It was found that CII and CIII attenuated at a faster rate than CI, as the resting
contrast levels increased from zero (-i.e. blank field). Once the resting contrast level is raised just above the threshold for pattern detection, CII and CIII are attenuated but CI amplitude remains fairly large, as the stimulus appears as a contrast change only.

Maffei and Fiorentini (1973) investigated the contrast dependency of striate cortex single units and found that in the cat, simple cells consistently showed a linear relationship between the response magnitude and the log contrast of a sinusoidal grating. Only a small proportion of complex cells showed a linear relationship and their response tended to saturate at relatively low contrast levels. As simple cells are exclusively present in the striate cortex, and as they show partial summation within the 'on' and 'off' regions of their location-specific receptive fields, Maffei and Fiorentini suggested that these units were involved in producing contrast-specific contributions to CI.

Kuba and Kubova, (1992) and Kubova et al. (1994) compared pattern-reversal with motion-onset VEPs and found that whereas the P100 component decreases in amplitude with decreasing contrast (becoming undetectable at a contrast of about 2% for motion-onset VEPs), N2 (or N145) amplitudes for both reversal and motion-onset VEPs did not vary significantly with contrast, above a contrast of 1.3%. It was also found that peak latencies increased with decreasing contrast and again this was more pronounced for P1 (P100) than for N2 for both reversal and motion-onset VEPs. These findings are in agreement with those of Müller and Göpfert (1988) that N2 arises from neurones that have high contrast sensitivity, and thought to reflect input from the magnocellular pathway.

The VEP to pattern-offset appears to depend more on the rate of change of contrast than does the VEP to pattern-onset (Spekreijse et al, 1973; van der Tweel, Regan and Spekreijse, 1969) as it was found that decreasing the rate of change of contrast did not affect the pattern-onset VEP but did considerably attenuate the pattern-offset main positivity. Furthermore, the behaviour of onset VEPs as a function of pattern presentation time (epoch)
was found to vary for different contrast levels (Spekreijse et al., 1973). They reported that most of the temporal integration preceded the saturation of VEP amplitude as saturation occurred at a shorter presentation duration when the contrast of the stimulus was increased. For example, a 20% contrast checkerboard presented for 20ms is similar in amplitude to a 10% contrast checkerboard that is presented for 40ms. This trade off holds up to durations of around 50-70ms, and these findings resemble psychophysical contrast thresholds (Kulikowski, 1977, Musselwhite and Jeffreys, 1982). Interestingly, this contrast/stimulus duration relationship only holds for onset VEP amplitude and not for latency. Latency was found to depend on contrast only (Musselwhite and Jeffreys, 1982). Pattern-offset VEPs also did not show any contrast/duration relationship (Spekreijse, van der Tweel and Regan, 1972).

The standing contrast level has been found to affect pattern-onset VEPs, whereas pattern-offset VEPs do not appear to be influenced by the standing contrast as such, but by the absolute magnitude of change in contrast (Spekreijse, van der Tweel and Zuidema, 1973). This has been interpreted as representing the essentially sustained behaviour of onset VEPs, and the relatively transient behaviour of offset VEPs.

Estévez and Spekreijse (1974) examined the relationship between reversal and on/off responses by creating a sequence of stimuli that were intermediate between pure onset/offset and pure reversal. They modulated two sets of checks in counterphase and, appropriate luminance levels were chosen so as to go by steps from onset/offset to a symmetric pattern reversal. In this way they aimed to trace the transition from onset/offset VEP components to reversal components via a series of small steps. In the pattern reversal modulation, a component with a latency of 107-134 ms was identified and this corresponded to the previously reported P100 component. This potential could be identified all the way to the pure offset condition. However, the peak of the negative component of onset responses with a latency of 120ms, became very attenuated and delayed whenever a small amount of contrast
disparity was present between the two modulated sets of check stimuli.

Estévez and Spekreijse's results showed that this attenuation is mainly due to the presence of an offset component that interacts with the onset response. Their data showed that for low contrast stimuli, the main contribution to the 107-134ms positivity (P100) of the reversal response seems to come from a 'contrast decrease' component which is followed by a smaller negative 'contrast increase' response. This increase component, however, has different properties from the mainly foveal negative CII component of the onset response. It was found that the checkerboard reversal response from foveal stimulation was not an algebraic sum of the corresponding increase and decrease responses of the pattern onset/offset stimuli. For extrafoveal stimulation, however, the algebraic sum of the increase and decrease responses compares quite well with the pattern reversal response. This evidence suggests that contrast increase and decrease responses differ widely in dynamic behaviour and the dependence on stimulus parameters is thought to portray a different cortical representation for the two responses. They concluded that pattern-reversal VEPs involve an interaction between pattern-onset and -offset VEPs, but predominantly reflects the pattern-offset response and is thus representative of an abrupt decrease in contrast. Jeffreys (1977) conducted similar studies and showed that the reversal main positivity appears to be a composite containing two superimposed positive peaks of different spatial distributions: an initial component corresponding to the onset CI and a succeeding component corresponding to the offset main positivity (P110); he thus concluded that the pattern-reversal response is a composite of pattern-onset CI and pattern-offset P110.

The aims of the experiments described in this chapter are a) to identify the differential behaviour of the pattern-onset/offset and -reversal VEP components with respect to stepwise changes in local luminance and contrast and b) to study the relationship between the VEP components of the three stimulus modes by tracing the transition of the VEP components
from the onset/offset mode, via a series of steps into reversal mode. Previous similar studies (Estévez and Spekreijse, 1974; Jeffreys, 1977) have investigated the full-field VEP components and predominantly investigated onset Cl, CII and CIII and the reversal and offset main positivity. Experiments in chapters 2 and 3 showed significant findings for the ipsilateral negativities for pattern-reversal and -offset, and for the contralateral onset P105. Thus, this study uses half-field stimulation to better differentiate the macular and paramacular components recorded on the ipsilateral and contralateral side of the scalp to the stimulated half-field.

5.2 Methodology

Fifteen healthy subjects were recorded (6 males, 9 females). Their ages ranged between 21 and 47 years (mean = 29 years). All had Snellen visual acuities of 6/6 or better, and none had a clinical history of visual problems.

A four-channel montage was used which was identical to that in the previous experiment (chapter 3). The electrodes were placed on a transverse row 5cm above the inion and 5 cm apart, with one electrode at the midline, and the others 5 cm either side of the midline electrode. A further electrode was placed at the inion. All 4 occipital electrodes were referred to a common mid-frontal reference (Fz).

The checkerboard stimulus was presented to the left half-field (0-12 degrees); the right half consisted of a uniform grey field of the same average luminance as the stimulus. A small ring (diameter subtending 1.5 degrees) at the centre of the vertical border of the pattern/blank interface provided a fixation spot. Three checksizes were presented (subtending 12, 50 and 80 minutes of arc at the subject's eye). As in the previous experiments, the left eye only was stimulated; the right eye was occluded with a pad.

Each subject was tested with 8 stimulus conditions for each of the three checksizes
studied— in total 24 stimulus conditions. Each stimulus condition consisted of the alternation of a constant high contrast checkerboard (A) with luminance levels of 11.5cd/m² for white squares, and 0.004cd/m² for black squares, with a second checkerboard (B), the contrast of which was systematically altered in fixed linear steps (Figure 5.2).

In experimental conditions 1 to 4, checkerboard A was alternated with checkerboard B, both were of identical spatial phase, but checkerboard B was of reduced contrast. The contrast of checkerboard B was reduced linearly after each stimulus condition recording until condition 4, when it becomes a uniform grey field. Hence checkerboard A (high contrast checks) is replaced by B until it becomes a grey field, and the stimulus checkerboard offset (Figure 5.1, steps 1 to 4).

![Diagram showing change in contrast and spatial phase for experimental steps 1 to 8. Checkerboard A is always of the same high contrast, whereas B varies in contrast and phase.](image)
The subsequent experimental conditions 5 to 8 also involved the alternation of constant high contrast checkerboard A with B. However, in these 4 conditions checkerboard B is of reverse phase. Checkerboard B in condition 5 is of low contrast and this is increased in linear steps over the next experimental conditions until step 8 is reached where checkerboard B is of equal high contrast to checkerboard A but of opposite spatial phase, and there is standard pattern-reversal stimulation (Figure 5.1, steps 5 to 8).

The recording epoch was 500ms. during which two VEP responses were obtained sequentially in a single run (with no pre-stimulus delay). The first VEP, which was recorded over the first 250ms represents the alternation from checkerboard B to A (i.e low to high contrast) and the alternation from checkerboard A to B (i.e. high to low contrast) was then recorded over the next 250ms. (Figure 5.3). In stimulus conditions 1 to 4 the sequence was essentially that of pattern-onset (the first 250ms.) followed by pattern-offset (over the next 250ms.) For stimulus conditions 5 to 8, spatial phases of checkerboards A and B were
reversed, contrast of B progressively increased, and, the VEP responses in both the first and second halves of the recording epoch represent pattern-reversal. The average of 64 such sequences was recorded for each checksize and stimulus condition. When necessary averaging was repeated (e.g. if subject was attending poorly, or responses were severely corrupted by myogenic artifact).

Figure 5.3: Diagram showing the two VEP responses (initial response I recorded over the first 250ms, and second response II recorded over the subsequent 250ms) obtained over the 500ms recording epoch. Depending on the experimental step response I may be a pattern-onset or -reversal VEP and, response II may be a pattern-offset or reversal VEP. The diagram depict steps 1, 4 and 8.

Ipsilateral and contralateral components were measured from the electrodes 5cm to the left and to the right of the midline, respectively. The following VEP components were identified and measured when possible: for pattern-onset, ipsilateral C1, CII, CIII and contralateral P105; for reversal, the ipsilateral N80, P100, N145 and contralateral N105; and for offset, the ipsilateral N85, P110, N165 and contralateral N115. Component recognition was based on their polarity and latency. Peak latency and peak-to-peak amplitude measurements were made for each component.
5.3 Results

Two VEP waveform responses (I and II) were recorded over a 500ms averaging period: the first (recorded over the initial 250ms.) is the response to the transition of a low contrast to high contrast checkerboard and the second is the response to the transition from high to low contrast checkerboard (see figures 5.1 and 5.3). The experimental steps 1 to 8 can be divided into two sections: The first, contrast change steps 1 through to 4, represent the progression from a low step contrast change, giving essentially a pattern-onset and pattern-offset VEP, culminating in a high contrast change pure pattern-onset and -offset VEP. The second, contrast change steps 5 through to 8, demonstrate the evolution of contrast change for reversal responses, reversing between high and low contrast checkerboards. The effects of contrast change, per se, on the different stimulus modes as recorded by these methods will be initially presented; The results for conditions 1 to 4 are described together in one section as they essentially describe the low to high contrast pattern-onset paradigm and high to low pattern-offset paradigm. Reversal is treated separately as it essentially represents the findings of experimental conditions 5 to 8, where there is reversal between low to high and, high to low contrast checkerboards. These sections are followed by a description of the overall results describing the transition between different VEP modalities.

5.3.1 Effects of contrast change on pattern onset/offset VEPs

In general, as the extent of step contrast change increased from experimental conditions 1 to 4 (i.e., towards a pure pattern onset/offset stimulus), the VEP responses became better-defined. Figure 5.4 shows the group average ipsilateral and contralateral VEP responses of the 15 subjects for experimental conditions 1 to 4, for the three checksizes used. Most of the individual subject responses behaved in the manner reflected in this group average figure. It can be seen that the evolution of better defined components with increasing
contrast change is best discerned when the smallest 12' checks were used. Pattern-onset ipsilateral CII and contralateral P105, and offset N85 essentially unrecordable at experimental step 1 with 12' checks, but enhance systematically from steps 2 through to 4. This is not the case when larger 50' and 80' checks are used. Figure 5.4 shows that even at experimental step 1 the larger checks (50' and 80') are producing clear onset (in particular CI and contralateral P105) and offset (P110) responses which at step 2, appear to already be near maximal in amplitude. Both the onset CII and offset N85 components, which continue enhancing with increasing contrast change for 12' checks, remain extremely attenuated throughout the experimental steps for checksizes 50' and 80', supporting the findings in chapter 3 which showed that these components are better seen for small checksizes suggesting their predominant macular origin. It is interesting to note that the contralateral onset P105 is sharply defined for 12' checks at experimental step 4, becomes broadened for 50' checks and appears bifid for the large 80' checks. This supports the results in chapters 3 and 4 which showed a broad and bifid P105 component for large checksizes which was ascribed to paramacular activity.

Multivariate analysis of variance (MANOVA) was carried out for the pattern onset and offset ipsilateral and contralateral responses with respect to checksize and the stepwise contrast change. A significant effect of checksize by contrast change was found (Pillais's test, p<0.06; Hotellings test, p<0.03). This result however, was attributable to only two of the VEP measures: ipsilateral pattern-offset N85 (p<0.0001) and P110 (p<0.003) amplitudes. The amplitudes of both N85 and P110 amplitudes were greater for the larger changes in contrast, and this in turn is marked for the smallest checksize used (12') (Figure 5.5 and 5.6). None of the pattern-onset components, nor any VEP latency measures contributed to the checksize by contrast change effect.
Both contrast change and checksize showed independent significant effects (Pillais' test, \(p<0.0001\)), however the effects of checksize alone will not be discussed any further in this chapter as they support those findings described in chapter 3 for the onset and offset components.

The highly significant effect of contrast change on amplitude was found for all ipsilateral and contralateral pattern-onset (CI, CII, CIII and P105: \(p<0.004\)) and pattern-offset (N85, P110, N165 and N115; \(p<0.007\)) components. Once again none of the latency measures of any onset or offset VEP components were significantly affected by contrast change. Figures 5.7 and 5.8 show the increase in amplitudes with increasing change in
contrast across the three checksizes, for the pattern-onset and -offset components.

For the smallest 12' checks, significant positive correlations were found between all the measured onset and offset VEP amplitudes, and increasing contrast change (Spearman's correlation coefficients: $0.34 \leq r \leq 0.64; p < 0.008$). Interestingly, the only exception to this
was onset CI amplitude \((r=0.1)\), which has been found to be small and poorly-defined to small checksizes and best seen for large checks (chapter 3). When larger 50' and 80' checks were used CI amplitude did correlate significantly with increasing contrast change (Spearman's correlation coefficient \(r=0.33, p<0.02\), and, \(f=0.36, p<0.006\), respectively). On the other hand, onset CII and offset N85 which were found to be predominantly macular in origin and thus enhanced by small checks (chapter 3), did not correlate significantly with increasing contrast change with the larger 50' and 80' checks.

5.3.2 Effects of contrast change on pattern reversal VEPs

Analysis for experimental steps 5 to 8 - that is for pattern reversal stimulation from small to high changes of contrast (pure pattern-reversal) was carried out. Responses became better-defined with increasing change in contrast, however this was not as marked as for the pattern onset/offset experimental conditions. Figure 5.9 shows the group average VEP
responses of the 15 subjects for experimental conditions 5 to 8. MANOVA of pattern-reversal VEP measures with respect to the interaction of contrast change by checksize did not reveal a significant effect (Pillais' test, p<0.6). However contrast change alone did have a significant effect (Pillais' test, p<0.02) as did checksize alone (Pillais' test, p<0.0001).

Figure 5.9: Group average ipsilateral and contralateral VEPs for experimental steps 5 to 8.

Univariate analysis showed that the significant effect of contrast change was most marked for both the amplitude and latency of the ipsilateral reversal P100 component (p<0.005) (Figure 5.10). P100 amplitude for all checksizes increased with increasing contrast change. P100 latency, on the other hand showed an overall decrease in latency with increasing contrast change. This latency decrease was fairly uniform when using 12' and 80' checks, however with 50' checks, there was an initial increase in latency with the first step
in contrast change (20%) after which latencies decreased uniformly with the shortest latencies occurring at the greatest contrast change (see figure 5.10). Although there appears to be a peak for P100 latency at experimental step 6 for 50' checks, there was no significant interaction between checksize and contrast change, but it is intriguing to note that the P100 component for 50' checks at experimental step 5, is broader and smaller compared with that for 12' and 80' checks (Fig 5.9).

No other component amplitudes showed significant changes with contrast change, although the latencies of both ipsilateral N80 and contralateral N105 showed significant results (p<0.0001), with a tendency for latencies to decrease with increasing contrast change (Figure 5.11).

Reversal VEPs recorded for low to high contrast change (during the first 250ms epoch) were compared with those recorded for high to low contrast change (during the second 250ms epoch) in order to ascertain whether there were any differences due to the direction of contrast change and/or the methodology for recording the two responses. MANOVA of component amplitudes and latencies showed that there was a significant effect (Pillais' test
p< 0.0001). Univariate analysis showed that all ipsilateral components of the second, high to low contrast change response were of significantly longer latency than those of the first, low to high contrast change response. Figure 5.12 illustrates these differences for 50' checks. However, no significant latency differences were found for experimental step 8, where both the first and second responses are identical (i.e. pure, high contrast reversal). This suggests that it is the direction of contrast change: the reversal from a high contrast to a low contrast checkerboard, which is prolonging component latencies, and not the order of recording. There was no significant difference in any of the amplitude measures.

Figure 5.11: Graphs of mean latencies with contrast change for the reversal ipsilateral N80 and offset ipsilateral N115 components.

Figure 5.12: Mean latency(ms.) change (+/- S.E.Mean) for ipsilateral reversal components for experimental steps 5 to 8, for low to high contrast change (red), and high to low contrast change (blue).
5.3.3 Observations on the transition between pattern-onset/offset stimulation to reversal stimulation.

Figures 5.13 and 5.14 show the group average ipsilateral and contralateral VEPs, respectively, for experimental conditions 1 to 8, checksizes 12', 50' and 80'. It can be seen that for experimental step 1, where the high contrast checkerboard A is replaced by checkerboard B which is of the same spatial phase and overall luminance, but of 60% lower contrast, the VEPs are very attenuated and not really discernible for the smallest 12' checks.

The second VEP waveform (recorded in the second half of the analysis epoch) shows the development of the reversal VEP from the offset VEP. If the individual potentials are traced through the 8 increasing steps of contrast change, it can be seen that offset and reversal VEP components are very similar in terms of polarity, latency and waveform configuration. The offset response does become better defined with increasing contrast change (i.e. it is best seen at stimulus step 4, when it is a pure high contrast offset stimulus), but in general, it appears to be better preserved and more clearly discernible than the first onset VEP response at the lower contrast change stimulus steps (steps 1 to 3). Reversal components were of similar morphology to offset components, with latencies being marginally more prolonged for offset potentials.

Amplitudes and latencies of potentials were checksize dependent. For example ipsilateral reversal N80 and P100, and offset N85 and P110, and onset CII were enhanced when the smallest 12' checks were used (Figure 5.13). The use of the larger 50' and 80' checks clearly showed the marked attenuation of offset N85, reversal N80, and onset CII components. The onset contralateral P105 also showed distinctive behaviour with checksize: The use of small 12' checks elicited a sharply defined P105 at around 105ms, best seen at experimental steps 4 and 5 (Figure 5.14, down arrows), whereas the contralateral onset P105 responses to 50' and 80' checks were markedly broadened and on certain occasions bifid in
appearance. Interestingly the reversal and offset contralateral negativities were very ill-defined and the reversal contralateral N105 was best seen with the use of large 80' checks (figure 5.14, up arrows). These effects of checksize on VEP components are consistent with the previous findings described in chapter 3.

The first VEP response (recorded in the first 250ms, of the recording epoch) shows marked changes in the overall configuration of the VEP over the 8 experimental steps. The VEP becomes better-defined over the first four stimulus steps in contrast change, particularly, at step 4 which represents a high contrast pure onset/offset stimulus. Steps 5 to 8 represent the evolution of high contrast pattern-reversal complex, with a clear ipsilateral N80, P100 and N145 and a less conspicuous contralateral N105 component, being seen in experimental step 8.

The pattern-onset C1 component for all three checksizes, becomes enhanced with increasing contrast change, and is maintained throughout steps 1 to 8, becoming better-defined and larger after step 4, and evolving into the standard pattern-reversal P100 main positivity. Using 12' checks for contrast stages 1 to 4, CII and contralateral P105 are relatively well-defined; for the larger checks CII is relatively attenuated, whereas CI and to a lesser extent CIII are enhanced.
Figure 5.13: Group average ipsilateral VEPs of experimental steps 1 to 8 showing the transition from low contrast change pattern-onset/offset through to pure pattern-reversal mode of stimulation for different checksizes. For each condition to left of the dotted line, responses are for low to high contrast, and those to the right of the dotted line are for high to low contrast.
Figure 5.14: Group average contralateral VEPs of experimental steps 1 to 8 showing the transition from low contrast change pattern-onset/offset through to pure pattern-reversal mode of stimulation for different checksizes. Responses to the left of the dotted line are for low to high contrast, and those right of the dotted line are for high to low contrast change. The onset P105 responses in experimental steps 4 and 5, are indicated by the down arrows, and, the reversal N105 responses in experimental steps 7 and 8, are indicated by the up arrows.
In nearly half the subjects (7/15), 80' check stimulation showed a slightly different pattern of evolution: the main ipsilateral positivity seen in steps 5 to 8 (i.e. the reversal P100, recorded in the first 250ms) appears to be a composite of the two ipsilateral onset positivities from steps 1 to 4 (i.e. CI and CIII). The 80' reversal P100 in these subjects is broader than usual and appears to arise because of the attenuation and eventual disappearance of the CII component. Figure 5.15 is an example of such responses from one subject. Interestingly, in this subject, the VEPs recorded in the second 250ms recording epoch (i.e. responses to high to low changes in contrast) which represent the evolution of a pattern-reversal from pattern-offset VEP, also appear bifid for experimental steps 6 to 8, although for steps 1 through to 5, the VEP mainly consists of a broad positivity. When smaller checksizes were used (12' and 50'), the onset CII remains prominent, and for steps 5 to 8, the P100 response is better defined and the CII appears to eventually evolve into the ipsilateral N145 reversal negativity.

The transition from onset CI/CIII to the reversal P100 with 80' checks was not well reflected in the group average responses (figure 5.13). This is likely to be due to the fact that in the greater number of subjects (n=8), the CII did not fully attenuate and thus remained apparent in the group average, albeit to a minor extent. There were no significant differences between the individuals who showed such responses with 80' checks and the remainder of the subjects in terms of age, sex and visual status.

