STUDY OF THE CHOLINERGIC FACTORS AFFECTING THE FLASH AND PATTERN REVERSAL VISUAL EVOKED POTENTIALS

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The effects of cholinergic agents undergoing clinical trials for the treatment of Alzheimer's disease and the anticholinergic agent scopolamine, were investigated on the components of the flash and pattern reversal visual evoked potentials (VEPs) in young healthy volunteers. The effect of recording the flash and pattern reversal VEPs for 13 hours in 5 healthy male volunteers, revealed no statistically significant change in the latency or amplitude measures. Administration of the muscarinic agonist SDZ 210-086 to 16 healthy male volunteers resulted in the reduction of the flash N2-P2 and pattern reversal N75-P100 peak-to-peak amplitudes. These effects on the flash VEP occurred at both doses (0.5 and 1.0 mg/day), but only at the higher dose on the pattern reversal VEP.

Administration of the antimuscarinic agent scopolamine to 11 healthy young male volunteers, resulted in a delay of the flash P2 latency but no effect on the pattern reversal P100 latency. The pattern reversal N75-P100 peak-to-peak amplitude was also increased post dosing. The combination of scopolamine with the acetylcholinesterase inhibitor SDZ ENA 713 resulted in no significant effect on the flash and pattern reversal VEPs, suggesting that the effects of scopolamine may have been partially reversed. Topical application of scopolamine in 6 young healthy volunteers also resulted in no statistically significant effects on the flash and pattern reversal VEPs.

The selective effect of scopolamine on the flash P2 latency but not on the pattern reversal P100 latency, provided a model whereby new cholinergic agents developed for the treatment of Alzheimer's disease can be investigated on a physiological basis. In addition, the results of this study led to the hypothesis that the selective flash P2 delay in Alzheimer's disease was probably due to a cholinergic deficit in both the tectal pathway from the retina to the visual cortex and the magnocellular path of the geniculostriate pathway, whereas the lack of an effect on the pattern reversal P100 component was probably due to a sparing of the parvocellular geniculostriate pathway.

KEY WORDS: Visual Evoked Potentials, Cholinergic drugs, Scopolamine, Alzheimer's Disease.

"It was the best of times, it was the worst of times...."

(Charles Dickens. A Tale of Two Cities.)

This thesis is dedicated to

my mother Mrs. Hansaben Padhiar

and

in loving memory of my dear father Mr. Amarsingh G. Padhiar.

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CHAPTER 1

Introduction

Alzheimer's disease (AD), is a devastating condition which occurs in later life. The disease is also known as presenile dementia when age of onset is between 45-65 years, and senile dementia when age of onset is over 65 years (Katzman 1986). The primary symptoms include loss of memory (particularly recent) and impaired cognitive function. However due to the heterogeneous nature of the disease, the disease has been difficult to diagnose (Mayeux et al. 1985, Neary et al. 1986). The diagnosis generally tends to be through differential diagnosis, using various psychometric tests, brain imaging, laboratory tests and EEG.

Currently there are no totally effective modes of therapy in AD. Treatment strategies include alleviating secondary symptoms of the disease such as depression and anxiety (Yesavage 1983). There are consistent reports of a reduction in cholinergic innervation (Wurtman 1985; Whitehouse et al. 1981; Mann 1985), although it is not the only neurotransmitter system affected by the disease (Appel et al. 1988; Price 1986;Cowburn et al. 1989). Various studies have also shown a correlation between a decline in cholinergic function and cognitive deficits (Perry et al. 1978; Bartus et al. 1982; Coyle et al. 1983). Hence the main form of drug therapy has been concentrated on improving the cholinergic function in patients with AD. However, this has met with little success, mainly due to the toxic side-effects associated with cholinergic agents (Drachman 1985). Therefore new products are continually being developed which will enhance the central cholinergic transmission in AD, without toxic side-effects.

Between 1981 and 1986, Harding and co-workers carried out numerous studies on the flash and pattern reversal visual evoked potentials (VEPs) in patients suffering from AD. The unusual finding of a delay in the major positive component of the flash VEP (P2) co-existing with a pattern reversal VEP (P100 component) of normal latency was reported in patients with AD (Doggett et al. 1981; Harding et al. 1981). These studies led to the possible use of the flash in conjunction with the pattern reversal VEP as possible diagnostic aids for the disease (Harding et al. 1984; Wright et al. 1984a; Harding et al. 1985; Wright et al. 1986). In addition, the anticholinergic agent scopolamine has been shown to mimic the flash P2 delay co-existing with a normal pattern reversal VEP, mimicking the observation in patients with AD (Bajalan et al. 1986). This suggested that the flash but not the pattern reversal VEP, was under the influence of acetylcholine.