When using the larger 50' and 80' checks, pattern-onset responses as obtained in steps 1 to 4, contained a prominent CI component without a consistent preceding negative potential. However, in the majority of subjects (n=13/15; 87%), 12' checks elicited a negative component which preceded CI for the first 4 contrast change steps. It became most prominent from steps 4 to 8 and evolved into the ipsilateral reversal N80 negativity. This negative component has also been described by other authors and labelled as Co (Kulikowski, 1977; Drasdo, 1980). Figure 5.16, shows such a VEP example for one subject (note also the large

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negativity recorded in the second half of the recording epoch: this component which is the offset N85 that evolves into the reversal N80 component is known to be enhanced with small checksizes (chapter 3)). This transition from onset Co to reversal N80 for 12' checks was also evident in the group average responses as most of the subjects showed this phenomenon (figure 5.13).

Figure 5.15: Ipsilateral VEPs to the largest 80' checks of steps 1 to 8 from a subject showing a small onset CII component (step 4 - up arrow) which attenuates further so that CI and CIII components appear to merge and evolve into a broad and bifid reversal positivity, P100 (down arrows).
Contralateral VEPs recorded in the second 250ms recording epoch (high to low contrast change) were fairly similar throughout steps 1 to 8 (figure 5.14). The contralateral main negativity was best seen for stimulation with the large checks (80') and was better-defined for more marked contrast change. The offset negativity (N115) was most prominent as contrast change increased from step 1 to 4; and with progression through steps 5 to 8, the reversal negativity (N105) again became more prominent, and decreased in latency.

Figure 5.16: Ipsilateral VEPs to the smallest 12' checks of steps 1 to 8 from a subject who had a Co pattern-onset component which subsequently evolved into the reversal N80 component (arrow)
Contralateral VEPs for low to high contrast conditions (recorded in the first 250ms) were similar to findings for the ipsilateral responses, in showing marked changes in response configuration. As mentioned previously, the contralateral pattern-onset P105 was most sharply-defined for experimental steps 4 and 5 and with small 12' checks. When larger checks were used, this positive component broadens and becomes bifid. The preceding negativity to the onset P105, becomes more prominent and evolves into the contralateral reversal component, N105 and the P105 itself is prolonged and evolves into the second positivity of the contralateral PNP reversal complex. This reversal contralateral N105 component was best seen for the large 80' checks (figure 5.15).

5.4 Discussion

These findings indicate that contrast change significantly influenced the amplitudes and morphology of pattern-onset, -reversal and -offset components. Furthermore the influence of checksize on VEP amplitudes with contrast change was clearly evident for most of components and was statistically significant for offset N85 and P110 amplitudes. Conversely, effects on latencies were not significant for any pattern onset and offset components but were significant for reversal ipsilateral N80 and P100, and contralateral N105. The degree of amplitude change with contrast appears to be greatest for offset, followed by onset and then reversal. Latency changes on the other hand, are greatest for the reversal responses.

Amplitude increase with augmented contrast change has been reported by several studies (Jeffreys, 1977; Spekreijse et al., 1973). However, Jeffreys (1977) reported the various onset components had different response characteristics: Onset CII and CIII were not as dependent on contrast change as CI, with the CIII component (and to a lesser extent CII) building up more rapidly with contrast increase and saturating at lower contrast levels. Our CI results showed similar trends when larger checks were used (80' checks) with a strong
tendency for increasing amplitude with increasing contrast change, however, this effect was not significant for small 12' checks. Experiments described here showed that CI amplitudes are greatest when large checks were used (chapter 3) and were not markedly affected by central scotoma (chapter 4). Small checks lead to an increase in pattern-specific stimulation. This mainly activates parvocellular pathways, whereas with large check stimulation, there are reduced pattern elements per unit area and increased luminance effects which would preferentially activate the magnocellular pathways. As CI has been reported to be predominantly contrast-dependent as opposed to pattern-dependent (Jeffreys, 1977), the attenuated CI results following 12' check stimulation in these experiments are thus in line with previous findings.

All offset and onset components were significantly enhanced in amplitude with increasing contrast change, whereas only the reversal P100 was significantly thus affected. None of the offset and onset VEP latencies were significantly influenced by contrast whereas, the majority of the reversal components decreased significantly in latency with increasing contrast change. These differences between reversal and onset/offset modes could be due to motion related mechanisms that may be present following reversal stimulation but not offset or onset stimulation. Spekreijse et al (1985) proposed that reversal stimulation invokes motion-onset and motion -offset mechanisms. Kulikowski (1977, 1978) suggested that reversal invokes both motion and pattern detecting mechanisms. Large patterns elicit predominantly transient mechanisms which depend on contrast change. Local luminance changes associated with alternation of black and white checks would be more evident with large checksizes, reflecting predominantly magnocellular activity. Van der Tweel and Spekreijse (1968) suggested that pattern-reversal VEPs to large checks have two constituents one related to the check edges, and the other related to check luminance change. This local luminance contribution is more evident for large check VEPs than for small check VEPs and
reflects mainly magnocellular contribution. They have shown that reversal VEPs are markedly attenuated when the edges of small checks were occluded, thus reflecting pattern/contour contribution to the small pattern reversal VEP (reflecting parvocellular activity). Thus response properties suggest that even though there may be similarities between reversal and offset VEPs, offset responses reflect a greater transient, magnocellular contribution, whereas reversal responses are likely to reflect a more even mixture of magnocellular and parvocellular contributions.

As contrast change decreased, pattern-offset VEPs showed greater rate of latency increase than pattern-onset VEPs. Onset and offset VEP latencies were not significantly altered with contrast change, although there was a tendency for latencies to decrease with increasing contrast change; Reversal N80, P100 and contralateral N105 latencies however were significantly decreased with increasing contrast change. Both N80 and P100 components have been shown to be of predominantly macular origin in experimental and clinical studies (Halliday et al., 1979, Kurita-Tashima et al., 1991, Bodis-Wollner et al., 1992, Shawkat et al 1993). Experiments investigating checksize and scotoma effects (chapters 3 and 4) also indicate that these two components are dominated by contributions from the macular pathway and seen best to small checksizes. The significant results for reversal N80 and P100 indicate that at high contrast change where pattern definition is greater, latencies (and amplitude in the case of the P100 component) differ significantly from those obtained at low contrast change stimulation, where pattern-edge detection mechanisms are less prominent and the main contribution is from the local luminance change. This is in contrast to the pattern-onset and -offset modes where all of the VEP components showed significant amplitude enhancement with increasing contrast change.

Pattern VEP and psychophysical studies in humans suggest the presence of two mechanisms, one sensitive to high contrast the other to low contrast: Several authors have
established that pattern VEPs can be used to predict contrast thresholds (e.g. Campbell and Maffei, 1970; Kulikowski, 1977; Cannon, 1983). However, above threshold there are conditions under which the VEP does not correlate with psychophysical measures. For instance, many subjects show VEP amplitude versus spatial frequency plots that at certain frequency ranges (particularly 2-4c/deg.) have a bimodal function with a low amplitude point mid range (Tyler and Apkarian, 1985; Strasburger et al, 1986; Bach and Joost, 1989). This 'notch' in the spatial frequency/VEP amplitude relationship has been attributed to signal cancellation taking place at specific spatial frequencies where two different neuronal subsystems interact (Strasburger et al., 1993; Kulikowski and Tolhurst, 1973). Kulikowski and Tolhurst (1973) reported two independent thresholds for temporally modulated gratings, one for the detection of movement and the other for detection of pattern structure. The movement detecting channel favoured low spatial contrast, whereas the pattern detection channel favoured high spatial frequency. It was found that at low spatial frequencies subjects were twice as sensitive to contrast reversal as they were to an onset/offset stimulus. As the contrast change for reversal is twice that for onset/offset, these findings were thought to show that low spatial frequency mechanisms mediated transient-like detectors which relied on change of contrast between two consecutive phases of the stimulus as opposed to maximum overall change in contrast. As spatial frequency is increased, there is a transition in the relative sensitivity to reversal and onset/offset and at 6c/deg they are equally detectable.

Strasburger et al. (1993) tested the hypothesis that the notch is a manifestation of the interaction between sustained and transient mechanisms. They compared VEPs and psychophysical thresholds for 8Hz contrast reversal (vertical sine wave gratings) with those for onset/offset stimulation at 8Hz and 16Hz., using a wide range of spatial frequencies. They found that psychophysical contrast thresholds showed that 8Hz reversal is effective for both transient and sustained mechanisms, whereas the 16Hz onset/offset was a poor stimulus for
transient mechanisms. A principal component analysis for VEP amplitudes supported this as it showed two independent factors contributing to the 8Hz reversal response but only a single source contributing to the 16Hz onset/offset VEP. Furthermore it was found that whereas there was a 'notch' in the amplitude/spatial frequency function for the 8Hz reversal stimulus (interaction between sustained and transient), the 16Hz onset/offset stimulus did not usually exhibit a notch, thus suggesting its single source (sustained mechanism only). Thus experimental evidence indicates that an onset/offset stimulus, particularly of low contrast and spatial frequency predominantly engages transient mechanisms compared with a reversal stimulus with the same physical characteristics.

The results concerning the transition from offset VEP components to reversal VEPs support the findings of Kriss and Halliday (1980) who reported that reversal and offset VEPs have essentially similar morphology and properties, and those of Estévez and Spekreijse (1974) who experimentally changed contrast and traced the major positivity (reported as having a latency of 107-134ms) from the offset mode to the reversal mode (i.e. the P110 or P100). The present study showed that the preceding negativities (N85 in the case of offset and N80 in reversal) and the subsequent negativities (N165 in the case of offset and N145 in reversal), as well as the contralateral N115 and N105 negativities also show close similarities between reversal and offset VEPs.

Pattern-reversal components were significantly more prolonged for experimental steps 5 to 8, when the stimulus consisted of a high to low contrast changed compared with low to high contrast change (figure 5.12). This finding is in keeping with previous studies that have reported VEP latencies to be influenced by contrast (Kulikowski, 1975, Musselwhite and Jeffreys, 1985, Jakobsson and Johansson, 1992), with higher contrast giving shorter latencies.

Estévez and Spekreijse (1974) concluded that pattern-reversal VEPs involves an interaction between pattern-onset and -offset VEPs, but with a closer affinity to offset.
Jeffreys (1977) came to similar conclusions but suggested that the pattern-reversal response is a composite of pattern-onset CI and the main pattern-offset positivity (P110). The results of the present study are in line with these conclusions as the onset CI could be traced through to the reversal P100 component. Ochs and Aminoff (1980) hypothesized that the pattern reversal waveform could be produced by the effect of pattern adaptation on the pattern-onset response: When onset VEPs were recorded immediately after adaptation to the stimulating pattern, the main negativity (CII) was lost and the latency of CI was increased by 14ms, and thus became similar to the pattern-reversal P100 component, both in terms of morphology and latency. Ochs and Aminoff thus suggested that adaptation to the pattern, as found in the conventional reversal VEP paradigm, causes the waveform differences between pattern-reversal and pattern-onset. In the present study it was found that in approximately 50% of subjects, the pattern-onset CII (when using large checks) disappears as the transition from onset to reversal progresses and hence the remaining broad positivity, which would be a combination of the original CI and CIII, does appear with a later latency than CI and subsequently resemble the conventional reversal P100 component (figure 5.14). CII is very much a pattern-dependent component and is enhanced by small pattern elements (Jeffreys, 1977), and the data in the present study also showed that the smallest checks elicited the largest CII components (chapter 3). When large 80' checks were used, the CII was less prominent and attenuated as the onset mode changed to the reversal mode, leaving the main reversal P100 broadened with an increased peak latency. The negativity (possibly N145) that followed this broadened P100 component was generally ill-defined. However, if smaller checks were used, CII became prominent, and the preceding positive (CI) waveform appeared better defined - as CI transformed into the reversal P100, the CII evolved into a well-defined ipsilateral N145 reversal negativity. Previous findings looking at the effects of checksize and central scotoma (chapters 3 and 4), have shown that the reversal N145 behaves in a similar
fashion to the P100 component and was significantly attenuated with small central scotomata and when large 80' checks were used, indicating a primarily macular origin.

When the smallest 12' checks were used, a marked negative component was found to precede the onset Ci component in the first 4 contrast change steps, becoming more prominent from steps 4 to 8 and evolving into the reversal N80 negativity. This onset pre-CI negativity seen for small checks, around 70-120ms, has been described by Kulikowski (1977) and Drasdo (1980), who refer to it as 'Co'. Both authors found it was prominent in the midline electrode for small checksizes. Very small checks (< 10') were reported to be optimal at eliciting Co, and this may explain why not all subjects produced a definite Co component as the checks in our experiments were essentially somewhat larger than optimal. However, it is interesting that when this macular-derived ipsilateral onset Co component was present (contrast change steps 1 to 4), it clearly evolved into a larger reversal N80 component (contrast change steps 5 to 8). Other reports have also shown that N80 is primarily a macular-derived potential (Kurita-Tashima et al., 1991, Bodis-Wollner et al., 1992), and the experiments involving the effects of checksize and central scotoma, support a macular origin for this component (chapters 3 and 4).

The transition of contralaterally recorded VEP components, from the pattern-onset mode to pattern-reversal has not been previously described. Half-field stimulation is necessary to highlight these responses. If the contralateral onset positivity (P105) is traced from the onset mode (steps 1 to 4) through to the pure reversal mode (steps 5 to 8), it is seen to attenuate as the preceding negativity remains as the reversal N105 component (figure 5.13). The contralateral onset P105 has been reported to have both macular and paramacular origins, as it was recorded from the central 2-6 degree field (Jeffreys and Axford, 1972). Previous studies (chapter 4) showed that the contralateral P105 was significantly attenuated by occluding the central 0-1.5 hemi-field, but it was also largest when relatively large checks
(50') were used (chapter 3) which suggests mixed macular and paramacular origins. These experiments have also shown that the morphology of the P105 component varies depending on whether macular or paramacular activity is targeted. The macular-derived P105 component, present to small checksizes (12') is sharply defined, whereas larger checks elicit a later, broadened and frequently bifid positivity (see figure 5.14: experimental steps 2 to 4, 50' and 80' checks).

5.5 Conclusions:

The present study showed that increasing contrast change enhances VEP amplitudes. This was significant for all pattern-onset and -offset components, but only for the reversal P100. Latencies had a tendency to decrease with increasing contrast change and this was significant for reversal ipsilateral N80 and P100, and contralateral N105 components. These results suggest that a pattern onset/offset stimulus is preferentially activating magnocellular mechanisms. A reversal stimulus, on the other hand, involves both pattern and motion processing systems and is likely to engage both magno and parvocellular mechanisms: large checksizes and low contrast change reflect the former, and small checksizes and high contrast change reflecting the latter.

Tracing the different VEP components from the onset modality through to the pure reversal modality appears to indicate that the onset ipsilateral Cl is closely related to the reversal P100, particularly for the smaller checksizes; with larger checks, the reversal P100 appears to be derived from an amalgamation of both onset CI and CIII components. When using small checks, the primarily macular-derived reversal N80 component appears to evolve from the ipsilateral onset Co component; if large checks are used, then Co is not evident and N80 is usually attenuated. The prominent contralateral onset P105, which is thought to reflect macular and paramacular contributions attenuates during the reversal modality leaving a small

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negativity, the N105 component which is predominantly a paramacular-derived potential. Offset ipsilateral NPN complexes and the contralateral negativity (N115) could be traced through to the reversal modes of stimulation, indicating that these components are similar in terms of stimulus related characteristics.
6. Comparison of Pattern-Onset, -Reversal and -Offset VEPs in Amblyopic Children

6.1 Introduction

The investigations concerned with assessing the effects of checks size, scotoma and contrast change demonstrated the relative macular and paramacular contributions to the ipsilateral and contralateral components of pattern-onset, -reversal and -offset VEPs. The sequential VEP technique was then applied to a clinical population of amblyopic children. Experimental evidence indicates that subjects with an amblyopic eye have selective deficits that primarily affect macular vision with reduced sensitivity mainly involving the parvocellular pathway. Amblyopia and squint is one of the most common causes of poor vision in children in developed countries, with an incidence of 2-5% of the general population (Graham, 1974). Onset, reversal and offset VEPs were used to assess vision of amblyopic and fellow eyes of amblyopic children in order to gauge the relative clinical effectiveness of these three stimulus modes.

Brief overviews on development of pattern VEPs in normal children, and in amblyopes are presented first. In addition, resumés of experimental findings in animals are given to provide details of the pathophysiological processes associated with amblyopia.

6.1.1 Pattern VEPs in Children

Several studies have monitored visual development using pattern-onset or -reversal VEPs, though there appears to be very few reports concerning the development of pattern-offset VEPs. Spekreijse (1978) and DeVries-Khoe et al (1982) described longitudinal studies on the maturation of pattern-onset VEPs and reported the waveform developed into puberty:
The characteristic positive-negative-positive complex with normal peak latencies: Cl (~80msec.), CII (~120msec.) and CIII (~180msec.) became evident from about 16 years of age. The pattern-onset VEP of infants consists of a single positive peak with a latency of about 150msec (Spekreijse, 1978). It appears that CII is absent in infants. Spekreijse (1983) reported that the prevalence of CII in a population of 214 children aged 2 months to 12 years was 0% in the first 5-10 months post term, and increased to about 40% at 20 months, and to approximately 100% by 100 months of age (8 years).

The early absence of CII suggests that neonates have a poorly developed contour mechanism (pattern perception), which is thought to stem from the extrastriate areas 18 and 19. This conclusion is supported by the relative attenuated CII component elicited when the stimulus pattern is de-focused in adult observers (Jeffreys, 1977). De Vries-Khoe and Spekreijse (1982) reported two developmental periods: a phase of fast development from birth to about 8 months, followed by a slower improvement phase up to puberty. The initial fast phase is the same for both threshold (smallest check to produce a VEP), and optimal pattern to produce the largest VEP, indicating that the amplitude versus checksize curve merely shifts along the horizontal axis towards the smaller checks, without a change in shape. De Vries-Khoe and Spekreijse (1982) have proposed that the basis for the initial fast phase in the first eight months of life, are passive developmental retinal processes that take place in the fovea in the first half year of life. Firstly, non-receptor structures, including the retinal ganglion cells move away from the foveal pit, and secondly, the average angular separation of foveal cones diminishes on the one hand, because the cones grow taller and thinner, and on the other hand, because they become more densely packed.

The second slower phase of improvement is evident in the slope of amplitude versus checksize plot: The slope becomes less steep with age, and by puberty the intersection of the horizontal axis approaches one minute of arc. This slower maturation phase is reflected by
the maturation of CII which is highly checksize dependent. Other evidence indicates that CII is of extrastriate origin (Jeffreys and Axford, 1972 b), and thus the improvement in CII may well reflect the maturation of extrastriate cortex.

Ossenblok and Spekreijse (1991) used dipole localization to deduce that two dipoles could account for the generation of the three pattern-onset components in adults (see page 22, for fuller description). The same research group (Ossenblok, Reits and Spekreijse, 1992) also concluded that striate activity dominates the onset VEP of the younger children (under 8 years) and extrastriate activity contributes to the onset VEP in the adult. It is suggested that the VEP of young children is dominated by a single dipole, whose position and orientation localises it to area 17. The waveform evolves from a single positive deflection with a peak latency around 130ms in the under 8 year olds, into a positive (CII) / negative (CIII) complex, with peak latencies of around 80, 100 and 150ms. in children aged 8 to 16 years. The predominance of striate activity in the young child, and the delayed contribution of the extrastriate activity agrees with evidence indicating the relative immaturity of areas 18 and 19, compared with 17 (Yakovlev and Lecours, 1967).

Tari et al (1984) recorded the pattern-reversal and -onset VEPs in a group of 38 normal children, aged 5 to 15 years. Analysis of latency and amplitude demonstrated an inverse relationship with increasing age: The CII latency decreased with age, whereas onset CII, CIII, and reversal component amplitudes all increased with age. The pattern reversal VEPs were largest around 12 years of age, whereas onset VEPs were largest in 10 year old children.

Sokol and Jones (1979) found that the mean latency of the pattern reversal VEP for small checks (<15'), does not reach adult levels until 5 or 6 years of age, whereas the mean latency to large checks (>60') attained adult values by 4 months of age. Moskowitz and Sokol (1983) recorded 439 children, aged 1 month to 5 years, and reported that pattern-
reversal VEP waveforms became more complex with age (increased number of components) - the peaks of components became sharper and better defined, and latencies reduced progressively (Figure 6.1).

![Developmental changes in the human visual system as reflected in the latency of pattern reversal VEPs (large checks=60', small checks=15'; 15x18 deg field size; electrode 1cm above inion). Note positivity upwards.](image)

From Moskowitz and Sokol, 1983.

In adults, peak latency of the VEP varies as a function of spatial frequency, with latency increasing as spatial frequency increases. The latency of all components of the pattern-reversal VEP decreases with maturation. At one month of age, the VEP to large checks has a simpler waveform than that of adults, and consists of a broad positive wave. Responses to very small checks (<15') are usually not detectable. At two months, the main positive wave (P100) to large checks is preceded and followed by negative potentials. By four months of age, N85 and N145 are very distinct for large checks, and present to small
checks. By the age of 4 years, VEP responses are very similar to those of an adult.

The decrease in VEP latency that takes place during the first year of life can be explained in part by anatomical changes in the retina, LGN and cortex and, they complement the maturational changes observed for other visual behaviours such as preferential looking acuity (Birch et al., 1983), contrast sensitivity (Atkinson et al., 1977), binocularity (Boothe et al., 1985) and color vision (Teller and Bornstein, 1985; Morrone et al., 1990). Receptors in the retinal macular area continue to differentiate postnatally, and are mature by about 4 months of age (Abramov et al., 1982). Nakayama (1968) and Magoon et al. (1981) have reported rapid increases in myelination of the optic nerve up to 2 years of age. Hickey (1977) reported that human LGN cells show a rapid increase in size during the first 6 to 12 months after birth, and attain adult-like dimensions by the second year of life. Parvocellular neurons were found to mature more quickly than magnocellular neurons, reaching adult size at 12 and 24 months, respectively. Striate cells receiving parvocellular LGN input also mature sooner than those receiving magnocellular LGN inputs. In macaque, neurons subserving the peripheral visual field have virtually adult levels of contrast sensitivity and acuity at birth, however, those subserving the fovea show a sixfold developmental improvement in both contrast sensitivity and acuity similar to adult levels, and this is reflected in psychophysical measures of acuity and contrast sensitivity development (Blakemore and Vital-Durand, 1981, 1982). Based on infant visual development studies, it appears that parvocellular systems for colour and form representation appear to be operational at an earlier postnatal stage than magnocellular based systems for relative position, motion and depth (Atkinson, 1992).

6.1.2 Amblyopia

Amblyopia is defined as loss of vision due to interruption of normal visual
amblyopia. It is derived from the Greek word for 'dullness of vision' and may be unilateral or bilateral. Von Noorden (1974) proposed a classification of amblyopia based on developmental factors. The main subgroups based on his classification were amblyopia occurring in association with strabismus, anisometropia and form vision deprivation.

Strabismic amblyopia is thought to develop as an adaptive mechanism against visual conflict caused by diplopia and overlap of mis-aligned images. In anisometropic amblyopia, the images falling on the two foveae originate from the same visual object, however, they are of different sharpness, or if the refractive error is corrected, of different size (aniseikonia). The dissimilarity between the two foveal images is incompatible with foveal fusion, and the adaptive mechanism is to suppress the image at the cortical level from one eye (usually the more ametropic eye). Form vision deprivation amblyopia occurs as a result of deprivation of normal visual experience early in life. This most commonly is due to corneal opacities, congenital cataracts, ptosis or other visual obstacles to normal retinal stimulation. The amblyopia is more severe after unilateral than after bilateral visual deprivation.

Clinical observations indicate plasticity associated with maturation of the visual process. It is during the maturational period for stereopsis that the young strabismic patient has the neural plasticity of sensory adaptation. After the age of about 9 years, suppression of vision from the amblyopic eye does not occur and patients can suffer persistent double vision. Thus visual abnormalities that have their onset in the first few months of life have the capability of being partly or wholly reversed, but for a limited period only (so called the critical or sensitive period).

The accepted treatment for amblyopia is occlusion of the fellow, better vision eye; thereby encouraging visual improvement in the weaker amblyopic eye. The best acuity results tend to be achieved by children who are occluded before the age of 4 years. The
treatment is less effective between 4 and 9 years of age and relatively ineffective after the age of 9 years. A possible complication of early patching is the development of occlusion amblyopia in the better vision eye, however this is usually reversible.

There have been numerous psychophysical studies of amblyopes to investigate their subnormal visual performance. For example, amblyopes commonly have elevated contrast thresholds for grating detection (Hess and Howell, 1977; Levi and Harwerth, 1977; Bradley and Freeman, 1981, Holpigion et al., 1986) and poor vernier acuity (Levi and Klein, 1982; Bradley and Freeman, 1985). Psychophysical investigations of amblyopic subjects (e.g. using random dot stereograms) have demonstrated impaired stereopsis, and other aspects of binocularity (e.g. Cooper and Feldman, 1978; Henson and Williams; 1980; Schor et al., 1983; Holopigian et al., 1986).

6.1.3 Neuro-anatomical and physiological basis of amblyopia

Much of our knowledge concerning basic physiological mechanisms in amblyopia have been derived from experimental studies, single unit electrophysiological recordings and neurohistological studies, mainly in cat and monkey.

During the first months after birth, binocular connections are very vulnerable and can be disrupted by visual inequalities between the two eyes. In experiments involving lid suturing at an early age, Hubel and Wiesel (1963) found that very few cortical cells were driven by the sutured eye, with the great majority being activated by the unsutured eye. Binocular deprivation caused less disruption of cortical connections than did monocular deprivation, and binocularity still persisted.

Hubel & Wiesel (1965) produced divergent squints in kittens by sectioning the medial rectus muscle in one eye at about 8-10 days after birth. Single cell recordings revealed that there was a severe decline in the number of cells in striate area 17 that were driven from
both eyes. They concluded that this loss was due, not to loss of cells but rather to changes in the overall ocular dominance distribution. It was apparently the lack of synergy between the afferents from the two eyes that caused the ocular dominance of the cells to change with an overall increase in the numbers in monocularly driven cortical cells. Despite the marked loss of binocular activation by neurons in area 17, the cats with divergent squint showed no behavioural abnormalities suggestive of defective vision. It is presumed that this was because the squint was divergent and the cats developed the ability to fixate with either eye alternately.

Ikeda et al (1974) suggested that it is particularly the sustained retinal neurons that have been deprived from adequate stimulation during early development, and the sustained pathway becomes less effective. Ikeda and Tremain (1978, 1979, 1980) studied the sustained X cells in the area centralis of the retina and the binocular properties of visual cortical cells in control cats and in cats reared with divergent or convergent squint of one eye, or defocused with atropine in one or both eyes. Recordings were made from both the striate cortex, to give an estimate of the proportion of binocularly innervated cortical cells, and from the lateral geniculate nucleus so as to compare the spatial resolving power (acuities) of geniculate neurons receiving their input from the normal retina with that of cells stimulated by the equivalent area of the retina in the squinting eye. Poor responsiveness of X cells was found only in the amblyopic deviating eye. These sustained X cells showed poor contrast sensitivity. Analysis of the sensitivity loss at different spatial frequencies showed that the higher spatial frequencies showed the greatest deficits - a result similar to that found for strabismic amblyopia in humans. In the atropinized kittens, Ikeda & Tremain (1980) simulated the effects of anisometopia. Atropine dilates the pupil and paralyses accommodation, so that the image received is blurred. Atropinized eyes produced less intense responsiveness by the sustained X cells in the area centralis. These results indicated
that amblyopia occurred in the esotropic eye when there was no alternating fixation, and in all atropinized eyes. Comparison of the number of cells driven by each eye showed that in normals the numbers were very similar as they were in cats with convergent or divergent squints with alternating fixation and no amblyopia. But in cats with amblyopia, the number of cortical cells driven by the normal eye was greater than that driven by the amblyopic eye.

Ikeda and Tremain (1978) and Ikeda and Wright (1976) proposed that protracted exposure of the fovea or area centralis to defocused images during the sensitive period is the common factor responsible for the effects of visual deprivation and strabismus. They argue that the defocused image provides an inappropriate stimulus for sustained ganglion cells in the central retina. The weak (or absent) firing of these ganglion cells leads to retarded or arrested growth of LGN cells. The geniculate cells therefore lose normal contrast sensitivity and visual acuity response properties. The inadequate growth and development of the geniculate cells leads to a deficiency in the normal development of the axon terminals at the visual cortex thus allowing input from the normal eye to dominate.

Macaque monkeys appear to have a similar visual system to that of humans and many studies have now used the monkey to provide a closer model. Blakemore, Garey and Vital-Durand (1978), and Crawford et al (1975), studied macaque monkeys and noted that monocular lid suture at about 4 weeks of age for periods of 2 to 4 weeks, caused a marked shift in ocular dominance towards the non-deprived eye at all layers of the striate cortex. Extension of the period of deprivation accentuates the shift in eye dominance, and, with prolonged monocular deprivation for the first two years of life, dominance by the normal eye is virtually complete. When the start of monocular deprivation is delayed until 11 to 16 months of age, there is no detectable influence on layer IVC, but outside IVC there still appears to be a small shift in ocular dominance. Prolonged deprivation in adults has no detectable effect.
Work by Von-Noorden (1970), Baker et al (1974) and Hubel et al (1977), have shown that prolonged monocular lid suturing in monkeys, during the first few weeks of life, leads to cell shrinkage in the layers of the LGN associated with the deprived eye. Furthermore a clear change in the relative sizes of the ocular dominance strips in layer IVC, with a shrinkage of stripes receiving input from the deprived eye and a corresponding expansion of those associated with the normal eye also occurs. These changes appear more marked when deprivation is instigated at 2 weeks of age rather than later.

Many factors have been proposed to account for the effects of visual deprivation. It has been postulated that during the critical period the afferent paths from the two eyes compete for control over cortical cells (Guillery, 1972, 1973; Wiesel & Hubel 1965, Hubel et al, 1977). Geniculocortical axons arising from adjacent laminae are thought to compete for synaptic access to binocular cortical neurones. Unilateral visual deprivation upsets this balance of competition and the reduced cell growth in the LGN is a consequence of the unbalanced axonal development and the smaller numbers of active synapses made by each axon. Hubel et al (1977) have suggested that the same competition mechanism may also explain the deprivation effects that occur in layer IV. The end result of deprivation depends on the amount of overlap of the terminals that still existed at the time of eye closure. This explains the greater severity of the effects of early closures and indicates that once the process of segregation is complete, eye closure will have little or no effect. This competition hypothesis predicts that bilateral lid suture should be less effective than monocular lid suture, since in the former case all the geniculate laminae would be deprived equally.

Sloper (1987) questioned the assumption that cells related to the open, normal eye are unaffected by the closure of the other eye. He compared the sizes of cells of both deprived and undeprived laminae of experimental animals with corresponding LGN cells in normal animals. The LGN in rhesus monkey consists of 6 laminae of which the inner two
consist of larger cells (magnocellular laminae) compared with the outer four laminae which contain smaller cells (the parvocellular laminae). These two sets of cells have been found to react differently to visual deprivation under certain conditions (Schiller and Colby, 1983; Shapley et al., 1980; Sloper, 1987). Sloper found that the initial effect on LGN cells following monocular lid closure at birth was not shrinkage of deprived parvocellular cells, but enlargement of cells in the undeprived laminae. This hypertrophy was responsible for the apparent difference in size seen between deprived and undeprived parvocellular cells. Less hypertrophy occurs in magnocellular laminae. The increased size of the undeprived parvocellular cells is maintained until about 8 weeks of age, when the undeprived parvocellular cells shrink back to normal size. However, the deprived parvocellular cells now also shrink in parallel and so little change in relative size is apparent. By 3 months of age the undeprived cells are of normal size and the deprived cells are very shrunken. This parallel shrinkage has not been seen in the magnocellular cells.

6.1.4 Pattern VEPs in Amblyopia

Many studies have reported that amblyopes show reduced pattern VEP amplitudes for high spatial frequency stimuli. Spekreijse, Khoe and Van der Tweel (1972) described a case study on a single strabismic amblyope. Using pattern reversal stimulation (5' and 20' checksizes, 3 degree display size) they found that, for a range of temporal frequencies (2Hz to 30Hz), the amblyopic eye VEP had a higher contrast threshold than did the fellow eye for all test frequencies. The amblyopic eye had a higher contrast threshold than the fellow eye for low frequency stimuli (2Hz to 17Hz), when presenting with 20' checks, but the same threshold as the fellow eye when presenting at high temporal frequency (20Hz to 30Hz). Pattern onset/offset stimulation with 20' checks was also studied. Both onset and offset VEPs were much reduced in amplitude from the amblyopic eye when compared to the fellow
eye. The onset/offset response of the amblyopic eye resembled that of the fellow eye elicited using an annulus (3-5 deg) stimulus field, thus suggesting that the VEP from the amblyopic eye may have a predominant contribution from the macular region. The amblyopic eye also had a reduced VEP amplitude as compared with the fellow eye for a large range of checksizes, from 2' to 100'. The fellow eye gave largest onset VEP amplitudes for 12' checks, while the amblyopic eye gave largest VEP amplitudes for 50' checks.

Sokol and Bloom (1973) compared monocular VEPs from non-amblyopic and amblyopic eyes in 15 subjects, using pattern reversal stimuli (15' checks, 18 x 18 degree display size, 12 reversals/second). All subjects had smaller VEPs from the amblyopic eye. Checksize effect was studied in one subject who showed a maximum VEP amplitude for 15' checks from the non-amblyopic, and a maximal amplitude for 60' checks from the amblyopic eye.

Mayles and Mulholland (1980) studied 62 strabismic patients aged 2 to 12 years. The stimuli were checksizes varying from 8' to 22', with a reversal frequency of 4Hz. For 8' checks there was a significant difference between normals and those with poor vision, however, for the 22' checks, there was no significant difference. It was found that amblyopic eyes did not produce a typical N80-P100-N145 reversal complex. The most common variation was the loss of the N145 component, postulated to be the outcome of the merging of the P100 with a later positive component. The difference in latency delay for the eyes with good vision and those with worse than 6/24 vision was significant for 11' and 22' checksizes.

Wanger and Nilsson (1978) compared ten amblyopic patients with ten normal subjects. Monocular and binocular pattern reversal stimulation, with 23'checks reversing at 1.4Hz was used. Seven of the ten patients showed a considerable amplitude asymmetry to
monocular stimulation or lack of normal increase of amplitude to binocular stimulation. Two patients displayed prolonged latency on stimulation of the amblyopic eye.

Sokol (1977) studied the pattern-reversal VEP in a 62 year old amblyopic adult. Acuities were 20/20 in the normal eye and 20/400 in the amblyopic eye. For a 12 degree field, the normal eye showed a maximal amplitude with 15' checks, and the amblyopic eye showed a maximal amplitude with 60' checks. There was a significantly larger signal at the 60' checks from the amblyopic eye compared with the normal eye. For a 6 degree field, the normal eye also peaked at 15'. Amplitudes for small checks (7.5' and 15') remained unchanged as field size decreased, but there was a drop in amplitude for the large checks. For the amblyopic eye, the maximal amplitude occurred with 30' checks. For a 3 degree field, both normal and amblyopic eyes showed a maximal amplitude for 15' checks, and there was no difference between the two eyes at other check sizes. This would seem to indicate that the increase of VEP amplitude in response to large checks in the amblyopic eye, (for both 12 and 6 degree fields), was the result of a greater contribution from the paramacular area in the amblyopic eye. To demonstrate this possibility, 12 and 6 degree fields, with the central 3 degree field blanked out were used, and it was found that the amblyopic eye made a greater contribution to the VEP response than did the normal eye. Also, to determine if regions outside the central 6 degree of the amblyopic eye showed any increase in their contribution for large check sizes, a 12 degree field with the a central 6 degree scotoma was presented to each eye and a large drop in the signals was found for the normal eye, especially for small checks. However, for the amblyopic eye, there was a drop in the signals obtained on stimulation with large checks. From these tests, Sokol concluded that regions outside the central 6 degree are essentially similar for the normal and amblyopic eyes, therefore, VEPs produced by the amblyopic eye receive a significant contribution from a retinal region that lies outside the central 3 degrees, but within the central 6 degrees.
Arden and Bernard (1979) studied 28 normal and 71 amblyopic children, aged 4 to 11 years. Of the amblyopic children, 56 had undergone occlusion therapy. It was found that in the amblyopic eyes, the latency of the VEP was increased and the amplitude was decreased, roughly in proportion to the loss of visual acuity. The VEP was found to be normal in the non-amblyopic eyes of the children who had never been treated by occlusion therapy. It was found that occlusion affected the fellow eyes of amblyopes, and increased the VEP latency, so that the response of the fellow eye was delayed beyond that of the amblyopic eye. It was found that after the end of occlusion, the VEP usually returned to normal, however, in a subgroup of patients with prolonged occlusion (12 amblyopes with full time occlusion), the change had not completely reversed to normal when tested one year later. Sokol (1983) measured the P100 latency of the pattern reversal VEP for small checks (15') in 68 normal and 32 amblyopic children. The amblyopic children showed longer P100 latencies in their amblyopic eye than in their fellow eye. Sokol suggested that there may be a selective loss of contrast-specific evoked potential mechanisms in amblyopia.

Animal studies have shown that amblyogenic factors predominantly lead to deficits in the parvocellular mechanisms and human VEP studies of amblyopia demonstrate attenuation of VEPs, mainly to macular-derived, small check (high spatial frequency) stimulation. This relatively selective deficit in amblyopic eyes can thus be used to investigate the macular/paramacular, and magno/parvocellular contributions to components of pattern VEPs. A comparison of pattern-onset, -reversal and -offset modes of stimulation, taking into account ipsilateral and contralateral half-field components appears not to have been previously investigated in amblyopes.
6.2 Methodology

6.2.1 Subjects

Three subject groups were studied: the first consisted of 11 control young adults aged 25 to 38 years (mean 31 years; 5 males, 6 females); the second of 20 control children aged 6 to 16 years (mean 11 years; 12 males, 8 females) and the third group were 18 young amblyopes, aged 7 to 16 years (mean 11 years; 7 males, 11 females).

Control subjects had Snellen acuities of 6/5 or better, and none had a clinical history of visual problems. All amblyopic children had a full orthoptic examination prior to electrophysiological testing. Fourteen were strabismic amblyopes (esotropes) and 4 were anisometropic amblyopes. Acuities of the non-amblyopic eyes at the time of recording were 6/5 or 6/6, with the exception of two strabismic patients, whose acuities were 6/9. Acuities of the amblyopic eyes ranged from 6/36 to 6/6 (mean acuity 6/12). The level of binocular vision was assessed using the Randot stereotests. Binocular single vision was absent in 13 patients (72.2%) and only grossly present in the remaining 5 cases (Randot to 200 seconds of arc). None had latent or manifest latent nystagmus.

All of the amblyopic children had undergone occlusion therapy with good levels of compliance. Age at which occlusion was started ranged from about 2 to 6 years (mean 4.2 years), and the mean number of years of patching was 2.5 years. The extent of occlusion was somewhat variable but tended to be for longer periods in the first few months of therapy (e.g. 6 hours per day) compared with later (e.g. 1/2 hour per day). Only one patient was still having patching therapy of 1 hour per day, at the time of VEP recording, whereas the remaining amblyopes had ended their occlusion therapy at least 2 years prior to testing. The variability in occlusion history of the amblyopes did not lend itself to statistical analysis with respect to VEP measures.
6.2.2 Technique

The stimulating and recording technique used for this experiment was the same as that described in chapter 2. However, as the majority of subjects tested were younger children, the recording time was shortened by using 4 test checksizes only. Compliance with the testing procedure was good: the children were old enough to understand the task and were encouraged throughout the testing procedure to perform well. Furthermore, none of the subjects had profound amblyopia and had acuities which were sufficient to maintain fixation on the target. None of the amblyopes had nystagmus or other fixation instability. Checkerboard patterns were presented to the left half-field (0-12 degrees) of each oscilloscope screen and recording methodology was as described previously (chapter 2). The four checksizes used subtended 12, 20, 50 and 80 minutes of arc at the subject's eye. Binocular, left and right eye responses for each of the four checksizes were recorded; the test order was randomly varied from subject to subject.

6.3 Results

6.3.1 Effects of age - comparison of normal children and adults

Multivariate analysis of variance (MANOVA) was used to compare VEP amplitudes between the adult control group, and the child control group across checksizes. There was no significant difference between groups across the checksizes (Pillais' test, F=0.388, p=1.00), nor in an overall comparison between groups (F=0.784, p=0.741). However, checksizes within each group showed a highly significant effect (F=2.54, p<0.0001), in agreement with previous findings in chapter 3. Similar results were found for MANOVA of VEP latencies: there was no significant difference between groups by checksizes (F=0.958, p=0.584) and checksizes had a significant effect on VEP latencies (F=10.214, p<0.0001). However, unlike the amplitude comparison, the effect of group alone did show a significant
effect on VEP latency (F=5.137, p<0.001), and this was marked for all onset ipsilateral and contralateral components, for reversal N145, and for offset N85 and N165.

Results concerning the effects of checksize alone will not be discussed any further in this chapter, as this is covered in detail in chapter 3.

Control subjects were divided into 3 age groups on the basis of changes expected by previous studies on pattern-onset VEP maturation in these age groups (Ossenblok, Reits and Spekreijse, 1992): i/8 years and under (n=3), ii/8-16 years (n=17), and iii/ over 16 years (adult controls, n=11). Evidence indicates that pattern reversal components are adult like by the age of 4 years, and all our subjects were over the age of 5 years (Moskowitz and Sokol, 1983). There is no pattern-offset maturation data in these age ranges.

![Figure 6.2: Scatter plots of significant onset (A) and offset (B) components with age.](image)

Significant negative correlations were present between these age groups and amplitudes of pattern-onset ipsilateral CIII (Spearman's $r=-0.677$, $p<0.0001$), and contralateral P105 ($r=-0.556$, $p<0.0001$), and the amplitudes of all ipsilateral offset components: N85 ($r=-0.309$, $p<0.01$), P110 ($r=-0.481$, $p<0.0001$) and N165 ($r=-0.387$, $p<0.0001$).
No significant findings were found for any pattern-reversal components. Figures 6.3 and 6.4 show the mean amplitudes of the onset and offset components, respectively, across the checksizes for the 3 age bands. Analysis of variance showed significant differences between the age groups for these component, with the younger age groups showing larger amplitudes (Kruskal-Wallis: CIII, $X^2=66.0$, $p<0.0001$; P105, $X^2=47.06$, $p<0.0001$; N85= $X^2=11.23$, $p<0.005$; P110= $X^2=35.8$, $p<0.0001$ and N165= $X^2=24.6$, $p<0.0001$).

![Figure 6.3: Histogram of mean onset ipsilateral CIII and contralateral P105 amplitudes across age groups.](image)

Similar analyses were conducted on VEP latencies with respect to three age groups. Significant positive correlations were present between increasing age bands and latencies of all ipsilateral onset components (Spearman's $r=0.499$ to $0.568$, $p<0.0001$), ipsilateral reversal N145 ($r=0.275$, $p<0.003$), and ipsilateral N165 ($r=0.35$, $p<0.0001$). A significant negative correlation was present between increasing age groups and contralateral onset P105 latency ($r=-0.317$, $p<0.0001$). The distribution of latencies across age for the above significant components can be found in figure 6.5 for onset and figure 6.6 for reversal and offset.
Comparing these component latencies between the three age bands, significant findings were present for all ipsilateral and contralateral onset components (Kruskal-Wallis, $X^2=30.6$ to 37.4; $p<0.0001$), reversal N145 ($X^2=10.1$, $p<0.007$), and offset N165 ($X^2 = 14.6$, $p<0.0008$).