New drug treatments usually undergo various clinical trials before approval can be obtained for its use. Since there is a selective deficit in the flash but not the pattern reversal VEP in patients with AD, which is also mimicked in healthy individuals with the anticholinergic agent scopolamine, it would not be unusual to test potential efficacy of a new cholinergic drug with the use of the flash and pattern reversal VEPs. In addition, the flash and pattern reversal VEPs are thought to represent different visual processing systems (Halliday 1982; Regan 1989), although the exact nature of the visual processing occurring from the level of the retina to the visual cortex is still not fully understood. Hence a study was proposed whereby the flash and pattern reversal VEPs are incorporated into clinical trials of cholinergic drugs, with the following main objectives:

1. To investigate the effectiveness of the flash and pattern reversal VEPs in clinical studies investigating new cholinergic agents developed for the treatment of AD.

2. To examine further the nature of the generators of the flash P2 and pattern reversal P100 components in relation to cholinergic function.

The thesis begins with three chapters on the background literature relating to the proposed studies. The first of these, chapter 2 examines the visual pathway, as this is the sensory system under investigation. Chapter 3 describes VEPs especially to the flash and pattern stimuli, as these are the tools used in the investigation. Thirdly, chapter 4 hopes to provide an understanding of the disease process for which the agents to be studied have been developed. The ensuing chapters then describe a series of investigations, each with their own rationale to answer a specific question. Finally, chapter 10 summarises the results of the studies undertaken and leads to a discussion relating to the objectives mentioned above.

CHAPTER 2

Anatomy and Physiology of the Visual System

2.1 Anatomy of the visual pathway

The visual pathway (fig. 2.1) as described by Polyak (1957) commences at the retina which contains the photoreceptors (rods and cones). The visual information is transmitted to the cortex by the axons of the retinal ganglion cells forming the optic nerve. The right and left optic nerve converge at the optic chiasma where fibres from the nasal half of each retina cross to the opposite side of the brain. Temporal fibres continue ipsilaterally, together with crossed fibres from the contralateral optic nerve, as the optic tract, leading to either the lateral geniculate nuclei (LGN), the superior colliculi, the nuclei of the accessory optic tract or the pretectal region of the brainstem (fig. 2.1). The optic tract from the LGN runs through the optic radiation not only to the primary visual cortex but also to the secondary visual cortex.

2.1.1 Retina

The retina as the sensory organ of the eye, is responsible for converting light energy (perceived in humans as electromagnetic radiation at wavelengths between 400-750 nm), into electrical impulses which can be interpreted by the brain. Ontogenically, the retina arises as part of the diencephalon and may be considered as an extension of the central nervous system (CNS).

The primate retina (fig. 2.2) is morphologically characterised by a distinct laminar organisation, containing six major cell types in five distinct layers (Cajal 1893). These layers, proceeding from the distal surface to the pigment epithelium toward the proximal surface, include the photoreceptor layer (PRL), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), ganglion cell layer (GCL), and optic axon layer. The main neuronal retinal cell types comprise the photoreceptors, bipolar, horizontal, amacrine, and ganglion cells. In addition there are neuroglia, the majority of which are specialised radial glia known as Müller cells.

The photoreceptor cells include the rods and cones, of which there are an estimated 75-150 million rods and nearly 6-7 million cones in the human retina. The receptor density (number of receptors per unit area) changes with retinal eccentricity such that the density of cones is greatest in the centre of the fovea and decreases towards the periphery, while that of the rods is greatest in periphery (usually 5 mm from the fovea

Figure 2.1. Schematic diagram of the main neural pathways involved in primate vision. (From Willmer 1982).