![Figure 6.4](image1.png) Figure 6.4 Histogram of offset ipsilateral component amplitudes across age groups.

![Figure 6.5](image2.png) Figure 6.5: Histogram of reversal N145 and offset N165 latencies across age groups.
In summary, no significant difference in VEP amplitude between the children and adults were found, however if age is divided into three categories (<8, 8-16 and >16 years), then differences were found for onset CIII and P105 amplitudes, and all ipsilateral offset component amplitudes (>16 year olds having smaller responses). VEP latencies differed significantly between the age groups, with VEPs being prolonged in the oldest age group, for all ipsilateral onset components, offset N165 as well as reversal N145, however, onset contralateral P105 showed the opposite and was shorter in the adult group.

6.3.2 Comparison of amblyopic with fellow eye in amblyopic children

For the majority of VEP components, amplitudes were smaller and latencies more prolonged from the amblyopic eye when compared with the fellow eye (Figure 6.7). MANOVA of the onset, reversal and offset VEPs with respect to checksize and viewing eye showed no significant checksize by eye interaction effect, but significant independent effects of viewing eye (Pillars test, F= 2.159, p<0.012) and checksize (F=2.88, p<0.0001).
6.8 shows the group average VEP responses of all 18 amblyopic subjects.

Univariate results showed that only certain VEP components contributed to the effect of viewing eye on the overall results. For VEP amplitudes, only two pattern-onset components showed a significant difference between amblyopic and fellow eyes, and this was seen for ipsilateral CII (F=6.871, p<0.02) and contra'lateral P105 (F=5.46, p<0.03)-figure 6.9. On the other hand, for latency, only pattern reversal ipsilateral components, showed significant differences between fellow and amblyopic eyes (N80: F=9.83, p<0.001; P100: F=14.639, p<0.001; N145: F=9.04, p<0.005) - figure 6.10.
Figure 6.8: Group average ipsilateral and contralateral VEPs (n=18) of the amblyopic and non-amblyopic eyes across the 4 checksizes.

Figure 6.11 shows the mean P100 amplitude and latency changes with checksize for the amblyopic and fellow eyes. This graph and the statistical analysis showed that there was a tendency for the P100 amplitude from the fellow eye to show a tuning effect and be largest for 50' checks. However, this effect may have been somewhat smeared by the group averaging and was not conspicuous in figure 6.8. Figure 6.11 shows that the P100 response from the amblyopic eye did not demonstrate a tuning effect and continued to increase with increasing checksize, whereas the fellow eye peaked for 50' checks. Latency, on the other hand, decreased with increasing checksize for both eyes in parallel.
6.3.3 Comparison of amblyopes and normally sighted subjects

The VEP measurements from the amblyopic and fellow eyes of amblyopic children were compared with the corresponding eyes of control, normally sighted children of the same age range, using the Mann-Whitney U-test. Significant differences (p<0.05) were found between the amplitudes of the amblyopic eye and corresponding control eye for onset CII,
CIII and contralateral P105, reversal N145, and offset N165. Mean amplitudes and standard deviations for these components, together with respective 'P' values can be found in Table 6.1. Significant differences were also present between the latencies of amblyopic eyes and corresponding control eyes for onset CII and contralateral P105, reversal N80, P100 and N145, and offset N85 and N165. Mean values can be found in Table 6.2.

Interestingly, there were also some significant differences when the fellow eye of the amblyopic children were compared to the corresponding normal eye of children in the control group. These were for reversal P100 amplitude (mean fellow= 4.4μV±3.4, control=5.4 ±3.3; p<0.001) and offset N165 latency (mean fellow 156.8ms ±23.2, control=147.7ms ±16.8, p<0.007).

<table>
<thead>
<tr>
<th></th>
<th>Amblyopic Eye</th>
<th>Control Eye</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset CII</td>
<td>2.2 uV ± 2.2</td>
<td>3.7uV ± 3.7</td>
<td>0.046</td>
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<tr>
<td>Onset CIII</td>
<td>6.7 uV ± 5.1</td>
<td>11.4 uV ± 6.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Onset P105</td>
<td>9.9 uV ± 5.3</td>
<td>13.2 uV ± 6.3</td>
<td>0.002</td>
</tr>
<tr>
<td>Reversal N145</td>
<td>3.8 uV ± 3.0</td>
<td>6.02 uV ±3.5</td>
<td>0.000</td>
</tr>
<tr>
<td>Offset N165</td>
<td>4.1 uV ±3.2</td>
<td>5.5 uV ± 3.2</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Table 6.1: Means and standard deviations of VEP component amplitudes that differed significantly between amblyopic and control eyes.
<table>
<thead>
<tr>
<th></th>
<th>Amblyopic Eye</th>
<th>Control Eye</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset CI</td>
<td>57.9 ms ±22.2</td>
<td>48.9 ms ±16.9</td>
<td>0.018</td>
</tr>
<tr>
<td>Onset P105</td>
<td>136.9 ms ±20.3</td>
<td>126.3 ms ±13.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Reversal N80</td>
<td>74.2 ms ±15.3</td>
<td>63.4 ms ±10.6</td>
<td>0.000</td>
</tr>
<tr>
<td>Reversal P100</td>
<td>108.1 ms ±17.8</td>
<td>96.1 ms ±9.5</td>
<td>0.000</td>
</tr>
<tr>
<td>Reversal N145</td>
<td>147.2 ms ±25.2</td>
<td>136.9 ms ±16.2</td>
<td>0.030</td>
</tr>
<tr>
<td>Offset N85</td>
<td>80.9 ms ±18.1</td>
<td>74.2 ms ±13.8</td>
<td>0.030</td>
</tr>
<tr>
<td>Offset N165</td>
<td>159.1 ms ±27.1</td>
<td>147.9 ms ±16.8</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Table 6.2: Means and standard deviations of VEP component latencies that differed significantly between amblyopic and control eyes.

6.4 Discussion

6.4.1 Effects of Age

Age appears to have a greater effect on VEP latency than on amplitude in the population studied (age range 6 to 38 years). Furthermore, pattern-onset and -offset components were more influenced by age than pattern-reversal components. It is interesting that if subjects are simply divided into children < 16 years and adults > 16 years, then there were no differences in VEP amplitudes between the two groups, however if the children are further sub-divided into 8 years and under, and, 8-16 years of age, then both onset (ipsilateral CIII and contralateral P105) and offset (all ipsilateral components) VEP amplitudes were significantly larger in the younger age groups compared with the adult group (>16 years).

The only reversal parameter to be affected by age was the ipsilateral N145 latency,
which increased with increasing age. This trend is similar to that obtained for all ipsilateral onset components and offset N165. Contralateral onset P105 deviated from this trend of positive correlation as it showed a significant decrease in latency in the adult group compared with the children.

The majority of previous studies on the development of VEPs with age have used either pattern-onset or -reversal and there appear to be few reports of maturational changes of offset VEPs. Furthermore, most studies have concentrated on younger age groups (in the first year of life and under 5 years of age) or have compared young adults with elderly (>60 years) subjects. Pattern-reversal VEPs are reported to reach adult size, latency and morphology by the age of 4 years of age (Moskowitz and Sokol, 1983). Since our child population was older than this, it is not surprising that pattern-reversal components did not vary significantly between the adults and children, (with the sole exception of N145 latency).

Studies of the effect of age on pattern-reversal VEPs have shown that P100 latency increases with increasing age: Celesia and Daly (1977) studied 15 to 70 year old volunteers and found a linear increase in mean latency with age (increasing from 93ms to 103ms over this period). Allison et al (1979) reported significant increases in reversal P100 latency after 50 years of age only. The results from the present study of 6 to 38 year olds thus are in agreement with previous reports that show no significant P100 latency changes in later childhood and early adulthood. Allison et al (1984) described the effects of age on other reversal components; they found a small, linear increase in latency of the N80 component over the entire age range of 4 to 95 years (<0.05ms/year). The N145 latency was found to decrease with age and reached adult levels at about 30 years, after which it increased again after 50 years of age. However, the results for N145 latency from this study shows the opposite: an increase in latency with age.

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Wright et al. (1985) studied flash, pattern onset-offset and reversal VEPs across the age span, and showed that the flash and onset-offset VEPs had age-related changes in morphology, whereas, the reversal waveform remained constant. Reversal amplitudes were found to be high in teenagers, but then reduced in the second decade, and remained relatively constant thereafter. Latencies increased with age for both pattern reversal P100 and onset CII. This was ascribed to reduction in retinal illuminance due to decrease in pupil size with age. Kriss et al (1984) compared pattern-onset, -reversal and -offset VEPs between young adults (mean 29 years) and elderly adults (mean age 79.8 years) using different checksizes. They found a general tendency for prolonged latencies in the older group, and for the later components of each VEP mode to have a greater increase in latency compared with the earlier components. Kriss et al found that onset components were relatively more prolonged than reversal and offset components in the older group. Although the age range in our study was very different, we also found more onset components than reversal or offset components showing increases in latency with age.

Kriss et al (1984) found amplitudes of all reversal components, offset N85 and P110, and onset CIII, to be significantly larger in the younger age group compared with the older group; however, onset CI and CII showed the opposite trend and were larger in the older group. Once again our results, though derived from a different age range, gave similar results; we found onset CIII and all offset ipsilateral components to be larger in the younger age group. Reversal components did not show significant changes. Furthermore, onset contralateral P105 (which was not described by Kriss et al., 1984, as half-fields were not done) was significantly larger, as well as earlier, in the children compared with those over 16 years of age. Interestingly, P105 latency shows an opposite significant trend to the ipsilateral onset CI, CII and CIII components, which suggests different origins and developmental profiles for this component.
Skull thickness, temperature and head size have been postulated as contributing to sex differences in VEPs, with females having 20% larger and 3-5ms faster responses (Stockard et al., 1979; Halliday et al., 1982; Kriss et al, 1984). It is also known that head circumference continues to increase until about 18 years of age, with greater increases in the younger age groups. It thus seems plausible that the larger amplitudes and shorter latencies for the majority of components seen in our younger subjects could be attributable to their immature smaller head size.

Ossenblok et al (1992) reported that striate area 17 activity dominates the onset VEP in children under 8 years of age, whereas extrastriate (areas 18 and 19) activity dominates the onset VEP in older children. These findings were based on a study of 10 children aged 6 to 16 years. It was found that in children under 8 years of age, a positivity at around 130ms dominates the onset VEP and this changes to a negative-positive (CII-CIII) complex with latencies at around 100 and 150ms between the ages of 9 and 16 years. The emergence of adult-like CII and CIII, (both components thought to be of extrastriate origin- Jeffreys, 1971; Jeffreys & Axford, 1972 a,b; Maier et al, 1987) from a single positivity supports the neuroanatomical evidence relating to neuronal changes in the striate cortex which is not completed before puberty (Schadé and Van Groenigen, 1961; Huttenlocher, De Courten, Carey and Van der Loos, 1982; Carey and De Courten, 1983). There is also evidence that areas 18 and 19 are relatively immature compared with area 17 (Yakovlev and Lecours, 1967). The number of synapses in the striate cortex is largest between 8 months and 2 years of age, after which there is a decline in synaptic density per neurone until the age of about 11 years when the numbers decrease to around 60% of the maximal value (Huttenlocher et al, 1982; Carey and De Courten, 1983). Such changes which represent the maturation of the striate cortex are likely to influence the VEP.

The results from the present study does not provide much support for this shift from
a predominantly striate-originated VEP to an extrastriate-one with age: There were no
significant findings for CII amplitude, although CIII did increase in latency with age and the
contralateral P105 component, which is thought to be of striate origin (Jeffreys & Axford,
1972 a,b) did decrease in amplitude with age. Lack of more significant results may be due
to our small number of under 8 year olds (n=3), and, possibly due to our use of a left half­
field stimulus: Ossenblock, De Munck et al (1994) showed that extrastriate activity becomes
more pronounced on stimulation of the left half of the visual field and this could possibly
mask the transition from striate to extrastriate dominated VEPs.

6.4.2 Effects of Amblyopia

There were more VEP components showing significant differences in amplitude and
latency when amblyopic eyes were compared with the corresponding eyes of control
subjects, as opposed to comparing the amblyopic eyes with the fellow eyes within the same
amblyopic group of subjects. Only onset ipsilateral CII and contralateral P105 were
significantly attenuated in the amblyopic eyes when compared with fellow eyes in the
amblyopic subjects. On the other hand, only reversal ipsilateral N80, P100 and N145 were
significantly prolonged from the amblyopic eyes when compared to their fellow eyes. If
VEPs from amblyopic eyes are compared to VEPs from control subjects, then as well as the
above mentioned components, reversal N145, and offset N165 amplitudes are also
significantly attenuated, and onset CII, CIII and contralateral P105 and offset N85 and N165
are prolonged.

These differences and the fact that significant differences were also found between
the fellow eyes of amblyopic subjects and corresponding eyes of controls, suggests that the
fellow eye of amblyopes is not totally normal and may also be slightly amblyopic. All our
amblyopic subjects had had occlusion therapy. Occlusion amblyopia and attenuated VEPs
in the fellow eyes of patients undergoing patching therapy has been reported (Kriss et al., 1994), and Arden and Barnard (1979) found that VEPs were larger and of shorter latency in the fellow eyes of amblyopes who have never had occlusion therapy, compared with those who had been patched. Further evidence of the involvement of fellow eyes comes from animal neuroanatomical studies which have shown that LGN parvocellular cells of both visually deprived and undeprived eyes are affected (Headon et al, 1985; Sloper, 1987). We found that reversal P100 (amplitude) and offset N165 (latency) were affected in the fellow eyes.

The subnormal findings for the fellow eyes of amblyopes has important clinical implications in amblyopia therapy. These findings suggest that amblyopic patients undergoing occlusion therapy need to have both eyes regularly and carefully assessed to ensure that the visual performance of the fellow eye does not deteriorate with therapy to the amblyopic eye. Moreover, the younger the child, the more rigorous care is needed in monitoring them, as their visual system has greater plasticity and would be more susceptible to amblyogenic factors. The extent of patching prescribed (hours per day) would thus need to be flexible and reviewed regularly to ensure minimal detriments to the fellow eye. The use of VEPs clinically would be useful in monitoring amblyopia, particularly in the very young, and in those who are developmentally delayed and can not adequately perform optometric testing. The amblyopic eye and fellow eye VEPs can be compared with each other to assess interocular differences and, this can be monitored over the period of occlusion therapy to ensure a) that the occlusion therapy is helping improve the amblyopic eye responses and, b) that the fellow eye responses do not deteriorate with therapy. However, it is important not only to compare amblyopic to fellow eyes, but also to compare the eyes of amblyopes with those of age matched laboratory controls.

The reversal P100 component is thought to reflect macular pathway activity
(Blumhardt et al, 1978; Haimovic and Pedley, 1982; Yiannikas and Walsh, 1983). We found (as described in chapter 3), that ipsilateral reversal components were sensitive to checksize and were larger in amplitude and more prolonged in latency when elicited by small checksizes. The reversal P100 component was particularly sensitive to small (0-1.5 deg) central scotoma (as found in chapter 4). The ipsilateral reversal components, in particular the P100 component has properties indicating macular activation, and when small checks are used, likely to reflect predominant activation by parvocellular mechanisms.

Our results agree with previous reports of attenuated pattern-reversal and -onset VEPs from amblyopic eyes (Arden, Barnard and Mushin, 1974; Regan, 1977; Sokol and Bloom, 1973, Spekreijse, Khoe and Van der Tweel, 1972) and suggest that the predominant contribution to the amblyopic eye VEP is from the paramacular areas. This is most clearly seen when comparing amblyopic and fellow eye changes of P100 VEP amplitude with checksize (figure 6.11): The fellow eye shows a tuning effect similar to that reported for normals in chapter 3, with largest amplitudes at 50' check stimulation, whereas amblyopic eyes show a steady increase in amplitude with increasing checksize, so that the largest responses are found for the largest 80' checks. In fact, at this checksize, mean amblyopic eye amplitudes are greater than that for the fellow eye - a similar finding was reported by Sokol and Bloom (1973) in one subject, where VEPs to 60' checks were greater in the amblyopic eye than in the fellow eye. It is interesting that the VEP tuning effect found in control eyes is different to the psychophysical findings reported in the literature in which sensitivity is higher for smaller element sizes (typically 4-8 cycles/deg., depending on stimulus variables such as field size). The relationship between psychophysical and electrophysiological measures are only approximate, but this has been shown to be useful when testing pre-verbal children. Several factors are likely to contribute to differences between electrophysiological and psychophysical measures, these include: VEPs are mass
responses recorded at the scalp (i.e. attenuated compared to cortical surface) and generated
mainly from primary visual areas, whereas psychophysical measures are behavioural
responses that involve primary and higher cortical areas; psychophysical measures usually
involve threshold detection, whereas in this case VEP tuning assessment the maximal
responses are most often being gauged.

Sokol (1977) used experimental scotomata and different field sizes to test amblyopic
and normal eyes. He concluded that the VEP from an amblyopic eye arises from outside the
central 3 degrees of the visual field. It has been shown that the central 1 degree field
contributes to onset CII, and that P105 is derived from the central 2-6 degrees; also CII and
CIII have been found to be less dependent on contrast and more on pattern detail (Jeffreys,
1971; Jeffreys and Axford, 1972, a,b). Furthermore, our results from experimental scotoma
studies (chapter 4) show that a central 0-1.5 degrees scotoma significantly attenuated reversal
P100 and N145 as well as onset contralateral P105. Based on the above results, we expected
onset CII, CIII, contralateral P105, reversal P100 and N145 to be affected in amblyopic eyes.
On the other hand, onset CI, is predominantly a luminance dependent component (Jeffreys,
1971; Jeffreys and Axford, 1972, a,b) and the analysis in chapter 3, showed that it is
maximal with the largest checksizes. Data in chapters 3 and 4 showed that contralateral
reversal N105 and offset N115 were not significantly affected by central scotomata, and were
maximal to the largest checksize and were thus primarily of paramacular origin. It was
postulated that onset CI and the contralateral components N105 and N115 primarily reflect
magnocellular activity, and thus we would not expect them to show any difference between
normal and amblyopic eyes.

On the whole, findings from this study support these expectations. The amplitudes
of onset CII, CIII and P105, and reversal N145 were significantly attenuated in amblyopic
eyes. One further component that we had not anticipated to be degraded in amblyopic eyes
when compared to control eyes was, the offset N165. However, offset N165 has been postulated to be of similar origin to reversal N145 (Estévez & Spekreijse, 1974; Kriss & Halliday, 1980), and thus may be affected in a similar manner in amblyopia. Significantly prolonged VEP latencies in amblyopic eyes occurred for all ipsilateral reversal components, and for N85 and N165 offset components. It was surprising that offset P110, was not like the preceding and proceeding negativities (N85, N165) in showing similar trends to the reversal NPN complex (as would be expected from the work cited above by Estévez & Spekreijse, 1974; Kriss & Halliday, 1980).

Prolonged and broadened onset contralateral P105 was found in amblyopes (figure 6.8). This possibly represents the paramacular 'sub-component' of P105 that was described in chapters 3 and 4. This paramacular P105 was found to occur later and appear broadened or bifid for stimulation with large checksizes and with introduction of central scotomata. The sharply defined early macular P105 seen with small check stimulation in normal eyes (chapter 3) would not be produced by amblyopic eyes where macular vision is compromised.

6.5 Conclusions

The pattern VEP changes that were found in amblyopic eyes support the previous findings regarding the macular/paramacular derivation of pattern-onset, -reversal and -offset components and their probable indication of parvocellular/magnocellular pathway activation. Amblyopic eyes have deficits primarily affecting the central field (3 degrees) of vision resulting in both the attenuation and prolongation of certain VEP components that are generated macularly.

Ipsilateral pattern-reversal components were all compromised in amblyopic eyes. Furthermore, offset ipsilateral N85 and N165 were similarly affected. Onset components, were also affected and this was particularly conspicuous for the macular-derived CII and
P105 components. If amblyopic eyes are compared with fellow eyes, then pattern-reversal stimulation is the best mode for accentuating the differences between the affected and unaffected eyes. Offset stimulation, in this case, showed no significant differences, and onset stimulation showed some differences, but these were not as significant as those for reversal. However, if an amblyopic eye is compared with the corresponding eye of a control subject, then all ipsilateral and contralateral onset, and all ipsilateral reversal and offset components are affected either in amplitude and/or latency. In spite of the fact that all 3 modes of stimulation appear to distinguish amblyopic from normal VEP responses, the results for pattern-reversal components reached higher levels of statistical significance.
7. Interocular Interaction Reflected in Pattern-Onset, -Reversal and -Offset

VEPs in Normal and Amblyopic subjects

7.1 Introduction

The visual evoked potential has been used to assess binocularity, but the relationship between the monocular VEPs to the binocular VEP is not well understood. Comparison of pattern VEPs to binocular and monocular stimulation has been performed in a number of studies (e.g., Campbell & Maffei, 1970; Wanger & Nilsson, 1978; Srebro, 1978), most have found that in normal subjects, the binocular pattern VEP is of greater amplitude than either of the monocular responses. The amplitude of a binocular VEP is reported commonly as being 1.4 times the mean monocular VEP (Campbell & Maffei, 1970; Wanger and Nilsson, 1978). However, the extent to which the binocular response is larger than the monocular response varies with stimulus conditions (such as spatial frequency, contrast and temporal frequency). Most reports describe that the binocular response is only partially larger than the monocular response - partial summation (e.g., White & Bonelli, 1970; Harter et al, 1973; Vaegan et al., 1980), whereas others report facilitation, that is the binocular VEP amplitude is greater than the sum of the monoculars (Ciganek, 1970; Srebro, 1978, Apkarian, Nakayama & Tyler, 1981) and even zero summation (the same size as the monocular response) has been found (Inoue, 1966).

There is variability in the findings of results obtained from normals with those from subjects with defective binocular vision (Apkarian, Levi & Tyler 1981; Harter et al, 1977; Vaegan et al, 1980). Thus the suitability of the VEPs in the clinical assessment of binocular vision disturbances, remains somewhat questionable. Most previous studies have used full-field grating or checkerboard reversal stimuli and have confined their analysis to the main positive component (P100). However there is evidence that pattern-onset stimulation, in
particular may be more effective than pattern reversal in demonstrating visual pathway
disease (Apkarian, 1991, 1994). The aim of this study was to compare changes in pattern-
onset, -reversal and -offset VEPs to assess their relative effectiveness in indicating binocular
interaction. As in the other experiments, responses to half-field stimulation were recorded
using a range of checksizes in order to separate the contributions from macular and
paramacular areas of the visual field. VEPs were recorded in normals and in amblyopic
subjects who have poor macular vision.