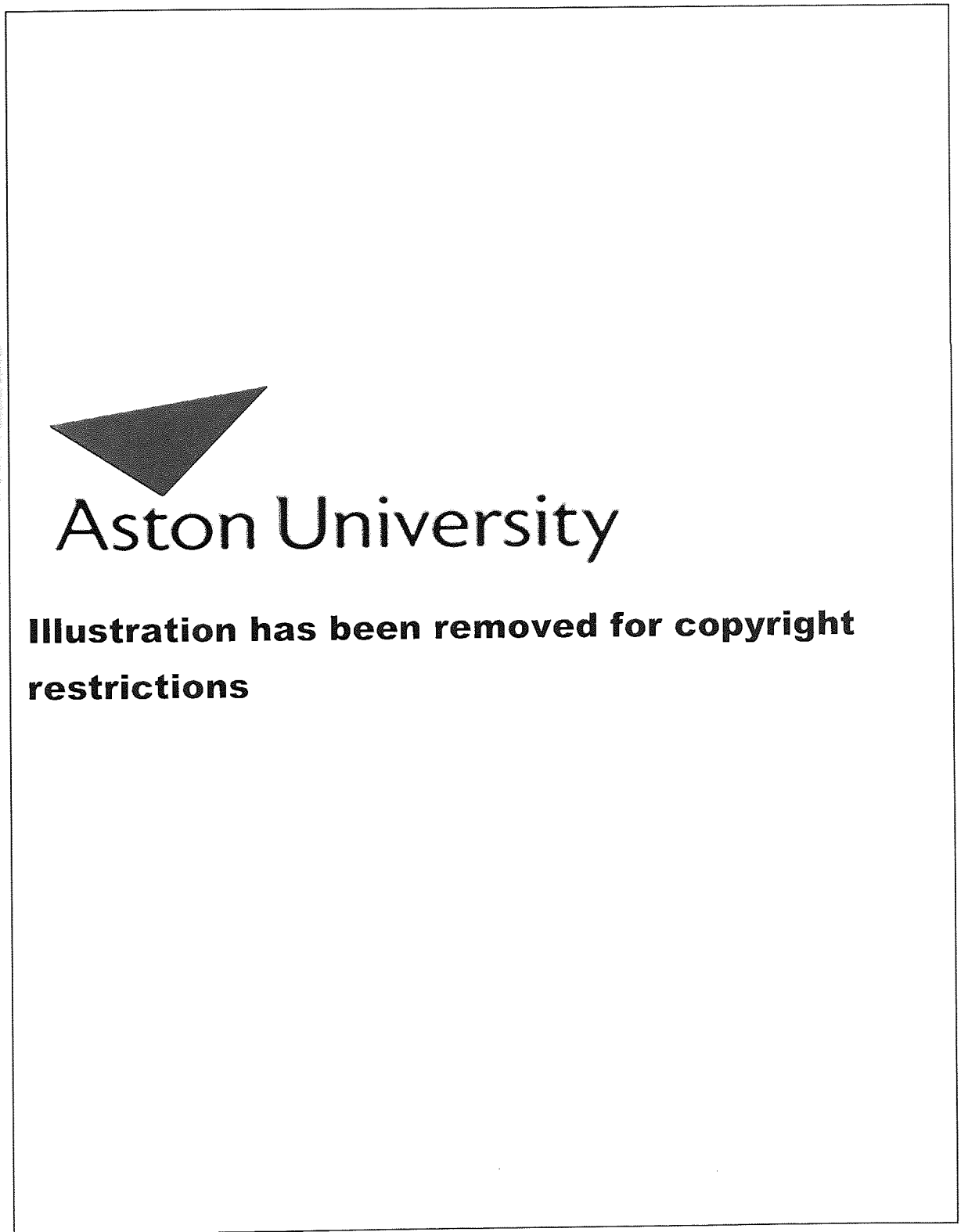


Figure 2.2. Schematic diagram of the primate retina. The terminals of the photoreceptors are greatly enlarged to show their connections with other cell types. CC, choroidcapilaris; BM, Bruch's membrane; RPE, retinal pigment epithelium; VM, Verhoeff's membrane; OS, outer segments of photoreceptors; IS, inner segments of photoreceptors; OLM, outer limiting membrane; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; OFL, optic fibre layer; ILM, inner limiting membrane; FD, flat diffuse bipolar; FM, flat midget bipolar; HI, H1 horizontal cell; HII, H2 horizontal cell; ID, invaginating diffuse bipolar; IM, invaginating midget bipolar. (From Kaplan et al. 1991).

or 20° of visual angle), and decreases towards the fovea. The fovea centralis contains no rods and is the location of highest visual acuity (Davson 1990).

The primate retina contains three types of cones, the red-sensitive (or L-cones) with maximum absorption near 566 nm, the green-sensitive (or M-cones) with maximum absorption near 535 nm, and blue-sensitive (or S-cones) with maximum absorption near 415-420 nm. The rods have a maximum absorption near 500 nm (Kaplan et al. 1991).

Visual information usually flows from the photoreceptor cells to bipolar cells and then to the ganglion cells which then transmit the signals to the brain. Lateral spread of information is achieved via the horizontal and amacrine cells (the interneurones) and also back to the distal neurones (bipolars, other amacrine, rods and cones) through the interplexiform cells (Pearlman 1981).

The ganglion cells constitute the output layer of the retina and their axons, initially unmyelinated, run through the retina to the papilla, across the sclera, through the lamina cibrosa before forming the optic nerve. Ganglion cells display signal convergence whereby they can be connected, either directly or indirectly with many receptors, bipolar cells, horizontal cells, and amacrine cells. This signal convergence is greater towards the periphery of the retina and depends not only on the size of the dendritic tree of the ganglion cell but also on the spatial extent of the lateral processes of the horizontal and amacrine cells, which are also responsible for lateral inhibition of electrical signals (Davson 1990). Signal divergence occurs whereby a single photoreceptor may be connected to several bipolar cells which in turn connect to several ganglion cells. However, since there are 125 million receptors to only about 1 million ganglion cells, the primate retina predominantly displays signal convergence (Davson 1990).