7.2 Methodology

The techniques employed for VEP recording and the control and amblyopic subjects
involved in this study are identical to those in the previous chapter 6.

7.3 Results

7.3.1 Binocular / Monocular Amplitude Ratios

There were no significant differences when comparing VEPs obtained from the left
and right eyes of normal subjects nor when comparing binocular/left eye ratios with
binocular right/eye ratios - (The use of the word 'binocular' indicates that both eyes are
stimulated and does not imply any level of binocular single vision). 'Binocular:monocular'
amplitude ratios were thus calculated as the 'binocular/mean left and right eye amplitude' for
normal subjects; and, 'binocular/non-amblyopic eye amplitude' for amblyopic subjects.

Both partial summation (1<ratio<2) and facilitation (ratio >2) were found in normal
adults and children across all VEP components. Mean binocular/monocular ratios ranged
from 1.05 to 1.99 (mean= 1.38 ±0.72). The reversal P100 component showed the least
variability and gave ratios ranging from 1.45 to 1.71 (mean= 1.59 ± 0.57). Ratios for reversal
N145 extended from 1.04 to 1.71 (mean= 1.32 ±1.04), and, those for reversal N80 extended
from 0.99 to 2.9 (mean= 1.83 ±2.2). Pattern offset components were less consistent than the reversal components with ratios extending from .79 to 5.97 (mean= 1.84 ±2.32).

Figure 7.1: Binocular and monocular VEP responses of a normal subject. The negative ipsilateral main components have labelled for onset (CII), reversal (N80) and offset (N85) responses.

There were no statistical differences between the VEP findings of normal children and normal adults, so the two control groups were combined into a single normal group. Figure 7.1 shows the binocular and monocular responses of a normal subject. Note that the binocular response is consistently larger (by ≈ 25%) than the monocular response. Note also that some components, (in particular, onset CII, reversal N80 and offset N85) tend to decrease in amplitude with increasing checksize.
In amblyopic subjects, stimulation with the smallest checks (12') produced ratios of near 1 for all the VEP components. Low ratios were also obtained for the reversal P100 component elicited by 20' and 50' checks which gave mean values just over 1 (1.08, 1.07 respectively). This implies that for small check stimulation, the binocular VEP is predominantly reflecting contribution from the non-amblyopic eye. Figure 6.7 in the previous chapter, shows the non-amblyopic eye responses are of similar size to the binocular responses.

Multivariate analysis of variance (MANOVA), for ratios of all VEP components with checksize and subject group as factors, showed an effect for subject group (normal vs amblyopic). This was highly significant (Pillai's test: F=4.49, d.f.=10; p<0.0001). Further examination of the univariate results revealed that it was the reversal P100 (F=27.7 p<0.0001) and the onset contralateral P105 (F= 7.3; p<0.008) ratios that showed the greatest differences. Comparing binocular/monocular VEP amplitude ratios between amblyopes and controls, a significant difference (with full Bonferroni adjustment, p<0.001) was found for the reversal P100 components for the smallest 12' checks and for the moderate sized checks (20' and 50' checks). Onset VEP ratios (for ipsilateral CII and contralateral P105) also showed significant differences, but only for the smallest 12' checks (with full Bonferroni adjustment, p<0.0001 and p<0.001, respectively). Small checksizes are known to enhance macular-derived potentials (Halliday, Barrett, Blumhardt & Kriss, 1979; Kurita-Tashima, Tobimatsu Nakayama-Hiromatsu & Kato, 1991; Bodis-Wollner, Brannan, Nicoll, Svetlana & Mylin, 1992) thus, these results suggest that it is the poor macular function in amblyopes that predominantly affects interocular interaction. Likewise, the P100 potential which is known to reflect macular function, is the component that shows the greatest difference between normals and amblyopes for small and moderate sized checks (12', 20' and 50'), but not for the largest checks (80'). Figure 7.2 shows clearly that as checksize increases, the
difference in amplitude ratios for P100 amplitude ratios between the normals and amblyopes decreases.

All offset VEP components and the remaining onset (C1, CIII) and reversal (N80, N145, N105) components did not show any significant findings.

\[ \text{Figure: 7.2 Mean P100 ratio (+/- S.E. mean) for controls and amblyopes.} \]

\( (* \text{ denotes a significant difference between controls and amblyopes}) \)

N.B. Controls and amblyopes tested with the same 4 checksizes - points on graph have been offset horizontally to avoid overlap of S.E. bars.

7.3.2 Binocular-Monocular Latency Differences:

There were no significant differences in the latencies of any of the components when comparing normal adults and normal children. Thus for further analysis normal children and adults were combined and treated as a single group to compare with the amblyopes.

The mean binocular latencies of all pattern-onset and -reversal VEPs, were shorter or equal to the mean monocular latency (of either eye) for stimulation with all checksizes. This was statistically significant (paired t-test, with full Bonferroni adjustment, \( p<0.001 \)) for
onset P105 component for all checksizes and for onset CIII, 50' and 80' checks only. For
reversal stimulation, a significant difference (paired t-test, with full Bonferroni adjustment, 
p<0.001) was found for the N80 component, for all checksizes and, for the P100 component, 
checksizes 12', 20' and 50'. Offset components showed no such trend (Fig 7.3).

Figure 7.3: Group average binocular VEPs (thin trace) superimposed upon monocular left eye VEPs (thick trace) 
of all normal subjects (n= 31). Vertical lines are placed through components that gave statistically significant 
binocular-monocular latency differences (reversal, N80 and P100; onset P105 and CIII).
Amblyopes did not show significant differences between binocular and monocular findings for the non-amblyopic eye. Some trends were noticed but these did not reach statistical significance: binocular reversal and offset components were of marginally longer latency, whereas onset components tended to be of shorter latency when compared with those obtained from the non-amblyopic eye. When compared with VEP latencies from the amblyopic eye, all binocular onset and reversal VEPs were shorter. Ipsilateral pattern-offset components did not show significant latency changes; however, the contralateral N115 was prolonged from the amblyopic eye when compared with that elicited binocularly. Binocular and monocular group average VEPs of amblyopic subjects can be seen in chapter 6 (figure 6.8). It can be observed that latencies of VEPs for binocular and non-amblyopic eye stimulation are very similar, whereas those from amblyopic eyes are relatively prolonged.

The binocular-monocular latency differences were compared between the control and amblyopic groups. The difference in control subjects was calculated as the 'binocular latency-mean monocular latency', whereas for amblyopic subjects the 'binocular latency-non-amblyopic eye latency' was used.

MANOVA of binocular-monocular latency differences for all checksizes and subject groups was carried out. A significant effect of checksize by subject group was found (Pillai's test; F= 1.66, d.f.= 30; p<0.02). Examination of the univariate findings showed that the predominant contribution was from the reversal P100 latency differences (F= 3.27, p<0.03). Checksize alone was also significant (Pillai's test, F=1.5, d.f.=30; p<0.05) with reversal P100 and offset N85 latency differences contributing to the overall effect (F= 2.91, p<0.04 and F=4.0, p<0.01 respectively). The effect of subject group alone was significant too (Pillai's test: F=3.3, d.f.=10, p<0.001) predominantly due to the marked differences for reversal N80 and P100 (F=17.9, and F= 13.1 respectively, both p<0.0001) and offset N85 latency differences (F=5.4, p<0.03). Figure 7.4 summarises the mean latency differences for
reversal N80 and P100 components. The predominance of significant latency results for the pattern-reversal VEPs, and the relative insensitivity of pattern-offset, are in line with the amplitude ratio findings, highlighting pattern reversal as the more reliable means of differentiating between normals and amblyopes.

The effects of visual acuity, type of amblyopia (strabismic or anisometropic) and the presence or absence of gross stereopsis on the binocular/monocular amplitude ratios and latency differences were investigated in the amblyopic patients. There were no significant correlations between visual acuity and VEP amplitude ratios or with latency differences. MANOVA of these VEP measures with respect to type of amblyopia and stereopsis also did not reveal any significant effects.
7.4 Discussion

In general VEP latency, as opposed to amplitude, is recognised as a more stable and reliable variable for experimental and clinical assessment. Our systematic analysis, comparing the three modes of stimulus presentations for different check sizes, VEP components, and for both amplitude binocular: monocular ratios and binocular-monocular latencies, has confirmed that latency and amplitude are robust variables: the reversal P100 amplitude ratios and, binocular-monocular latencies of the reversal components (N80 and P100), were consistently and significantly different when comparing normal and stereoblind subjects.

Assessment of binocular interaction using the pattern VEP amplitude arose from experimental findings in animals and reports that single neurones respond to similar visual inputs to the two eyes, and were selectively sensitive to particular amounts of binocular disparity (Barlow et al 1967; Pettigrew et al 1968). There is a unanimity between studies regarding the existence of neurones responding to differing amounts of preferred disparity. Such cells produce large responses only at an optimal binocular disparity, and, in some studies the response at this preferred disparity is often much larger than the sum of the responses obtained on stimulation of each eye separately. Thus, it appears that there can be a considerable degree of facilitation in cortical cell responses and that the presence of neural facilitation may be related to the process of stereopsis. However, VEPs appear not to reflect well the single cell studies. The rarity of binocular facilitation reported for VEPs is in strong contrast to the frequent binocular facilitation revealed by single unit research (Pettigrew et al, 1968; Poggio and Fischer, 1977).

Pattern VEPs have been used to examine interactions associated with binocular vision by recording the VEPs from each eye individually and comparing these with those viewed binocularly (Srebro, 1978; Arden, Bernard and Mushin, 1974; Wanger and Nilsson, 1978;
Campbell and Maffei, 1970). There is general agreement that the amplitude of the binocular pattern reversal VEP is greater than the amplitude of either of the monocular responses. Although this result seems to hold for a large number of individuals with normal binocularity, the results for individuals with abnormal binocular vision due to strabismus or anisometropia have been variable. Srebro, (1978) reported binocular facilitation in normals and its failure when binocularity is disrupted. When viewing binocularly, the VEP was found to be 25%-30% greater in amplitude than the sum of the amplitudes for monocular viewing. This binocular facilitation was lost in patients with small angle esotropias and in normals whose binocular function is disturbed by a vertical prism placed over one eye. The binocular VEP amplitude in the esotropes and normals with prisms was generally only slightly larger than that observed when the normal eye viewed the target alone.

Apkarian et al. (1981) studied binocular interactions for a range of stimulus conditions including changes in spatial frequency and contrast. The degree of binocular interaction in normals (ratio of binocular to the average monocular response) ranged from zero summation to binocular facilitation. Facilitation was found to occur between about 1 and 3 c/deg. At the higher spatial frequencies, facilitation was absent and there was only partial summation. At low contrast levels and for uniform field flicker, there was no interocular rivalry yet the 'binocular' responses were of similar amplitude to the monocular responses (zero summation). It was found that the response amplitude and the degree of binocular summation and facilitation could vary markedly with slight variations in contrast. They concluded that the complexity of the various VEP interactions places severe limitations on the usefulness of binocular interaction studies. In our study, facilitation in amblyopes could not be assessed as binocular responses were compared to those from the fellow eye, and not to half the sum of the monocular responses, as in the study by Apkarian et al. (1981). Facilitation was rare in our normal populations: the two stimulus conditions in
normals that were significantly different from amblyopes resulted in facilitation (ratio >2) and these were reversal N80 (12') and offset N85 (20''); under identical stimulus conditions, the mean ratio values for amblyopes were 1.00 and 0.94 respectively, which essentially indicates 'zero summation'.

Most studies agree that for individuals with normal binocularity, the amplitude of the binocular VEP is approximately 1.4 times greater than the amplitude of either corresponding monocular response (Campbell and Maffei 1970; Wanger and Nilsson, 1978). Vaegan et al (1980) studied normal subjects using transient and sustained VEP stimulation modes. The sustained mode (2Hz.) involved the onset-offset of vertical gratings at the peak and high end of the contrast sensitivity function. However, the transient mode (8Hz) involved peripheral grating reversal at the low or peak of the contrast sensitivity function, with occlusion of the central 3 degrees to prevent foveal stimulation. The binocular VEP in the normals was found to be 1.4 times larger than the mean monocular response, however this was only true away from the peak of the contrast sensitivity function. At the peak, the binocular:mean monocular ratio was significantly larger (1.64). Interestingly, about half of the stimulus conditions we used in our study for the normal subjects resulted in ratios that were greater than 1.4. Ratio values of around 1.4 (>1.3>1.5) were predominantly found for onset CIII and P105, whereas the reversal P100 gave values around 1.6 (range: 1.54-1.66).

The attenuated binocular:monocular amplitude ratios in the amblyopic subjects when compared with the normal subjects support the other studies and imply reduced binocular interaction and minimal contributions from the amblyopic eye to the binocular response. This is a particularly consistent and significant finding for the pattern-reversal main positivity (P100). The link between maximal binocular:monocular ratios and peak contrast sensitivity functions could well explain this phenomenon where pattern-reversal (a more optimal stimulus than pattern-onset -offset for ganglion cell excitation) is the preferred mode for
elucidating the differences in binocular interaction between normal and amblyopic subjects.

Most human VEP studies on binocular interactions have chiefly taken into account amplitude, disregarding latency, which is known to show less inter- and intra-individual variation. One of the first reports on binocular-monocular latency differences was from Lesèvre (1982) who used 12' check reversals and recorded P100, N140 and P200 in binocular and monocular viewing conditions. She found that component N140 in the experiments was the only component of pattern reversal response that showed binocular summation and N140 was the component that had a decrease in latency observed for binocular responses compared to monocular responses, which was highly statistically significant. This N140 latency decrease was observed under all luminance and contrast conditions tested and this decrease had an average value of 10msec. The results in this thesis, however, showed significantly shorter binocular latencies for the reversal N80 and P100 components but not for the N145 component.

VEP latency is influenced by spatial frequency and contrast (Kulikowski, 1975, Musselwhite and Jeffreys, 1985; Jakobsson and Johansson, 1992) with higher contrasts giving shorter latencies. It is also known that binocular contrast sensitivity is higher than the monocular (Campbell and Green, 1975), so it would be reasonable to assume binocular VEP latencies to be shorter than those elicited by monocular stimulation (Bagolini et al, 1988; Knierim et al, 1985; McCulloch and Skarf, 1991, Johansson and Jakobsson, 1993). Johansson and Jakobsson (1993) found that normal subjects gave significantly shorter VEP latency during binocular than monocular viewing of a reversing sinusoidal grating at 4c/deg (but not at 8c/deg) with high and moderate contrast levels (1.0 and 0.316). However, stereoblind subjects had similar binocular and monocular latencies, though there was a tendency for the binocular latency to be more prolonged. There was a significant binocular-monocular latency difference between normal and stereoblind subjects. Investigations of
latencies in single unit recordings and VEPs in cats (Minke and Auerbach, 1972) revealed the presence of cells that responded faster on binocular stimulation than on monocular stimulation, and the latency of the surface VEPs appeared to be determined by the fastest single units. It was thus hypothesized that binocular stimulation activates separate, fast-conducting pathways that are not activated on monocular testing. In our study, we wanted to investigate whether such binocular-monocular latency differences varied depending on stimulus presentations, and to ascertain the most useful stimulus mode for differentiation between normal subjects and those with disrupted binocularity. Once again our results proved most significant for the pattern-reversal mode of stimulation. In normals, the binocular-monocular significant latency difference was not confined to the main P100 potential, but was also present for the N80 component.

Pattern-reversal VEPs (for both amplitude and latency measures) are more sensitive than either pattern-onset or offset at distinguishing between subjects with normal and defective binocularity. Pattern-onset and -offset VEPs had more inter- and intra-individual variability and smaller differences between normals and amblyopes. This was most conspicuous for all offset findings, and onset latency results. For reversal stimulation, the P100 component for amplitude ratios, and, both the N80 and the P100 latency differences gave significant differences between normals and amblyopes. Both these reversal components are known to be of predominantly macular origin and are enhanced by small checksizes (Halliday et al, 1979, Kurita-Tashima et al, 1991; Bodis-Wollner et al, 1992). The paramacular-derived contralateral reversal N105 potential showed no consistent trends in the subject groups. Checksize itself is a very important factor influencing the macular and paramacular VEP components. The checks that produce the clearest patterns subjectively, also produce the highest amplitude VEPs and decrease or increase of that ideal checksize produces progressively smaller responses (Harter and White, 1970). We found
significant differences between the normals and amblyopes for binocular:monocular P100 amplitude ratios for the small and moderate sized checks (12', 20' and 50') but not for the largest checks (80'). The mean amplitude ratios for amblyopes are around 1 for these checksizes, thus implying lack of binocular interaction and predominant contribution from the non-amblyopic eye.

The essentially negative findings for pattern-offset VEPs alongside the positive results for pattern-reversal VEPs are somewhat unexpected, as these responses are generally thought to be of similar origin (Estévez and Spekreijse, 1974). However, it is also known that the offset potentials can be smaller and more variable in waveform and latency (Harter, 1971; Spekreijse et al., 1973). All these factors could contribute to the lack of significant results and trends in the pattern-offset data.

One had expected to find more pattern-onset components showing significant differences between normal and abnormal interocular interaction. Detailed investigations concerning the physiological significance of pattern-onset components (Jeffreys, 1971; Jeffreys and Axford, 1972 a,b) have suggested that they differ in origin: CI and P105 being of striate origin, whereas CII and CIII of extrastriate origin. These conclusions were derived from studies of the influence of retinal location on the amplitude distribution of these VEP components. Stimulation of the central, foveal 1 degree field was found to contribute mainly to the CII component but virtually nothing to the CI and P105 components, which were primarily derived from the 2-6 degree region of the field (Jeffreys and Axford 1972 a). CII and CIII have also been found to be less dependent on contrast and more on pattern detail and the amplitude of these components parallel the subjective impression of the clarity of the contours of the stimulus pattern elements. CI has been found to be large when the stimulus comprises contrast change only, whereas under such conditions, CII and CIII were very reduced in amplitude. From such reported findings it was anticipated that both CII and
CIII would show significant binocular:monocular amplitude ratio differences between the normal and amblyopic groups, as these components would be selectively attenuated from amblyopic eyes.

7.5 Conclusions

These findings indicate that binocular VEP interaction in normals is best shown by potentials that have predominant contributions from macular pathway activity (reversal ipsilateral N80 and P100, and onset ipsilateral CII and contralateral P105, to small checksizes). Pattern-reversal stimulation appears to be the best means for studying the differences between normal subjects and those with defective binocularity. It is important that amplitude ratios of responses to small checks, as well as latency differences of responses to moderate sized checks are assessed together to optimally evaluate interocular interaction. These results indicate a possible role for the use of VEPs in investigating binocular function providing the appropriate methodology is used. This may be particularly useful in the assessment of the pre-verbal child.
8. General Discussion and Conclusions

8.1 Comparison of pattern-onset, -reversal and -offset VEPs

The experiments in this thesis were designed to compare responses to pattern-onset,-reversal and -offset, especially with respect to macular and paramacular contributions. The effects of changes in checksize, contrast and central scotomata were systematically investigated. The clinical usefulness of the triple onset/reversal/offset pattern stimulus was assessed by studying amblyopic subjects who have a deficit in macular vision, and, by comparing the interocular interaction, as assessed by binocular and monocular VEPs, in controls and amblyopes. The ipsilateral pattern reversal VEP, and in particular the P100 component, was the most sensitive to experimental manipulations designed to investigate differences in macular and paramacular activity, producing the most statistically significant results. Table 8.1 gives a summary of macular and paramacular VEP characteristics as derived from VEP studies in this thesis and by other (e.g. Behrman, 1972; Blumhardt et al., 1978; Todd Meredith and Celesia, 1982; Wanger and Nilsson, 1978; Sokol, 1983) and from physiological evidence (e.g. Daniel and Whitteridge, 1961; Harter and White, 1969; Schein, 1988).

The pattern-offset VEP generally showed the greatest amount of intra- and inter-individual variability with large standard deviations, fewer significant findings and was the least useful in distinguishing between macular and paramacular activity, and between normals and amblyopes. However, offset components did show changes similar to those observed in pattern-reversal VEPs. This supports the suggestion that the ipsilateral VEP complexes and contralateral negativities obtained with both stimulus modes are essentially similar and probably involve the same physiological origins (Kriss and Halliday, 1980). Pattern-onset VEPs, on the other hand, behaved quite differently to VEPs elicited by the
other two stimulation modes. Ochs and Aminoff (1980) suggested that adaptation to the pattern as occurs in the reversal mode causes the differences in waveform between pattern-reversal and onset (and presumably pattern-offset). They found that if pattern-onset is recorded immediately after pre-pattern exposure (subjects remained adapted to the checkerboard throughout the time of the VEP, its near continuous presentation was interrupted for 240ms. with a grey field, following which a single onset VEP was obtained and averaged), CII is attenuated and CI was increased in latency resembling the reversal P100. This did not occur if there was a 4 second interval (grey field) between the adapting pattern and the VEP stimulus (checkerboard presented for 240ms). However, the results from the present experiments do not support such a theory. The long recording epoch encompassing all three stimulation modes, with only a 300ms interval during which the pattern is off, would also act as an adaptive stimulus for pattern-onset, and the pattern-onset VEPs recorded in this thesis were very distinctly different from the reversal VEPs.