2.1.2 Optic nerve and tract

The human optic nerve contains about a million axons, mostly myelinated and surrounded by glial cells and connective tissue (Polyak 1957). Around 65% of fibres in the optic nerve represent the macula - the region of highest visual acuity. The optic nerve from both eyes meet at the optic chiasma at the base of the skull. Fibres from the nasal hemiretinas decussate such that each optic tract contains fibres from the temporal hemiretina of one eye, together with fibres from the nasal hemiretina of the other eye. The visual impulse from the right half of the visual field is exclusively conveyed along the left optic tract to the left cerebral hemisphere. The degree of

decussation varies between species and is a function of binocular vision, e.g. in the guinea-pig 99% of fibres cross leaving only 1% uncrossed, the rat has about 10% of uncrossed fibres, the opposum 20%, whilst the cat and primates, with frontally directed eyes have almost equal numbers of crossed and uncrossed fibres (Davson 1990). The uncrossed fibres are thought to subserve primarily stereoscopic perception of depth. Half the macula fibres also undergo decussation at the chiasma such that half the macula is represented in each optic tract.

The optic tract leads to the first central stations of the visual pathway - the lateral geniculate nucleus (LGN), the superior colliculi, the nuclei of the accessory optic tract, pretectal region of the brainstem, and the hypothalamus (Rodieck 1979; Pearlman 1981).

2.1.3 The geniculate pathway.

Approximately 80% of fibres from the optic tract go to the LGN -the major central relay station (Davson 1990). Each major sensory system has an important set of synaptic connections in the thalamus: the thalamic relay in the visual system being the LGN. The term "relay" implies that the function of the LGN is simply to pass information along in an unaltered form. However, the complex synaptic interactions occurring between the incoming optic tract fibres and the geniculate cells dispute this. The LGN consists of two subdivisions, a larger dorsal nucleus (dLGN) and a smaller ventral one (vLGN). Although both subdivisions receive retinal afferents, only the dorsal appears to convey visual information to the cerebral cortex (Brodal 1981; Davson 1990).

Brindley (1970) suggested that the function of the LGN was as a relay station and the advantage in interrupting the visual pathway, was to "discard unimportant information, compress it into fewer channels and/or modulate the flow of information to the cortex". In addition, non-visual inputs to the LGN could regulate the discarded information and modulate the rest such that it may be useful in changes in the state of attention. There is probably less than 20% of synaptic input to the dLGN that is retinal in origin, thereby providing a framework in which information can be modified and processed, (Sherman and Koch 1986).

2.1.3.1 Lateral geniculate nucleus

The LGN of both primates and cat consists of thin layers containing nerve cell bodies and nuclei, alternating with layers containing only nerve fibres and a few neuroglia cells, (fig. 2.3). In the cat, there are three cellular layers, called A (most superficial),

Figure 2.3. TOP: Lateral geniculate nucleus of cat. There are three layers of cells-A, A1, and C.
BOTTOM: Lateral geniculate nucleus of monkey showing six layers. The four dorsal layers, 3, 4, 5, and 6 are the parvocellular layers. The two ventral layers, 1 and 2, are the magnocellular layers. (From Kuffler et al. 1984).

A1 and B. Thuma (1928) originally described these layers, with layers A and A1 having very similar morphologies. Layer A and B receive input from the contralateral retina whilst A1 receives ipsilateral retinal input. Guillery (1969) however indicated a more complex lamination; layer B was replaced by three layers C, C1 and C2, with layers C and C1 being analogous with A and A1, i.e. receiving contralateral and ipsilateral input respectively and C2 receiving no retinal input. Generally the cat LGN laminae are termed A, A1 and C.

The primate dLGN displays six distinct layers of cell bodies, each separated by a narrow almost cell-free zone (fig. 2.3), (Pearlman 1981). The four most dorsal layers are the parvocellular layers containing relatively small cells, whilst the two most ventral layers are the magnocellular layers containing larger cells. The four parvocellular layers of the posterior aspect of the dLGN combine anteriorly to form two layers. The posterior dLGN contains the representation of the central 15^{o}-20^{o} of the contralateral half-field, with the remainder half-field represented in the more anterior four-layered segment. The layers are numbered from ventral to dorsal 1 to 6. Parvocellular layers 6, 4, and magnocellular layer 1, receive contralateral retinal input and parvocellular layers 3 and 5 and magnocellular layer 2 receive ipsilateral input (Glees and Clark 1941).

The dLGN also contains intrinsic neurones involved in intergeniculate integration of the afferent input. These neurones are believed to be inhibitory in nature. Hamori et al. (1983) found approximately 15% of cells in the magnocellular and 4% in the parvocellular layers were interneurones and do not project to the visual cortex. They propose that the role of the greater number of intrinsic neurones in the magnocellular layers of the dLGN was to maintain the transient responses of Y-like cells (section 2.3). In addition, the LGN receives heavy projections from many structures such as the brainstem, midbrain and the cortex. In the cat, almost 80% of the input to the LGN is of extraretinal origin, (Schiller 1986).

There are reports that only the large cells in the dLGN project to Brodman's area 18, whereas geniculate cells of all sizes project to area 17 (Garey and Powell 1971; Holländer and Vanegas 1977). There is further morphological evidence that large geniculate cells send dichotomizing branches to areas 17 and 18 (Holländer and Vanegas 1977). The physiological counterpart of the anatomical observation have shown that the large, fast-conducting relay cells in the dLGN (the Y-cells) project to both areas 17 and 18 by means of a branching axon and the smaller, slower-conducting relay cells (X-cells) project only to area 17.

The human dLGN is similar in basic structure, but there is a considerable degree of variability from one brain to the next in the details of the laminar patterns (Hickey and Guillery 1979). The basic function achieved by the lamination is to keep the retinal inputs separate. Thus integration of the inputs from the two eyes to give a single field of vision is a function of the cerebral cortex. Each geniculate layer contains a precise retinotopic map of the contralateral visual field. Retinal ganglion cells of different loci project upon distinct visuotopic points in the dLGN. However, not all areas of the retina are equally represented; proportionally much more of the dLGN is devoted to the central representation rather than the peripheral retina. Regions of greater ganglion cell densities send more axons to the brain and consequently dominate a greater portion of the central representation of the retina (Brindley 1970).

The neurones projecting from the parvocellular layers of the dLGN to the striate cortex tend to be thinly myelinated with restricted terminal arbors in the striate cortex (Hickey and Guillery 1979). Whereas neurones projecting from the magnocellular layers tend to be thickly myelinated with large terminal arbors in the striate cortex. (See section 2.1.5 for a fuller account of the geniculate projections to the cortex).

The vLGN is morphologically distinct and much smaller than its dorsal counterpart (dLGN). Its function is not clear, but receive inputs from the optic tract through collaterals relaying in the dLGN from the superior colliculi, from the juxta-visual areas of the cerebral cortex and also from the dLGN itself (Swanson et al. 1974). Efferent connections go to several subcortical nuclei such as the pretectal nuclei, the lateral terminal nuclei of the accessory optic system, the ventral portion of the suprachiasmatic nuclei of the hypothalamus and the superior colliculi. The vLGN input to the superior colliculi is confined to the deeper layers and there is no evidence for a direct cortical projection (Brodal 1981).

2.1.4 Non-geniculate pathways

It is estimated that approximately 20-30% of fibres in the optic tract in man go to other areas of the brain instead of the LGN, forming the non-geniculate pathway (Davson 1990). The majority of these fibres terminate in the superior colliculi, pretectal region and the hypothalamus. A few, called the accessory optic fibres go to a wide range of other regions in the brain.

2.1.4.1 Superior Colliculus

In monkey, up to 10% of retinal ganglion cells project to the superior colliculus- which is also known as the mesencephalic root of the optic tract (the diencephalic root

runs to the LGN), (Perry and Cowey 1984). The superior colliculus is composed of seven layers, but only the first three superficial layers receive visual information whilst the deeper layers receive auditory and somatosensory inputs and give rise to most of the efferent connections of the superior colliculus. There are over forty different structures projecting to the superior colliculus, but the main source of visual input is the retina, both directly and indirectly via the cortex. The direct retinal input is from the contralateral retina whereas the ipsilateral halves of each retina is mapped indirectly via the visual cortex (Wickelgren and Sterling 1969). The occipital input arise mainly from pyramidal cells of layer V in Brodman's areas 18 and 19 as well as 17 (Heurta and Harting 1984 and 1984a). There is also ipsilateral input from Brodman's area 8 which correspond to the frontal eye field, from which conjugate movements can be elicited. The superior colliculus receives several nonvisual afferents such as from the spinal cord, inferior colliculus which receives acoustic information, ventral LGN, cerebellum, substantia nigra, hypothalamus and the reticular thalamic nucleus (Brodal 1981).

The efferent fibres from the superior colliculus include some to the brainstem and spinal cord, the pontine nuclei and the reticular formation of the medulla, pons and mesencephalon and some to the inferior olive (fig. 2.4). The ascending fibres of the superior colliculus go to the pretectum, pulvinar, dLGN and vLGN (Huerta and Harting 1984). The notion that the tectum and dLGN form two distinct but parallel visual pathways (e.g. Schneider 1969), has figured prominently in the understanding of collicular function. However, these pathways are not entirely separate, since the superficial layers of the superior colliculus project to the dLGN (to the interlaminar zones, layers 3 and 6; in cat to the parvocellular layer C). The superior colliculus sends a projection to the pulvinar (or lateral posterior-pulvinar complex) which in turn projects to layer I of Brodman's area 17. Therefore, information from the superior colliculus can reach the striate cortex either via the tecto-pulvinar-cortical channel or via the tecto-geniculo-cortical pathway (Huerta and Harting 1984a).