<table>
<thead>
<tr>
<th>Macular-Derived VEPs</th>
<th>Paramacular-Derived VEPs</th>
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<tbody>
<tr>
<td>Enhanced by small checksizes.</td>
<td>Enhanced by large checksizes.</td>
</tr>
<tr>
<td>Attenuated by small central scotomata.</td>
<td>Not affected by small central scotomata.</td>
</tr>
<tr>
<td>Strongly influenced by contrast change.</td>
<td>Minimally affected by contrast change.</td>
</tr>
<tr>
<td>Selectively attenuated in amblyopic eyes.</td>
<td>Not affected in amblyopic eyes.</td>
</tr>
<tr>
<td>Good interocular interaction in normals. (Partial summation &amp; binocular-monocular latency differences)</td>
<td>Minimal interocular interaction in normals.</td>
</tr>
<tr>
<td>Poor interocular interaction in amblyopes (dissimilar to normals).</td>
<td>Minimal interocular interaction in amblyopes (similar to normals)</td>
</tr>
</tbody>
</table>

Table 8.1: Summary of macular/paramacular VEP differences.
It is interesting that for pattern-onset stimulation, the contralateral P105 showed consistent significant results for the different experimental paradigms, whereas the contralateral reversal N105, and offset N115, were variable and not always clearly discernible for all subjects. Table 8.2 summarises and highlights those components that were significantly affected in the different experimental conditions. Onset and reversal components were generally more sensitive than offset components to manipulation of the various experimental variables. Onset and reversal components showed a similar number of components giving significant changes for both controls and amblyopes. However, the reversal ipsilateral P100 and N145 components showed the greatest number of statistically significant findings across all the experimental paradigms. Furthermore, the reversal P100 component showed the least inter- and intra-individual variability. This supports clinical evidence that it is the most reliable potential for eliciting macular responses, however it is important to choose appropriate stimulus conditions, namely high contrast reversal using moderate to small checksizes to encourage macular contributions. The initial negative component of the ipsilateral reversal NPN complex (N80) has similar properties to the P100, indicating that it too has a major contribution from macular-derived activity.

The contralateral onset P105 component showed the largest number of significant findings compared with the other pattern-onset components, across all the various studies. It was also the only onset component to show significant differences in both amplitude and latency for the interocular interaction study and when comparing normal and amblyopic VEPs. Both the P100 and N145 pattern-reversal components showed significant amplitude and latency differences between normals and amblyopes. When the effects of checksize were investigated, the offset components behaved in a similar manner to the reversal VEPs. The latter observation is in agreement with the findings of other studies (Estévez & Spekreijse, 1974; Kriss and Halliday, 1980).
Several studies have found the reversal P100 component to be attenuated in amblyopic eyes (Sokol and Bloom, 1973; Wanger and Nilsson, 1978, Sokol, 1983), but there have been very few reports on the reversal N145 component, which has proved to be almost as sensitive as the P100 component in the experiments in this thesis. The N145 component was significantly influenced by checksize, attenuated by the smallest central scotoma and was significantly affected in amblyopic eyes compared with normal eyes. This supports the findings of Mayles and Mullholland (1980) who reported a marked attenuation of the N145 component in amblyopic eyes.

Ipsilateral onset components and offset components showed similar trends in amplitude following the introduction of experimental central scotomata and alterations in

Table 8.2: Overview of results. X denotes significant effect of stimulus variable on VEP component (amp.= amplitude; lat.= latency)
stimulus contrast. This dichotomy, which becomes evident when contrast change is investigated, with reversal on one hand and onset/offset VEPs on the other, may well be due to the inherent nature of the stimulus modes. Reversal stimulation contains a motion element as well as a pattern-specific element, whereas onset and offset do not have a motion component, but have pattern-detection as well as local luminance-change elements. Spekreijse et al. (1985) and Kulikowski (1977, 1978) have emphasized that motion detecting mechanisms are contributing to the pattern-reversal VEP. Spekreijse and colleagues described reversal VEPs in terms of motion-onset and motion-offset, whereas Kulikowski's conclusions were based on the psychophysical evidence that a reversing pattern has two different detection thresholds, one for motion or flicker and the other for detecting the pattern. Moreover, he reported that for coarse patterns, the reversal VEP can be attributed to movement processing, whereas for patterns of higher spatial frequency (>3c/deg.), both pattern and motion systems contribute to the reversal VEP. Van der Tweel and Spekreijse (1968) also suggested that large pattern reversal VEPs have been reported to have constituents: one related to the detection of the pattern (i.e. the sharp edges) and the other related to changes in local luminance within the individual check. This proposition could also be applied to pattern-onset and -offset: large check VEPs contain more luminance change constituents than pattern detection constituents, when compared with small check VEPs.

The evidence from the studies described in this thesis indicates that the reversal VEP complex as a whole is more effective than the onset responses in studying macular-derived responses. However, if one wants to obtain simultaneous macular- and paramacular-derived responses or pattern-specific and luminance-specific responses, then pattern-onset, half-field stimulation would be the more suitable stimulus of choice. Onset ipsilateral CII and contralateral P105 to small checksizes, would mostly reflect pattern-specific responses,
whereas CI and P105 to large checksizes would predominantly reflect luminance-specific and
paramacular responses. CI significantly increased in size as checksize increased, in contrast
to the behaviour of CII, due to the fact that larger checks invoke a greater luminance than
pattern contribution to the stimulus. Furthermore, CI was one of the few components still
preserved when the central field was occluded with a hemi-scotoma as large as 0-3°. These
findings support those of Spekreijse et al (1973) and Jeffreys (1977), who found CI, unlike
CII and CIII, to be unaffected by de-focusing and to pattern pre-exposure or adaptation, and
to increase in amplitude with increasing contrast beyond the level at which the pattern
contours become clearly detected. These factors imply that CI is predominantly a contrast-
dependent component whereas CII and CIII appear to be contour or pattern-dependent
components.

Contralateral reversal N105 and offset N115 were the two components that showed
the least significant findings. They appear to reflect paramacular activity, and, as they are
somewhat variable and not always recordable in all subjects, this may explain their poor
sensitivity in the different experiments. Onset CI, on the other hand appears to be relatively
consistent in normal subjects, and the results were significant when using checksize and
contrast change to test its strength at portraying luminance-specific activity.

Interestingly, it can be seen from table 8.2, that onset and reversal VEPs resemble
each other in that it is mainly amplitudes and not latencies which are significantly affected
by scotomata, whereas for pattern-offset both amplitudes and latencies are affected.
Experimental scotomata caused the most consistent effect across onset, reversal and offset
component amplitudes, with the exception of contralateral N105 and N115. The
unresponsiveness of these latter two components indicates their predominantly paramacular
origins. Both contralateral components N105 and N115 did not show significant changes
in amblyopic subjects, and this is to be expected in patients who have a visual problem
primarily related to macular function. The only other component that did not show either a latency or amplitude difference between controls and amblyopes was the offset P110, this was somewhat surprising as this component resembled the P100 component in so many other aspects. Although the trends for these two components were similar when comparing controls and amblyopes, the offset P110 results did not reach statistical significance which could be due to the greater inter- and intra-individual variability seen for all offset responses. Another possibility is that like the P100 component, the P110 is likely to reflect both macular and paramacular components, with the P110 component being mainly of a paramacular-derivation and would thus not be so affected in amblyopes.

Previous studies of normal subjects have reported that the variability in VEP latencies was significantly less than that in VEP amplitudes (Meienberg et al., 1979; Van Lith et al., 1978). It is therefore interesting to observe from table 8.2 that when comparing controls and amblyopes and the interocular interaction differences, the majority of significant results, involved component latencies to a greater extent (8 VEP components) as compared with amplitudes (5 components). Pattern-onset CI, CIII and contralateral P105, reversal N80, P100 and N145 and, offset N85 and N165 all showed significant latency differences between controls and amblyopes. All these components (except for CI) have been shown by the experiments in previous chapters and by the work of others (Jeffreys, 1977; Blumhardt et al., 1978, Kriss and Halliday, 1980) to have contour or pattern-specific contributions and associated predominantly with stimulation of the macular area. Changes of checksize predominantly affected component latency, whereas contrast change and scotoma mainly affected amplitude.
Although a large number of VEP amplitudes and latencies demonstrated statistically significant changes with the different experimental paradigms, the reversal P100 component showed the greatest degree of significance (most p-values of <0.0001) for the different experimental manipulations. Also, the scotoma experiments showed that reversal P100 and N145 (as well as onset contralateral P105 to small checks) were significantly affected when using the smallest (0-1.5 degree) scotoma. These observations all add support to the reversal P100 as being the most reliable component for distinguishing macular pathway responses.

The close association between the reversal and offset ipsilateral and contralateral components was also reflected in the contrast change experiments, when individual components were traced through from the reversal mode of stimulation to the offset mode. Figure 8.1 gives a diagrammatic summary of how components interrelate with each other and how they appear to transform from one stimulus mode to the next as found in chapter 5.
The broken arrow between onset CIII and P100 represents the association between these two components which is only evident when larger checksizes are used. P100 seems to be derived from the amalgamation of CIII and CI, or viewed from another angle, with large sized checks, the macular CII component attenuates to such an extent leaving CI joined to CIII as a bifid broad positivity (figure 5.15). It is interesting to note that the contralateral onset P105 did not truly have an analogous component in the reversal mode, although it could be traced to the positivity following the contralateral reversal N105 component.

The close resemblance in the behaviour of the reversal P100 and N80 components with the onset CII component and their apparent macular nature, suggested that these reversal responses could be traced from the onset CII component. However it was CI, the predominantly luminance-dependent component that could be traced through to the P100 component in the contrast experiments (Chapter 5). This therefore suggests that the reversal P100 has a certain luminance-specific contribution, which could be the motion element inherent in pattern-reversal stimulation. This would support previous theories by Spekreijse et al (1985) and Kulikowski (1977) who suggested motion-specific as well as pattern-specific elements in the pattern-reversal VEP.

Onset CII, which has been found to be predominantly of macular origin could be traced through to the reversal N145 component. Studies in the literature on pattern-reversal VEPs mainly describe the P100 component and there have been few investigations of the N145 component. The present experiments have found a close correlation between the behaviour of P100 and N145 components, with both showing the greatest extent of significant results when comparing normal and amblyopic subjects. The affinity of N145 with the onset CII component implies it is a more pattern-specific component than the P100 component.

CI and CIII components seem to combine to form a large broadened positivity which
can be traced through to the reversal P100 component and this appears to emerge with the attenuation of onset CII as checksize increases. It has been reported that CIII is a relatively pattern-dependent component (Jeffreys, 1971; Jeffreys & Axford, 1972 a,b), however the study in Chapter 3 shows a bimodal distribution for CIII amplitude with checksize, and this suggests that two mechanisms may be involved: a pattern specific, macular-derived contribution (best seen to stimulation with smaller checks), and a luminance-derived contribution (obtained with larger checks). The reversal P100 is like the onset CIII, as it also appears to have a luminance/motion-derived contribution as well as a pattern-derived contribution.

One can think of the reversal VEP morphology as the ipsilateral onset VEP minus the CII component. It is interesting that developmental studies of onset VEP in normal young children (Spekreijse, 1983) have shown that CII is absent in the first year of life and is present in only 40% of children aged 20 months. The onset VEP in these infants, who have a poorly developed contrast mechanism, is thus very similar in configuration to the reversal P100, which would also be reflecting predominantly luminance contributions in such young subjects. With increasing age, onset and reversal show maturational differences: In the onset response, the emergence of CII creates a positive/negative/positive (CI, CII, CIII) complex, whereas the pattern-reversal response has the same morphology but becomes more sharply defined and of shorter latency. The present studies involved older children (chapter 6), in whom CII had already emerged.

Like the P100 component, the reversal N80 appears to be pattern-specific and macularly-derived. This is supported by results of experiments in this thesis and other reports similarly support this notion (Halliday et al., 1979, Kurita-Tashima et al., 1991, Bodis-Wollner et al., 1992). The possible association between the reversal N80 and onset Co has not been previously described. Both components have properties indicating a foveal
origin. Co is found to be somewhat variable in size and not present in all subjects and thus not very amenable to systematic investigation.

The contralateral onset P105 appeared to be a robust component throughout the experimental studies in the thesis. It was the most reliable onset component for differentiating between controls and amblyopes, second to the reversal ipsilateral P100/N145 complex. Onset P105 component does not appear to have an analogous component in the responses to the reversal or offset modes. In the contrast change experiments it seems to be associated with the positivity that may be present immediately after the contralateral reversal N105 component. The contralateral reversal N105 appears to be a paramacular component which is conspicuous when the stimulus field and checksize is large and half-field stimulation is used. However, the main contralateral onset component, the P105, appears to be enhanced by macular simulation; It therefore seems that if the stimulus is macular-orientated then the P105 would dominate, but if the stimulus is changed to being a more paramacular-orientated one, then this positivity attenuates and the following negativity (i.e. N105) accentuates.

8.2 Physiological mechanisms underlying pattern VEP components

Individual VEP components are not likely to reflect exclusively one particular visual submodality or brain site. Visual stimulation will activate several subsystems and there will be generation of activity from a combination of systems and areas (e.g. magno and parvocellular systems, and striate / extrastriate origins), though particular areas may predominate according to the stimulus characteristics. There are conflicting reports in the literature regarding the origins of particular VEP components. As mentioned previously, CI has been thought to be generated in the striate cortex, whereas CII in the extrastriate (Jeffreys and Axford, 1972). However, Maier et al (1987), as well as Drasdo (1980)
proposed that Cl originated in area 18, with CII coming from area 17 and possibly 18.

Lesèvre and Joseph (1979) reported areas 19 and 18 as sources of CI and CII respectively.

It is also thought that striate activity dominates the immature VEP in young children (≤8 years), whereas extrastriate activity contributes in older subjects (Ossenblok, Reits and Spekreijse, 1992). This implies that onset CII which is not evident in infants and young children and emerges in later childhood, is likely to be of extrastriate origin. In contrast CI, which is conspicuous in the immature onset VEP, is thought to be of striate origin. This, therefore contradicts some of the above mentioned studies (Maier et al., 1987; Drasdo, 1980; Lesèvre and Joseph, 1979), which similar to those by Ossenblok and colleagues, are based on dipole localisation techniques and models.

Studies in this thesis have shown certain VEP components to different visual stimulation modes behave in a very similar manner. Experiments involving the transition from one stimulus mode to another have shown an apparent close link between particular components. In particular, onset CI - a supposedly striate-derived component (Jeffreys and Axford, 1972), appears to be transformed to the reversal P100 component - a supposedly extrastriate-derived component (Halliday and Michael, 1970) through the contrast change experimental steps that begin with low contrast change onset/offset and progress through to high contrast reversal stimulation (in Chapter 5).

As mentioned in the previous section (8.1), most components appear to have response properties which indicate they are of macular and/or paramacular origin. The response properties of the components with respect to changes in checksize, contrast and scotoma size, and, in their interocular interaction, as well as their behaviour in subjects with poor central vision and absent binocular function, indicate they either reflect macular or paramacular function (table 8.1 outlines the macular/paramacular VEP differences). Ipsilateral reversal and offset components, and onset CII are mainly representing macular contributions, whereas
contralateral reversal N105 and offset N115 and onset CI are predominantly paramacular in origin.

Certain components such as onset ipsilateral CIII and in particular, the contralateral P105 appear to have both macular and paramacular contributions, with one dominating over the other depending on the stimulus parameters. CIII was found to have a bimodal distribution when amplitude is plotted against checksize, which may be reflecting the activity of the two pathways. The contralateral onset P105 component consistently revealed its macular and paramacular configuration in the different experiments in this thesis. The macular-derived P105 is a sharply-defined positivity around 105ms. seen with small checks (< 35'), whereas, the paramacular-derived P105, is of a different morphology, being broad and often bifid (the second sub-component of the waveform occurring around 125ms). This broadened P105 remained conspicuous with the introduction of central scotomata of different sizes. Furthermore, it was one of the few identifiable components (from both the ipsi and contralateral side) still recordable when large 0-4.5° central scotoma was introduced, indicating its extra-macular origin.

The onset ipsilateral Co component was not identifiable in all control subjects, and was only apparent with the smallest check sizes used, thus indicating its predominant macular contribution. Its link with the reversal N80 negativity (from pattern-onset to pattern-reversal transition studies), further supports the foveal origins of this reversal component, as N80 was particularly enhanced with small check sizes.

The interesting behaviour of the VEP components which can be related to macular and paramacular activity can be compared with the relative distribution and attributes of the retinal parvocellular and magnocellular ganglion cells (P-cells and M-cells), respectively. P-cells have small cell bodies, receptive fields and dendritic fields and are very numerous constituting 80% of the ganglion cells in and near the fovea (Perry et al, 1984). M-cells,
however, are about 3 times larger, with large receptive fields and only constitute about 6-10% of ganglion cells in and around the fovea, increasing slightly to 8-10% at greater eccentricities and can be found throughout the retina (Silveira and Perry, 1991). The average densities of P- and M-cells have been measured by Perry et al. (1984) who found ganglion cells to be layered close to the fovea with two P-cells for every cone, with the density of M-cells outside the fovea being uniformly around 10% of the density of P-cells. Thus, P-cell mediated activity (parvocellular mechanisms) can be related to an extent to macular-derived activity, and M-cell mediated activity (magnocellular mechanisms) to paramacular-derived activity. Furthermore, there are also subcortical projections, as approximately 10% of the retinal ganglion cells (γ-cells) project primarily to the superior colliculus, and these have magnocellular-like characteristics.

Distinctions between magnocellular and parvocellular mechanisms were elaborated in the Introduction (Chapter 1), and are summarised in Figure 8.2. The magnocellular and parvocellular pathways appear separate at level of the retina and LGN. At the receiving cortical level V1, there appears to be a rearrangement into three streams: the parvo-blob (P-B), parvo-interblob (P-I) and magno stream. At the V2 level, these 3 streams are represented by the thin stripe, interstripe and thick stripe compartments, respectively. At the higher cortical levels the magno-stream is associated with V3 and MT, whereas V4 has been shown to contain different regions representing the P-I and P-B streams (De Yoe et al., 1988; Zeki and Shipp, 1989). The higher levels of the inferotemporal areas receive major inputs from V4, and, the posterior parietal areas receive major inputs from MT.

This hierarchical framework for the magnocellular and parvocellular streams is not exclusive as there is extensive cross projections between the two streams at all cortical levels (Felleman and Van Essen, 1990). For example: there are connections within V1 between the parvo-blob, interblob and magno-4B, connections also exist between the parvo-thin and
magno-thick stripes within V2, furthermore connections also exist between the predominantly magno-MT and parvo-V4 areas. It is therefore with some caution that individual VEP components can be labelled as reflecting a particular visual system: The inference to VEP component origins based on the findings from the studies in this thesis would thus only refer to the major dominant mechanism for a particular component.

The magnocellular system (involving, 4B-thick stripe-MT pathway, figure 8.2) is characterised by cells that are selective for movement perception, low spatial resolution and fast, low contrast motion. This system is also colour-blind and has very high temporal resolution, with cells responding quickly and this response decaying rapidly even when the stimulus is maintained. The parvocellular-interblob-pale stripe system, on the other hand, carries high resolution information about borders and colour and is selective for shape and orientation discrimination. The temporal resolution for this system is opposite to that of the magnocellular system in that it is slow, and this feature together with its selectivity for high spatial resolution renders it specific for the detection of high detailed patterns. The parvo- and magnocellular blob-thin stripe-V4 system (figure 8.2), conveys information regarding colour and shades of grey, is selective for low spatial resolution, high contrast, slow temporal resolution but not motion, shape discrimination or stereopsis. (Schiller, 1986).

Thus the parvo-blob stream is very wavelength (colour) selective, with the parvo-interblob stream being highly selective for orientation as well as wavelength and binocular disparity. The magnocellular stream is highly selective for direction of motion but it has been shown that some cells may also be selective for binocular disparity and orientation. Spatial frequency tuning however, has been found to occur in all streams, although more so in the parvo-blob and -interblob cells of V1 (Tootell et al, 1988). Magnocellular cells have greater contrast sensitivity than parvocellular cells (Schiller and Colby, 1983). Thus magnocellular cells respond to patterns at low contrasts, and at low to intermediate spatial...
frequencies, whereas parvocellular cells respond at high contrasts and spatial frequencies (Schiller, 1986).

Table 8.3 summarises the predominant origin of the pattern VEP components as surmised from the results of experiments in this thesis. The criteria upon which a component is designated as reflecting parvo- or magnocellular activity are based on its overall characteristic behaviour in the different experimental paradigms and based on the above facts regarding the magno/parvocellular characteristics. VEP components that appeared selective for small checksizes and foveal-field stimulation and showed maximal interocular interaction with significant attenuation in amblyopic eyes, are presumed to predominantly reflect the parvocellular system, whereas components that are enhanced by large checksizes, with a possible motion element to the stimulus (i.e., pattern-reversal), not affected by small central...
scotomata and pattern detail, not exhibiting significant interocular interaction and not significantly altered in amblyopic eyes, are presumed to primarily reflect magnocellular mechanisms.

<table>
<thead>
<tr>
<th>PATTERN VEP COMPONENT</th>
<th>SYSTEM (Parvocellular / Magnocellular)</th>
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<tbody>
<tr>
<td>Onset</td>
<td>Parvo</td>
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<td>Magno</td>
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<tr>
<td>Reversal</td>
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<td>Magno</td>
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<td>Offset</td>
<td>Parvo + Magno</td>
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<td>Parvo + Magno</td>
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<td></td>
<td>Magno</td>
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Table 8.3: Probable mechanisms reflected in the pattern VEP components.

It can be seen from table 8.3 the majority of components appear to mediate both parvo- and magnocellular mechanisms. For pattern-reversal and offset, all ipsilateral components may reflect both systems but the contralateral negativities appear to predominantly represent magnocellular mechanisms. On the other hand, pattern-onset, appears to be the only stimulus mode which as well as producing components of probable mixed magno and parvocellular origins, also evokes components that fundamentally behave as being parvocellular-derived (Co and CII), or magnocellular-derived (Cl).

The above conclusions were derived from the behaviour of the various components in the different experimental paradigms in this thesis. Ipsilateral reversal and offset components and onset CIII and contralateral P105 vary in their behaviour to changes in checks size, contrast, field, and extent of interocular interaction. Hence, when small checks,
high contrast, central field stimulation are used, VEP components are likely to reflect parvocellular mechanisms. However, if large checks, low contrast, peripheral field stimulation is used, and when amblyopic eyes are tested (where macular vision is compromised), VEP components are likely to reflect magnocellular mechanisms. Further evidence to combined origins of the reversal P100 component can be seen when amblyopes were compared with normals for the different checksizes (chapter 6): For the majority of checksizes (12', 20' and 50'), normals had larger P100 amplitudes, reflecting the main macular/parvocellular origins of this component, which is attenuated in amblyopic eyes; however, with the largest 80' checks, the P100 amplitude obtained from amblyopic eyes was greater than that obtained from the normal eyes (see figure 6.11), this may be explained by the fact that amblyopic eyes reflect a predominantly paramacular/magnocellular mechanism, with minimal macular/parvocellular contributions and hence are tuned to show a maximal response with large checks, whereas normal eyes reflect both mechanisms and show tuning with checksize variation. This supports the observations that moderate pattern defocusing increases the VEP amplitude for large reversal patterns while attenuating those for small patterns (Regan and Richards, 1973): large patterns reflecting paramacular/magnocellular activity, small patterns reflecting macular/parvocellular mechanisms, and hence defocusing of small checks (which target macular function) result in VEP attenuation.

Onset contralateral P105 appears to reflect two distinct underlying origins, depending on the stimulus parameters: The sharp macular-derived P105 recordable to small checks but dramatically attenuated with even the smallest of scotomata, and, the broad and bifid paramacular-derived P105. This bifidity may occur due to the emergence of the later limb of the 'W' waveform, which may represent the paramacular sub-component. This broadened P105 is elicited with larger checks (≤35') and is relatively well-preserved even when large central scotomata are introduced. The behaviour of the P105 component in amblyopic
children (chapter 6) shows that with small checks (12' and 20') it is barely discernible (figure 6.8), however, with larger 50' and 80' checks, it is relatively well-preserved when compared to other components of the three stimulation modes. This relatively well-preserved P105 recorded from amblyopic eyes with larger checks very likely reflects paramacular visual function.

The alteration in component morphology has not been previously described for the onset contralateral P105, but there have been relatively few studies of this component, as most investigations in the literature used full-field stimulation which would mask this component, or, have limited their use of checksizes (Kriss et al 1984; Jeffreys, 1977; Shagass et al 1976). However, Jones and Keck (1978) described the presence and interaction of two positive VEP sub-components to low spatial frequency gratings. They found a double peak in some subjects when larger field sizes and lower spatial frequencies (< 1c/deg).

The other components, namely onset Co, CI and CII and the contralateral reversal and offset negativities, are far more stimulus specific, in that under certain conditions, they are not elicitable. The onset components CII and more particular Co appear to be parvocellular-derived as they are essentially absent when the stimulus targets the magnocellular pathway (e.g. with low contrast, large checksizes, central scotoma and with poor macular vision). The reversal and offset contralateral negativities (N105 and N115), conversely are unaffected by these stimulus variables and moreover are enhanced by large checks and low contrast stimuli, thus suggesting magnocellular mechanisms.

It is known that at the level of the LGN, magnocellular cells conduct at fast velocities to the striate cortex, whereas parvocellular are slower (Schiller and Colby, 1983). It has been found in monkeys that the difference between the two pathways from retina to V1 is about 4 msec. (Lennie et al., 1990). Evidence to support the stimulus-dependent, combined magno- and parvocellular origins of the ipsilateral reversal and offset components comes
from the findings of latency changes with checksize described in chapter 3. The significant
decrease in latencies with increasing checksize implies that with larger checks, the
components are mediated via the faster conducting magnocellular pathways, whereas with
smaller checksizes, amplitudes are enhanced but latencies are increased, suggesting they are
mediated via the slower conducting, pattern-specific parvocellular pathways. This theory is
supported by several psychophysical reports that have shown that reaction times to the onset
of a grating of high spatial frequency exceeds that to the onset of a low spatial frequency

8.3 Proposals for future studies: Clinical and experimental applications

Having defined VEP components as macular or paramacular and having explored the
relative parvocellular and magnocellular contributions to these components, future studies
can be implemented in specific clinical populations to explore and support the findings in
this thesis. For example, in diseases that affect central vision such as maculopathy (e.g.
Stargardt's), cone dystrophy, macular hypoplasia and degeneration, optic nerve compression
(e.g. dysthyroid optic neuropathy), we would expect macular-derived components to be
selectively attenuated. Hence pattern-specific mechanisms, that is parvocellular mechanisms
would be implicated. Components such as onset Co, CII should be degraded, and if stimulus
variables are appropriately targeting macular function (high spatial frequency, central field),
then onset CIII and contralateral P105 and the ipsilateral reversal and offset components
would also be attenuated. In conditions that preserve central vision but the field is
constricted, such as in CSNB (congenital stationary nightblindness), the earlier stages of
gyrate atrophy and retinitis pigmentosa (and the numerous conditions it is associated with),
then the predominantly paramacular-enhanced components (onset CI and contralateral
reversal N105 and offset N115) should be involved. Enhancement of the reversal
contralateral N105 component has been documented in studies where the central-field is occluded with experimental scotoma (Blumhardt et al., 1989). Pattern-reversal studies in dysthyroid optic neuropathy patients showed involvement of macular fibres as the N80 and P100 were attenuated whereas the contralateral paramacular N105 components were enhanced (Shawkat et al., 1993). The use of VEPs in such clinical conditions is particularly useful in the paediatric setting, where one cannot carry out formal visual field or even visual function testing due to the young age of the child or to lack of co-operation. The studies of pattern-onset, -reversal and -offset in amblyopic children (Chapters 6 and 7), highlighted the importance of macular-derived VEP amplitude and latency assessment in the detection of poor central visual function and interocular interaction.

However, the developmental effects on VEPs should be taken into account, particularly for pattern-onset stimulation. Pattern-onset is favoured by some for use in young children (Apkarian, 1991), and it is the main initial positive component which is measured (Cl) as the negative CII only emerges in later childhood. One should therefore be cautious in interpreting visual function in young children, when CII has not yet developed. Our studies have shown that Cl is predominantly contrast and luminance-dependent as opposed to being a pattern-dependent component, whereas the reversal P100 is predominantly pattern-dependent but also has substantial contrast-dependent elements. It may therefore be more appropriate in young children to use pattern-reversal stimulation where the stimulus (in terms of spatial frequency and contrast) is varied to test the macular and paramacular systems. A direct comparison between conventional onset VEP and reversal VEP studies in young children is therefore difficult to make: the onset VEP waveform has yet to evolve further as CII develops into the well-known adult configuration. Furthermore, the experiments in this thesis have shown that onset CI and reversal P100 did not show similar trends and behaviour that would suggest a comparable physiological origin; instead, pattern-reversal P100 did
share similar characteristics with another onset potential: the contralateral P105 component. Both the reversal P100 and contralateral onset P105 were similarly attenuated in amblyopic eyes and showed comparable interocular interactions. However this implies that if pattern-onset stimulation is to be compared to reversal stimulation, then a half-field stimulus field is necessary to reveal the P105 component.

A potentially important use of different pattern VEP modes in a clinical setting, is in the investigation of the patient with nystagmus. Nystagmus has been classified into idiopathic, latent, neurological or sensory-defect (e.g. due to albinism, cone dysfunction, CSNB, optic nerve hypoplasia, cataracts) (Casteels et al., 1992). Nystagmus has been shown to degrade pattern-reversal VEPs more than pattern-onset VEPs (Kriss et al., 1989). If pattern-reversal and pattern-onset VEPs are recorded in patients who have nystagmus secondary to poor central vision, (such as in albinism, cone dysfunction and optic nerve hypoplasia), both VEP modalities are likely to be reduced. In albinism, latency changes have also been reported (Harding et al., 1986). However, theoretically, nystagmus that is not associated with a central visual problem (e.g. CSNB) should have a well-preserved pattern-onset response. A comparison of onset and reversal VEPs in idiopathic nystagmus patients may be less helpful, as such an early onset visual problem (the lack of a stable image on the retina) is likely to have rendered the eye amblyopic. The effects of eye movements and retinal image slip on VEP components can be studied in patients with pure latent nystagmus. These patients have minimal or no nystagmus when viewing binocularly, but the nystagmus becomes manifest when one eye is occluded. Thus the patient can be their own control and the effects of nystagmus on each of the VEP components can be studied. (The amblyopic patients studied in this thesis did not have latent nystagmus).

It may be interesting to repeat some of the experiments using several checksizes smaller than 12\', which would be better at targeting the parvocellular mechanisms and which
may further enhance some of the components such as Co and CII. Although the use of VEPs to very small checksizes may be affected by poor fixation and accommodation, particularly in young children or in older subjects (loss of accommodation with age is known to occur; Millodot and Newton, 1981). Further manipulation of the stimulus field may also be explored (upper/lower half-fields, sector and quadratic fields, small central-field) with different checksizes and multiple channel montage. This may add further information regarding the distribution, origins and characteristics of the VEP components of the three pattern VEP modes.

Sequentially presenting the three modes of pattern stimulation ensures similar subject and recording conditions as well as reducing testing time which is particularly useful when assessing children. Experiments in this thesis were confined to stimulation of the left half-field. However, if this method is used clinically, then both half-fields should be tested. To maintain reduced testing time and the advantages of identical recording conditions, a methodology similar to that described by Rowe (1981) who recorded left and right half-field VEPs sequentially in a single run, or, Jones et al., (1994) and Brusa et al. (1995) who described a novel method of interleaved checkerboard reversal stimulation of different areas of the visual field (left and right half-fields; central field, peripheral fields, and left and right hemisurround fields) may be adopted.

The overall comparison of the three modes of stimulation has shown that reversal and onset are superior to pattern-offset in providing reliable findings in normals and amblyopes. Pattern onset VEPs may be of greater interest experimentally as the components are more clearly delineated in being pattern or luminance dependent (reversal VEP components tend to be mixed). One of the major pattern-onset components which showed a significant difference between normals and amblyopes is the contralateral P105 component. Furthermore this component showed a consistent change in morphology depending on
whether the stimulus targets macular or paramacular mechanisms. This implies that to extract the maximum amount of information from pattern-onset VEPs, a half-field stimulus is essential as this P105 component is not usually evident with full-field stimulation. In all subjects studied in this thesis, the onset P105 component was always confined to the contralateral channels and was not evident in the midline channels. This may introduce a limitation in the use of this mode of stimulation in subjects where stimulus fixation point cannot be insured (e.g. in very young children and in developmentally delayed and uncooperative subjects). As mentioned previously another limitation in the use of pattern-onset in children, is the developmental changes in the waveform, i.e. the emergence of CII in later childhood which needs to be considered (Spekreijse, 1978, Wright et al., 1985). Pattern reversal however is somewhat more consistent, with a greater number of significant results than pattern-onset, and was better at separating groups of normals from amblyopic subjects. Furthermore, the reversal waveform is essentially the same throughout life (Wright et al., 1985), and as the most relevant components form the ipsilateral NPN complex, half-field stimulation is not essential as these components are well-defined with full-field stimulation.
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Appendix A: Supporting Publications and Presentations

ISCEV, 32nd Symposium, Banff, Canada (July 1994) - paper presentation (abstract)
Interocular interaction assessed by sequential pattern-onset, -reversal and -offset VEPs.
Shawkat FS & Kriss A (ISCEV grant award)


ARVO, 1997 Meeting, Florida, USA. (1997) - poster presentation (abstract)
VEPs to step changes in contrast from pattern onset/offset to reversal.
Kriss A and Shawkat FS

CVRS, 6th Meeting, Pisa, Italy (June 1997) - paper presentation (abstract)
Changes in macular and paramacular derived VEPS to pattern-onset, -reversal and -offset stimulation in amblyopia.
Shawkat FS, Kriss A and Taylor D

CVRS, 6th Meeting, Pisa, Italy (June 1997) - poster presentation (abstract)
Effects of contrast change: a means of studying the transition between pattern-onset, -reversal and -offset VEP components.
Shawkat FS and Kriss A

Objectives: The extent of interocular interaction for macular- and paramacular-derived VEPs as reflected in the responses to checkerboard onset, reversal and offset stimulation in normals and amblyopes was investigated. Methods: 31 normal adults and children and 18 amblyopic children were tested. Pattern-onset,-reversal, and -offset stimuli were presented sequentially in a single sweep. Using half-field stimulation, the latencies and amplitudes of components ipsilateral and contralateral to the stimulated half-field were measured for binocular and monocular stimulation of each eye using a range of checksizes (12, 20, 50 and 80 min.arc.).

Results: The ratios between binocular and monocular amplitudes for normals were compared with "binocular/good eye" amplitude ratios for amblyopes. The reversal, P100, ratio was found to differ significantly between normals and amblyopes for 12', 20' and 50' checks. Significant differences were also found when using the smallest 12' checks for onset CII, CIII and contralateral P105, reversal N80 and offset P110 potentials. Normals had binocular latencies that were significantly shorter than monocular latencies for onset P105, reversal N80 and P100 and to a lesser extent, onset CIII VEPs. Amblyopes did not show such trends. Comparing the binocular-monocular latency differences of normals with the binocular-non-amblyopic eye latencies of amblyopes, significant differences were found for reversal N80 (all checksizes), P100 (20' and 50') and N145 (20') and for onset P105 (50').

Conclusions: Binocular VEP interaction in normals is best shown using pattern-reversal and by potentials which represent macular pathway activity, i.e. components which are enhanced when small checksizes are used such as the reversal N80 and P100. The binocular-monocular latency differences in normals and amblyopes, suggest that this may be a useful tool for the assessment of binocular function, when used alongside binocular:monocular amplitude ratios for pattern reversal VEPs.
Interocular interaction for VEPs to half-field checkerboard stimulation was investigated in 31 normals and 18 amblyopes. Half-field pattern-onset, -reversal, and -offset stimuli (0-12 degrees; checksizes 12', 20', 50', 80') were presented sequentially in a single sweep. Latencies and amplitudes of components ipsilateral and contralateral to the stimulated half-field were measured. Binocular/monocular VEP amplitude ratios showed significant differences between amblyopes (binocular/good eye ratio) and controls (binocular/mean monocular ratio) using 12' checks for components sensitive to stimulation of the macular pathway (reversal N80 & P100, onset CII, CIII and contralateral P105, and offset P110). Reversal P100 also demonstrated statistical differences for 20' and 50' checks. In controls, binocular latencies were significantly shorter than monocular latencies for ipsilateral reversal (N80, P100), and both contralateral (P105) and ipsilateral (CIII) onset components. This was not seen in amblyopes. Interocular interaction in normals is best shown using pattern-reversal stimulation and by components which represent predominantly macular pathway activity (i.e. those enhanced by using smaller checksizes). In normals, but not in amblyopes, binocular VEPs tend to be earlier than monocular VEPs.
Association for Research in Vision and Ophthalmology - ARVO. Florida USA (May 1997)

VEPS TO STEP CHANGES IN CONTRAST FROM PATTERN-ONSET/OFFSET TO REVERSAL.
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Purpose. The relationship between the VEP components to half-field stimulation were studied by tracing the transition from onset/offset mode, via a series of contrast change steps into reversal mode. Methods. VEPs were recorded in 15 subjects from 3 trans occipital electrodes referred to a midfrontal electrode. The checkerboard stimulus was presented in the left half-field of the screen. Three checksizes were studied (12', 50' 80'). Eight contrast conditions for each checksizes were recorded. Each condition consisted of the alternation of a constant high contrast checkerboard A with a second checkerboard B. Checkerboard B was initially of identical spatial phase to A, but contrast was reduced systematically until B was a uniform grey field (onset/offset). In subsequent steps checkerboard B was of opposite spatial phase and contrast was systematically increased until B was of equal high contrast (full reversal). Occipital VEPs ipsilateral and contralateral to the stimulated half-field were recorded. Results. All ipsilateral and contralateral onset (Cl, CII, CIII and P105) and offset components (N85, P110, N165 and N115), and reversal P100 significantly enhanced with increasing contrast. Extent of amplitude change with contrast appears to be the greatest for offset, followed by onset and then reversal. Pattern-offset components could be traced through to the reversal components (offset N85-P110-N165 became reversal N80-P100-N145). Onset Cl and CII could be traced through to reversal P100 and N145, respectively. When small 12' checks were used, onset C0 could be traced through to reversal N80 component. Conclusions. Offset and reversal components are closely related and suggest similar physiological origins. Onset and reversal components differ in morphology but the onset CI appears comparable to the reversal P100 component. Onset C0 and reversal N80 components appear analogous, being conspicuous to small checksize stimulation and suggesting a predominant macular origin.
CHANGES IN MACULAR AND PARAMACULAR DERIVED VEPs TO PATTERN-ONSET, -REVERSAL AND -OFFSET STIMULATION IN AMBLYOPIA

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Pattern stimulation has proved itself to be an effective technique of studying the visual system in health and disease. However, there appear to be differences in the property of VEPs elicited to different forms of pattern stimulation, namely, pattern-reversal, -onset and -offset. We studied the macular and paramacular components of these 3 main modes of pattern VEP stimulation. The effects of checksize and experimental scotomata on onset, reversal and offset VEPs were initially investigated in control subjects. To evaluate the clinical efficacy of the different modes of pattern stimulation, the responses from amblyopic subjects (where macular function is compromised) were compared with those from controls.

Method: The three stimuli were delivered sequentially in a single recording epoch so that a direct comparison could be made for virtually identical subject and recording conditions. Half-field stimulation was adopted to better separate contributions from macular and paramacular areas of the visual field. Ten different checksizes (6' to 110'), and 4 central scotomata (subtending 0-1.5, 0-2, 0-3 and 0-4.5 degrees) were used. A total of 41 normal and 18 amblyopic subjects were studied.

Results: Ipsilateral reversal components, onset (ipsilateral CII and contralateral P105) and, to a lesser extent ipsilateral offset components were: significantly enhanced by using small checksizes and were susceptible to small central scotomata. These properties thus indicate that these components are predominantly of macular origin. Contralateral reversal and offset potentials (N105 and N115), and ipsilateral onset CI, were enhanced by large checks and relatively unaffected by central scotomata, suggesting they are predominantly generated by paramacular function. These trends were confirmed in amblyopes, in whom macular vision is compromised, as ipsilateral reversal components, onset CII and the contralateral P105 were significantly attenuated for stimulation of amblyopic eyes. Full statistical analysis of VEP components elicited by amblyopic eyes compared with fellow eyes demonstrated that pattern reversal showed the most conspicuous and reliable inter eye differences.

Conclusions: Ipsilateral reversal components, onset ipsilateral CII and onset contralateral P105 components appear to predominantly reflect macular function and are selectively attenuated in amblyopic eyes.
EFFECTS OF CONTRAST CHANGE: A MEANS OF STUDYING THE TRANSITION BETWEEN PATTERN ONSET REVERSAL AND OFFSET VEP COMPONENTS

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The relationship between the VEP components to half-field stimulation were studied by tracing the transition from onset/offset mode to the reversal mode by way of a series of contrast change steps.

Method: Pattern VEPs were recorded in 15 subjects from 3 trans occipital electrodes referred to a midfrontal electrode. The checkerboard stimulus was presented in the left half-field of the screen. Three checksizes were studied (12', 50' 80'). Eight contrast conditions for each checksize were recorded. Each condition consisted of the alternation of a constant high contrast checkerboard (A) with a second checkerboard (B) in which the contrast was systematically changed. Checkerboard B was initially of identical spatial phase to A, but contrast was reduced systematically until B was a uniform grey field (onset/offset). In subsequent steps checkerboard B was of opposite spatial phase and contrast was systematically increased until B was of equal high contrast (full reversal). Occipital VEPs ipsilateral and contralateral to the stimulated half-field were recorded.

Results. All ipsilateral and contralateral onset (CI, CII, CIII and P105) and offset components (N85, P110, N165 and N115), and reversal P100 significantly enhanced with increasing contrast. The extent of amplitude change with contrast appeared to be the greatest for offset, followed by onset and then reversal. Pattern-offset components could be traced through to the reversal components (ipsilateral offset N85-P110-N165 became reversal N80-P100-N145, and contralateral offset N115 became reversal N105). Onset CI and CII could be traced through to reversal P100 and N145, respectively. When small 12' checks were used, onset Co could be traced through to reversal N80 component. The contralateral onset P105 component did not have a comparable component in the reversal mode.