The superior colliculus appears to be more important for visual functions in lower than in higher vertebrate species. The superior colliculus is important for orientation of the head and eyes towards the source of an external stimuli whether it is visual, auditory or somatic. The capability of fixation on objects by the eyes is greatly impaired by the loss of the superior colliculi, although other conjugate movements of the eye may be preserved (Guyton 1981). The signals for fixation originate in the visual cortex (primarily in the extrastriate areas) then pass to the superior colliculus, from there to the reticular areas around the oculomotor nuclei and thence to the motor nuclei themselves. The superior colliculus is also important for rapid eye movements. The

Figure 2.4 Simplified diagram of the main afferent and efferent connections of the superior colliculus (left side of figure) and pretectal nuclei (right side). The various pretectal nuclei are not indicated for simplicity. Efferent connections are shown by heavy lines and afferent by thin lines. The connection shown by the broken line from pretectal nuclei to the Edinger-Westphal nucleus is considered part of the elementary reflex arc for the light reflex. Many connections are not included. For simplicity, all connections except the tectospinal tract are shown as being ipsilateral. (From Brodal 1981).

numerous interconnections of the superior colliculus indicates that this small area cooperates with many other regions and that it is not only related to visual function.

2.1.4.2 Pretectum

The pretectal region is another reflex centre and lies just rostral to the superior colliculus where the midbrain fuses with the thalamus. This region has been considered to be involved in the reflex arc of the pupillary light reflex. Certain retinal ganglion cells respond to overall changes in luminance of the visual field which send axons through the optic nerve and tract to synapse in the pretectal region (Brindley 1970). The axons then project bilaterally to preganglionic parasympathetic neurones in the region immediately adjacent to the somatic motor neurones of the third nerve nucleus (Kandel and Schwartz 1985). The axons from this region go out of the brain with the third nerve to innervate the ciliary ganglion where the postganglionic neurones innervate the smooth muscle of the pupillary sphincter.

Fibres from the cerebral cortex to the pretectal nuclei come mainly from the visual areas in the occipital lobe. There are also afferent connections from the superior colliculus, ventral LGN, possibly some from the posterior regions of the thalamus and from the pontine reticular formation (fig. 2.4). The efferent connections of the pretectum are to some extent reciprocal to their afferents. There are efferent connections to the superior colliculus, the ventral LGN, the accessory oculomotor nuclei, and the pulvinar, as well as to regions less immediately related to visual function, such as the reticular thalamic nucleus and parts of the interlaminar thalamic nuclei, the reticular formation of the brainstem, the hypothalamus and some other regions (Brodal 1981).

2.1.4.3 Accessory optic system

The accessory optic system consists of three small cell groups of crossed finely medullated axons which divide into the superior and inferior accessory tracts (Davson 1990). The inferior fasciculus is a group of fibres projecting in the medial terminal nucleus of the accessory optic system. The superior fasciculus is larger and more diffuse and terminates in the basal portion of the medial terminal nucleus. These nuclei receive afferent fibres from several regions, but not from the retina directly (Brodal 1981). These accessory oculomotor nuclei are thought to integrate vestibular impulses from the cerebral cortex and the superior colliculus, while input from the retina may contribute via intercalated stations such as the pretectal region, which receives afferents from the retina. This pathway may allow the retina to influence

large portions of the brainstem and thus become involved in visually guided activity over a large range.

There are probably many functions of this pathway but they have been difficult to ascertain. Some fibres respond to light falling on the retina, and some may be concerned in non-specific arousal of the brain and in controlling the release of gonadotrophins via the hypothalamus (Brindley 1970). Schiller (1986), suggested the function of the neurones of the accessory optic system was to process information about both the velocity and the direction of a moving stimulus.

2.1.4.4 Hypothalamus

The hypothalamus contain axons terminating in the suprachiasmatic nuclei and is responsible for control of circadian rhythms. The suprachiasmatic nuclei lie just above the optic chiasm and lateral to the third ventricle. Apart from retinal ganglion cells, the suprachiasmatic nuclei also receives input from the vLGN as well as other nonvisual inputs (Rodieck 1979).

Stephen and Zucker (1972) reported that the tonic effects of light on bodily function and the rhythmic effects which manifest in circadian rhythms, were influenced by the light input through the suprachiasmatic nuclei and pineal gland, whilst the rhythmic effects of continuous exposure to light and dark were influenced by the accessory system. However, the rhythmic activity of the suprachiasmatic nuclei itself does not depend on the visual input but that the diurnal cycle of light intensity, conveyed by visual input, is probably used to synchronise this rhythm (Schwartz and Gainer 1977).

2.1.4.5 Pulvinar

The pulvinar is a thalamic nuclear mass with close connections with the LGN, and projects to visual, auditory and somatosensory association areas in the cortex. It plays an important role in the processing of the visual information. It is generally regarded as a polysensory nucleus depending largely on the cerebral cortex for its sensory input and projecting to polysensory areas of the cortex involved in processing polysensory information a long way removed from the primary stimuli (Trojanowski and Jacobson 1976). A direct pathway from the retina to the pulvinar is a subject of debate in primates. But generally, the pulvinar depends on cortical and tectal input for the integrative visual activities that it mediates. Damage to the pulvinar can result in impairment of directed attention and pattern perception (Schiller 1986).

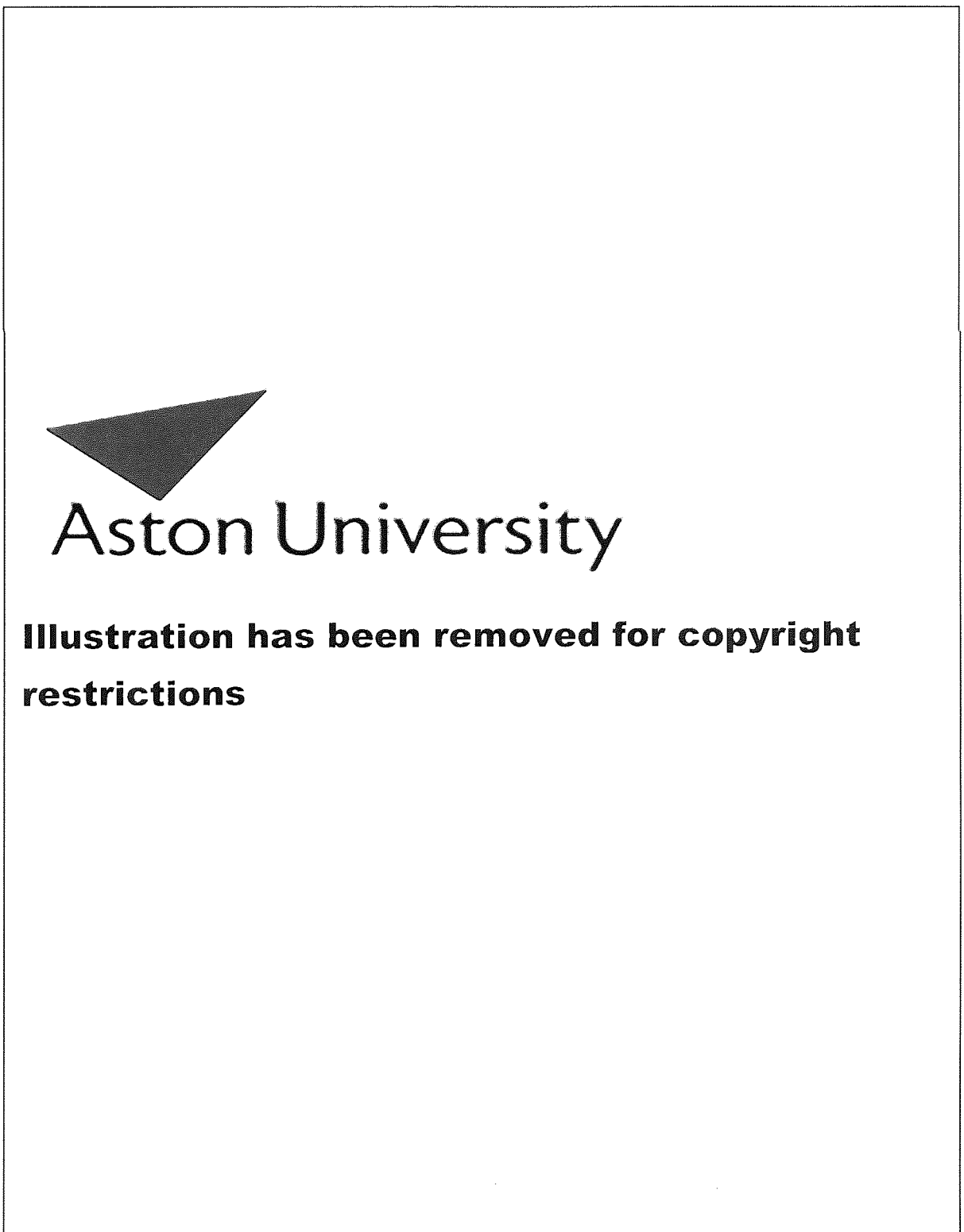
2.1.6 Visual cortex

Visual information is projected to the cortex from the dLGN through the optic radiation (fig. 2.1). After sweeping around the lateral ventricle, the fibres terminate on the lateral surface of both the temporal and occipital horns of the lateral ventricle (Kandel et al. 1984). Fibres representing the inferior retina travel in a broad arc over the temporal horn of the ventricle and loop into the temporal lobe before turning caudally to terminate in the occipital lobe. These fibres form the Meyer's loop and account for unilateral lesions in the temporal lobe affecting vision in the superior quadrant of the contralateral visual half-field. The geniculocortical fibres from the inferior part of the retina, terminate in the inferior region of the cortex lining the calcarine fissure, whereas fibres from the superior retina terminate on the superior region (fig. 2.5).