Conclusions. Offset and reversal components are closely associated suggesting they may be mediated by similar physiological mechanisms. Onset and reversal components differ in morphology but the onset CI appears comparable to the reversal P100 component. Onset Co and reversal N80 components appear analogous and have a predominant macular contribution as they are conspicuous to small checksize stimulation.
Interocular interaction assessed by VEPs to pattern-onset, -reversal, and -offset in normally sighted and amblyopic subjects

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Accepted for publication: 16 September 1996

Abstract

The extent of interocular interaction reflected in sequentially averaged VEPs to checkerboard onset, reversal and offset stimulation was investigated to assess the relative efficacy of the three modes of pattern stimulation. Thirty-one controls and 18 amblyopic children were studied. Components on the side of the scalp ipsilateral and contralateral to the stimulated left half-field were measured for checksizes 12', 20', 50' and 80'. Binocular:monocular amplitude ratios for normals were compared with 'binocular good eye' amplitude ratios for amblyopes. The reversal P100 ratio was found to differ significantly between normals and amblyopes for 12', 20' and 50' checks. Ipsilateral (C11) and contralateral (P105) onset components also differed significantly but for the smallest 12' checks only. In controls, onset components (P105 and C11) and reversal components (N80 and P100) showed significantly shorter binocular as compared with monocular latencies. These latency differences were not found in amblyopes. Our results show that interocular interaction in normals is best shown by potentials which predominantly reflect macular pathway activation, and are most conspicuous for reversal N80 and P100 components. Similarly, these components demonstrated the clearest differences when comparing binocular interaction effects between controls and amblyopic subjects. © 1997 Elsevier Science Ireland Ltd. All rights reserved

Keywords: Visual evoked potential; Reversal; Onset/offset; Binocular; Amblyopia

1. Introduction

The visual evoked potential (VEP) has been used to assess binocularity, but the relationship between the monocular VEP and the binocular VEP is not well delineated. A number of studies have compared pattern VEPs to binocular and monocular stimulation (Campbell and Maffei, 1970; Wanger and Nilsson, 1978; Srebro, 1978) and most have found that in normal subjects, the binocular pattern VEP is of greater amplitude than either of the monocular responses. The binocular VEP amplitude has been reported as being 1.4 times the mean monocular response (Campbell and Maffei, 1970; Wanger and Nilsson, 1978) and most have found that in normal subjects, the binocular pattern VEP is of greater amplitude than either of the monocular responses. The binocular VEP amplitude has been reported as being 1.4 times the mean monocular response (Campbell and Maffei, 1970; Wanger and Nilsson, 1978). However, the extent to which the binocular response is larger than the monocular response varies with stimulus conditions (particularly with spatial frequency, contrast and temporal frequency). Most reports state that the binocular response is only marginally larger in amplitude than the monocular response, that is, binocular:monocular ratios are greater than 1 but less than 2, (partial summation) (White and Bonelli, 1970; Harter et al., 1973; Vaegan et al., 1980). However, others have found facilitation, where the binocular VEP amplitude is much larger, that is, greater than the sum of the monocular VEPs (Ciganek, 1970; Srebro, 1978; Apkarian et al., 1981a). The absence of binocular summation, where the binocular response is the same size as either of the monocular responses, has also been described (Inoue, 1966). There is variability in the findings of reports comparing normals with amblyopic subjects (Apkarian et al., 1981b; Harter et al., 1977; Vaegan et al., 1980). Most previous studies in controls and patients have used full-field grating or checkerboard reversal stimuli, and have confined their analysis to the main positive component (P100). However, there is a suggestion that pattern-onset stimulation, in particular, may be more effective than pattern reversal in demonstrating visual pathway disorders.
A. Kriis / Electroencephalography and clinical Neurophysiology 104 (1997) 74-81

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We have recorded pattern-onset, reversal and offset VEPs sequentially in a single averaging run, thereby obtaining these 3 responses for nearly identical recording conditions (Kriss et al., 1984). A range of check-sizes were presented in one half-field in order to distinguish contributions associated with stimulation of macular and paramacular areas of visual field. Pattern reversal VEPs to half-field stimulation, show an apparently paradoxical lateralization: the main macular positivity (P100) is recorded over the side of the scalp ipsilateral to the stimulated half-field, and a paramacular-derived negativity (N105) over the contralateral side of the scalp (Barrett et al., 1976). Thus as ipsilateral and contralateral VEPs show important differences, we analyzed these responses separately to determine their particular sensitivity to interocular interaction. The majority of previous binocular VEP studies have measured amplitude changes only. We have examined both amplitude and latency measures, and have assessed the extent of binocular interaction for the three modes of pattern stimulation in controls and amblyopes with poor macular vision.

2. Method

2.1. Subjects

Three subject groups were studied: the first consisted of 11 normally sighted young adults aged 25-38 years (mean. 31 years; 5 males, 6 females); the second of 18 binocularly uncorrected amblyopes, 6-16 years (mean. 11 years; 12 males, 8 females), and the third group consisted of 18 young normally sighted children aged 7-16 years (mean. 11 years; 7 males, 11 females).

Normal subjects had Snellen acuities of 6/5 or better, and none had a clinical history of visual problems. All amblyopic children had a full orthoptic examination prior to electrophysiological testing. Fourteen were strabismic amblyopes (esotropes) and 4 were anisometropic amblyopes (nearsightedness). Acuity of the non-amblyopic eyes at the time of VEP recording was 6/36 or better in 13 patients (72.2%), and only grossly normal in the remaining 5 cases (mean acuity 6/12). The level of binocular vision was assessed using the Randot stereotests. Binocular single vision was totally absent in 13 patients (72.2%), and only grossly present in the remaining 5 cases (Randot to 200 s of arc). All of the amblyopic children had undergone occlusion therapy with good levels of compliance. Occlusion regimen was variable and ranged from 1/2 h to 6 h per day, for periods ranging from approximately 6 months to 7 years. Only one patient was still having patching therapy at the time of VEP recording.

2.2. Technique

Occipital VEPs were recorded using EEG silver/silver chloride electrodes, attached to the scalp with collodion. The electrode impedance was reduced by gentle skin scarification to below 10 kΩ. A 7-channel montage was used, consisting of a transverse row of five electrodes placed 5 cm above the inion and 5 cm apart, so that two electrodes were spaced at 5 and 10 cm to either side of the midline electrode. One electrode was located at the inion and another 2.5 cm above the inion. All occipital electrodes were referred to a common mid-frontal reference (Fz).

Subjects were seated on a height-adjustable chair, in a darkened room. The forehead was placed on a cushioned rest, and the seat height altered so that the subject’s eyes were aligned symmetrically in the mirror stereoscope. Two oscilloscopes (Hewlett Packard, 1321A, X-Y Display, P4 phosphor), each subtending 24° by 18.5°, were clamped together, at a distance of 1 m from the subject. These were viewed through the stereoscope so that the subject’s left eye viewed one oscilloscope and the right eye viewed the other oscilloscope.

Black and white checkerboard patterns were presented in the left half-field (0-12°) of each oscilloscope screen; the right half consisted of a uniform grey field of the same average luminance as the checkerboard. A small ring (diameter subtending 1.5°) at the centre of the vertical border of the pattern/blank interface provided a fixation spot. Four sizes of black and white checks were presented and these subtended 12, 20, 50 and 80 min of arc at the subject’s eye. The luminance levels were 11.5 cd/m² for white squares, and 0.004 cd/m² for black squares. Binocular left and right eye responses for each of the four check-sizes were recorded: the test order was randomly varied from subject to subject.

VEPs to pattern-onset, reversal and offset were acquired in a single epoch, lasting 900 ms. A 20 ms pre-stimulus interval preceded the stimulus sequence, the checkerboard then appeared for 300 ms, was replaced by the complementary pattern which was on for a further 300 ms (i.e. reversal) and then disappeared for 300 ms (pattern-offset). Previous studies using a similar stimulus paradigm (Kriss et al., 1984), have shown that 300 ms are a sufficient time interval between successive contrast changes to obtain reliable responses to the three stimulus modes. The average of 64 such sequences was recorded for each checksize and viewing condition. When necessary averaging was repeated (e.g., if subject was attending poorly, or responses corrupted by myogenic artifact).

As expected, ipsilateral and contralateral, half-field occipital VEPs were best seen in the lateral channels (Barrett et al., 1976). Components were therefore measured from the electrodes 5 cm to the left and to the right of the midline (Fig. 1). The following VEP components were identified and measured: for pattern-onset, ipsilateral CI, CII, CIII and contralateral P105; for reversal, the ipsilateral N85, P100, N145 and contralateral N105; and for offset, the ipsilateral N85, P110, N165 and contralateral N115. Component recognition was based on their polarity...
and latency range (see Jeffreys (1971), Halliday et al. (1979) and Kris and Halliday (1980), for details regarding component identification). Peak latency and peak-to-peak amplitude measurements were made for each component (Fig. 1).

3. Results

3.1. Binocular/monocular amplitude ratios

There were no significant differences when comparing VEPs obtained from the left and right eyes of normal subjects, nor when comparing binocular/monocular ratios with binocular/lefteye ratios. Binocular/monocular amplitude ratios were thus calculated as the 'binocular mean left and right eye amplitude' for normal subjects; and 'binocular/normal nonamblyopic eye amplitude' for anamblyopic subjects. Partial summation (1 < ratio < 2) and facilitation (ratio > 2) were found in normal adults, and children for all VEP components. The largest checksize (80') gave the most variable results in normals, ranging from facilitation (onset, CI) to minimal or absent interaction, i.e., ratios of, or near 1 (onset P100; reversal N80, N145; offset P105). Binocular/monocular ratios, for the onset components, in normal subjects ranged from 1.05 to 1.99 (mean = 1.38 ± 0.72). The P100 component elicited by pattern reversal stimulation showed the least variability and gave ratios ranging from 1.45 to 1.71 (mean = 1.59 ± 0.57). Ratios for reversal N145 extended from 1.04 to 1.71 (mean = 1.32 ± 0.04), and the offset for reversal N80 extended from 0.99 to 2.9 (mean = 1.83 ± 2.2). Pattern offset components were less consistent than the reversal components with ratios extending from 7.9 to 5.97 (mean = 1.84 ± 2.32).

There were no statistical differences between the VEP findings of normal children and normal adults, so the two control groups were combined and this amalgamated group was used to compare with the patients.

In amblyopic subjects, stimulation with the smallest checks (12') produced ratios of near 1 for all components. Low ratios were also obtained for the reversal P100 component elicited by 20' and 50' checks, which gave values of 1.08 and 1.07, respectively. This implies that for small check stimulation the binocular VEP is predominantly reflecting contribution from the non-amblyopic eye.

Multivariate analysis of variance (MANOVA), for ratios of all VEP components (checksize and subject group as factors), showed an effect for subject group (normal or amblyopic) only. This was highly significant (Pillai's test: F = 4.49, df = 10, P < 0.0001). Univariate results indicated that it was the reversal P100 (F = 27.7, P < 0.0001) and the offset contralateral P105 (F = 7.3, P < 0.008) ratios that showed the greatest differences.

A significant difference (with full Bonferroni adjustment, P < 0.001) was found between binocular/monocular amplitude ratios of amblyopes and controls for the reversal P100 components for the smallest 12' checks and for the moderate sized checks (20' and 50' checks). Onset VEP ratios (for ipsilateral CI and contralateral P105) also showed significant differences but only for the smallest 12' checks (with full Bonferroni adjustment,
Fig. 2. Mean P100 amplitude ratios (± 2 S.E.M.) for the different check­sizes in normal and amblyopic subjects. The two groups are significantly different for all checksizes except for the largest 80' checks. Significant differences (**P < 0.001) between controls and amblyopes are indicated by asterisks.

P < 0.0001 and P < 0.001, respectively). The P100 potential which is known to reflect macular function predominantly (Halliday et al., 1979), is the component that shows the greatest difference between normals and amblyopes for small and moderate sized checks (12', 20' and 50'), but not for the largest checks (80'). Fig. 2 shows that as checksize increases, the difference in amplitude ratios for P100 amplitude ratios between the normals and amblyopes tends to decrease.

There were no statistically significantly differences for offset VEP components, and the remaining onset (CIII, CIII) and reversal (N80, N145, N105) components.

Table 1

Mean binocular and monocular VEP latencies (ms) that are significantly different in normal subjects (paired t test, with full Bonferroni adjustment, P < 0.001).

<table>
<thead>
<tr>
<th>Component</th>
<th>Binocular</th>
<th>Left Eye</th>
<th>Right Eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIII 50'</td>
<td>141.2 ± 23.8</td>
<td>155.1 ± 29.8</td>
<td>154.7 ± 28.6</td>
</tr>
<tr>
<td>CIII 80'</td>
<td>143.5 ± 16.1</td>
<td>152.5 ± 20.3</td>
<td>151.9 ± 16.7</td>
</tr>
<tr>
<td>P105 12'</td>
<td>118.0 ± 16.9</td>
<td>122.8 ± 16.5</td>
<td>123.1 ± 16.7</td>
</tr>
<tr>
<td>P105 20'</td>
<td>113.9 ± 18.1</td>
<td>119.4 ± 15.7</td>
<td>122.1 ± 20.2</td>
</tr>
<tr>
<td>P105 50'</td>
<td>119.4 ± 14.6</td>
<td>124.7 ± 13.6</td>
<td>125.6 ± 16.1</td>
</tr>
<tr>
<td>P105 80'</td>
<td>120.1 ± 15.2</td>
<td>126.6 ± 15.6</td>
<td>125.7 ± 13.5</td>
</tr>
<tr>
<td>Reversal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N80 12'</td>
<td>69.4 ± 5.3</td>
<td>73.2 ± 9.1</td>
<td>76.1 ± 10.6</td>
</tr>
<tr>
<td>N80 20'</td>
<td>64.2 ± 6.1</td>
<td>67.2 ± 5.9</td>
<td>68.3 ± 4.5</td>
</tr>
<tr>
<td>N80 50'</td>
<td>58.6 ± 8.7</td>
<td>62.0 ± 7.4</td>
<td>63.0 ± 7.4</td>
</tr>
<tr>
<td>N80 80'</td>
<td>57.5 ± 10.1</td>
<td>60.2 ± 8.6</td>
<td>62.8 ± 8.8</td>
</tr>
<tr>
<td>P100 12'</td>
<td>102.2 ± 7.0</td>
<td>106.3 ± 8.6</td>
<td>106.4 ± 7.9</td>
</tr>
<tr>
<td>P100 20'</td>
<td>93.1 ± 6.9</td>
<td>98.1 ± 8.3</td>
<td>100.4 ± 6.9</td>
</tr>
<tr>
<td>P100 50'</td>
<td>90.7 ± 6.7</td>
<td>95.6 ± 7.4</td>
<td>93.7 ± 6.0</td>
</tr>
</tbody>
</table>

3.2. Binocular/monocular latency differences

There were no significant differences in the latencies for any of the components when comparing normal adults and normal children. Thus for further analysis data of normal children and adults were combined and this amalgamated group was compared with the amblyope group. There were also no significant differences in latencies when comparing left and right eyes of control subjects.

The mean binocular latencies of all pattern-onset and -reversal components, were shorter or equal to the mean monocular latency (of either eye) for stimulation with all checksizes. This was statistically significant (paired t test, with full Bonferroni adjustment, P < 0.001) for onset P105 component for all checksizes and for onset CIII, 50' and 80' checks only. For reversal stimulation, a significant difference (paired t test, with full Bonferroni adjustment, P < 0.001) was found for the N80 component, for all checksizes and, for the P100 component, checksizes 12', 20' and 50'. Offset components did not show such trends (Fig. 3, Table 1). There were no significant differences when comparing binocular-left eye and binocular-right eye latency differences in controls.

Amblyopes did not show significant differences when comparing binocular with non-amblyopic eye latencies. However, all onset and reversal VEP latencies from the amblyopic eye were consistently prolonged compared with the binocular response (Fig. 4).

The binocular-monocular latency differences were compared between the control and amblyopic groups. The difference in control subjects was calculated as the 'binocular latency-mean monocular latency', whereas for amblyopic subjects the 'binocular latency-non-amblyopic eye latency' was used.

MANOVA of binocular-monocular latency differences
for all check sizes and subject groups was carried out. A significant effect of check size by subject group was found (Pillai's test: $F = 1.66, df = 30, P < 0.02$), mainly ascribable to the reversal P100 latency differences ($F = 3.27, P < 0.03$). Check size alone was also significant (Pillai's test: $F = 1.5, df = 30, P < 0.05$) with reversal P100 and offset N85 latency differences contributing to the overall effect ($F = 2.91, P < 0.04$ and $F = 4.0, P < 0.01$, respectively). Subject group alone was also significant (Pillai's test: $F = 3.3, df = 10, P < 0.001$) due to the contributions of reversal N80 and P100 ($F = 17.9$ and $F = 13.1$, respectively, both $P < 0.0001$), and offset N85 latency differences ($F = 5.4, P < 0.03$). Fig. 5 summarises the mean latency differences for reversal N80 and P100 components. The predominance of significant latency results for the pattern-reversal VEPs, and the relative insensitivity of pattern-offset, are in line with the amplitude ratio findings, highlighting pattern reversal as the more reliable means of differentiating between normals and amblyopes.

The effects of visual acuity, type of amblyopia (strabismic or anisometropic) and the presence or absence of gross stereopsis on the binocular:monocular amplitude ratios and latency differences were investigated in the amblyopic patients. There were no significant correlations between visual acuity and either VEP amplitude ratios or latency differences. MANOVA of these VEP measures with respect to type of amblyopia and stereopsis also did not reveal any significant effects.

4. Discussion

Assessment of the pattern reversal P100 latency is acknowledged to be a reliable parameter in clinical practice. Our study, comparing the three modes of pattern stimulation showed reversal VEPs, namely the reversal P100 amplitude ratios and, binocular-monocular latencies of the reversal components (N80 and P100), were the most sensitive in differentiating between normals and amblyopes, and reaffirms the usefulness of reversal stimulation in clinical testing. For onset stimulation, the contralateral P105 component, appears to be the best component at demon-

strating interocular interaction, as gauged both by amplitude ratios and latency differences. There are inconsistencies between previous binocular evoked potential studies. Variations in stimulus and recording conditions across laboratories are the most likely to lead to discrepancies. Apkarian et al. (1981a) carried out extensive investigations of binocular interactions using pattern onset VEPs. They reported that the degree of binocular interaction in normals ranged from no summation to binocular facilitation (ratios > 2). Facilitation was found to occur optimally between about 1 and 3 c/deg. At the higher spatial frequencies, facilitation was absent and there was only partial summation. In our study, facilitation was not detectable for reversal stimulation in amblyopes, however, in normals, facilitation was found for reversal N80 and offset N85. Under identical stimulus conditions, the mean ratio values for amblyopes were 1.00 and 0.94 respectively, which essentially fall within our criterion of 'zero summation'. Most studies agree that for individuals with normal binocularity, the amplitude of the binocular VEP is approximately 1.4 times greater than the amplitude of either corresponding monocular response (Campbell and Maffei, 1970; Wanger and Nilsson, 1978). Vaugan et al. (1980) found the binocular VEP in the normals to be 1.4 times larger than the mean monocular response, however, this was only true for spatial frequencies away from the peak of the contrast sensitivity function. At the peak, the binocular:monocular ratio was significantly larger (1.64). Interestingly, in our study, in which a high contrast level was used, about half of the stimulus conditions for the normal subjects resulted in ratios that were greater than 1.4, with the reversal P100 having values around 1.6 (range: 1.54-1.66). The attenuated binocular:monocular amplitude ratios in our amblyopic subjects, particularly when using the smallest checks, support previous studies and imply reduced binocular interaction and minimal contributions from the amblyopic eye to the binocular response. VEP latency is influenced by spatial frequency and contrast (Kulikowski, 1977; Muselwhite and Jeffreys, 1985; Jakobsson and Johansson, 1992), with higher contrasts giving shorter latencies. It is also known that binocular contrast sensitivity is higher than the monocular (Campbell and Green, 1985). Thus these factors may be contributing to shorter latencies for binocular viewing, compared with monocular viewing, in normal subjects (Bagolini et al., 1988; Knierim et al., 1985; McCulloch and Scarf, 1991).

Lesèvre (1982) assessed binocular-monocular latency differences using 12' checks. She measured pattern reversal P100, N140 and P200 components, and found that component N140 in particular showed binocular summation, and a significant decrease in latency for binocular stimulation as compared with monocular stimulation. The latency decrease was, on average, about 10msec and was observed under all luminance and contrast conditions tested. Johansson and Jakobsson (1993) studied normal subjects and found significantly shorter pattern-reversal P100 latency for binocular viewing as compared with monocular viewing when using 4 c/deg sinusoidal gratings. However, amblyopic subjects had similar binocular and monocular latencies. Investigations of single unit recordings and VEPs in cats (Minke and Auerbach, 1972) reveal the presence of cells that respond faster to binocular stimulation than to monocular stimulation. The latency of the surface VEPs was closely associated with the responses from the faster single units. It was thus postulated that binocular stimulation preferentially activates the faster conducting pathways that are not activated on monocular testing. However, another factor to consider is that the shorter binocular latencies may reflect a reduction in the integration time by binocular units. Our data indicate that pattern-reversal is more effective than pattern-onset or -offset at distinguishing between subjects with normal and defective binocularity. Pattern-onset and -offset VEPs show more inter- and intra-individual variability compared with reversal P100 and N80 components. Both these two components are known to be of macular origin and are enhanced by small checksizes (Haliday et al., 1979; Kurita-Tashima et al., 1991; Bodis-Wollner et al., 1992). Checksize is an important factor in influencing both macular and paramacular VEP components. Optimal checksize progressively increases as more peripheral retinal areas are stimulated. The relative insensitivity of peripheral retinal areas to small checks is ascribed to the larger receptive field organisation of the periphery compared with central retinal areas. Moreover, the cortical magnification factor (defined as millimetres of cortex per degree of visual angle) decreases with increasing retinal eccentricity (Daniel and Whitteridge, 1961). We found significant differences between normal and amblyopic amplitude ratios for the smaller checks, indicative of the poor macular function in the amblyopes. The negative findings for pattern-offset VEPs alongside the positive results for pattern-reversal VEPs are unexpected, as these two response modes are thought to be generated by similar mechanisms (Estèves and Spekreijse, 1974). However, it is also known that the offset potentials can be of more variable waveform and latency, and of smaller amplitude compared with pattern-reversal VEPs (Harter, 1971; Spekreijse et al., 1973). This may account for the lack of significant offset results. A more unexpected finding was that pattern-onset was also relatively insensitive. Detailed investigations concerning the physiological significance of pattern-onset components (Jeffreys, 1971; Jeffreys and Axford, 1972b) have suggested that they differ in origin: CI and P105 are thought to be of striate origin, whereas CII and CIII are of extrastriate origin. These conclusions were derived from studies of the influence of retinal location on the occipital amplitude distribution of the VEP components. Stimulation of the central,
foveal 1 degree field was found to contribute mainly to the C1 component and minimally to the Cl and P105 components, which were primarily derived from the 2–6° region of the field (Jeffreys and Axford, 1972a). Cl and CII were found to be less dependent on contrast and more on pattern detail. In distinction, Cl had marked sensitivity to changes in contrast only. On the basis of these findings, we anticipated that both Cl and CII would show significant amplitude ratio differences when comparing normals and amblyopes, as these components should be markedly affected in amblyopia. Instead, the contralateral P105 component appeared to be the most reliable potential at differentiating between normals and amblyopes. This indicates that when using pattern-onset stimulation, half-field stimulation and analysis of VEPs from lateral channels are essential in order to clearly distinguish the more sensitive P105 component recordable over the contralateral hemisphere.

To conclude our data show that normal binocular VEP interaction is best shown when the appropriate small stimulus checks are used to accentuate the components which primarily represent macular pathway activity. Differences between normal subjects and amblyopes are greatest for macularly-derived VEPs. Pattern-reversal stimulation appears to be the better stimulus mode for accentuating such differences. It is important that small checks (25° or less) are used, and that both amplitude ratios and latency differences of the predominantly macularly-derived reversal N80 and P100 potentials components are evaluated.

Acknowledgements

We would like to thank Dr. A.M. Halliday for useful discussions, Mr. J. Pitman for technical help and The Iris Fund for financial support.

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