The visual cortex receives input from not only the LGN, but also from the various subcortical nuclei. Tigges and Tigges (1985) reported that at least twenty subcortical structures project directly to the visual cortex. This in turn results in a wide range of neurotransmitters systems involved in the visual system (see section 2.4). Examples of some of the subcortical projections in the macaque monkey include, the locus coeruleus, raphe nuclei, nucleus annularis, nucleus centralis superior, pontine reticular formation, mesencephalic reticular formation, nucleus linearis, lateral hypothalamus, nucleus basalis of Meynert, nucleus of the diagonal band of Broca, claustrum, nucleus basalis lateralis amygdalae, medial interlaminar nucleus, pulvinar, nucleus lateralis posterior, nucleus limitans, interlaminar thalamic nuclei, and nucleus ventralis anterior.

Cell bodies of the cerebral cortex are arranged in layers separated by relatively cell free zones containing mainly the dendritic and axonal processes of the cells. The cortex is divided into cytoarchitectonic areas based on the differences in the appearance of the cortical layers, of which the occipital lobe is the most distinctive. Different staining methods reveal different aspects of the cortical layers, for instance Nissl (aniline dye) stains cell bodies, axons may be visualised by means of a myelin stain and finally the pigment content of cells is revealed by pigment-specific stains. The cortex reveals six layers, which have been labelled with Roman numerals by Brodman (1909) and Vogt and Vogt (1919) and later modified by Hässler (1966). From the cortical surface to the underlying white matter these layers are I the molecular layer, II the corpuscular layer, III the pyramidal layer, IV the granular layer, V the ganglionic layer, and VI the multiform layer. Layers III and IV are further divide into sub-layers e.g. layer IV is further subdivided into layers IVA, IVB, and IVC and the latter is subdivided further

Figure 2.5. Projection of the right visual field on the left visual cortex in humans. (From Brindley 1970).



into IVCalpha and IVCbeta. In general, the more superficial layers are the source of commissural and association connections within the cortex, while the deeper layers are the sources of corticofugal connections.

There are two main types of cortical neurones: pyramidal cells and nonpyramidal cells. The main differences are in the lengths of the axons and shape of the cell bodies. Pyramidal cells which make up approximately 60% of the neural population of the visual cortex and are present in all layers except layer I. Pyramidal neurones have longer axons and a cell body shaped like a pyramid the apex of which points towards the cortical surface giving rise to a single apical dendrite, while the base of the cell gives rise to several dendrites spreading in a radial fashion (Pearlman 1981). The pyramidal cell axon usually descends to the subcortical white matter and projects either to another cortical site or to a subcortical target such as the LGN or superior colliculi.

The nonpyramidal cells comprise a heteromorphic population. A common feature of these nonpyramidal neurones is their axons are distributed entirely within the visual cortex. They are classed as multipolar, bitufted and bipolar, and based on the frequency of spines they are further subdivided into spinous, sparsely spinous and spine-free. The multipolar neurones make-up 60% the nonpyramidal neurones and predominate in layer IV. The majority of multipolar neurones in turn are termed stellate cells. Axons of the stellate cell, ramify locally to form intracortical connections. The dendrites of the stellate cells arise around the circumference of the cell body in a star-shaped pattern. The spiny stellate cell is restricted to cortical layer IV where most of the LGN fibres terminate, usually on the dendritic spines (Lund 1973). In addition, there are other neurones such as double bouquet cells, chandelier cells, basket cells, and crescent cells, as well as neuroglial cells (Pearlman 1981).

Within the cortex, in addition to the lamination pattern, there are different patterns of cytochrome oxidase (CO) staining that exists in both the striate and extrastriate cortices, (Livingstone and Hubel 1982). CO is a mitochrondrial enzyme whose presence is thought to represent differences in chronic neuronal activity. In the striate cortex, CO labelling reveals small round, dense "blobs" on a lighter background, mainly in layers II and III. The spaces between the dense staining is called the "interblob" region (Livingstone and Hubel 1982). In the extrastriate cortex, there are three subdivisions distinguishable by CO staining, arranged in stripes. Two types of dark staining stripes, alternate as thin and thick stripes, all separated by paler staining interstripes (Hubel and Livingstone 1989).

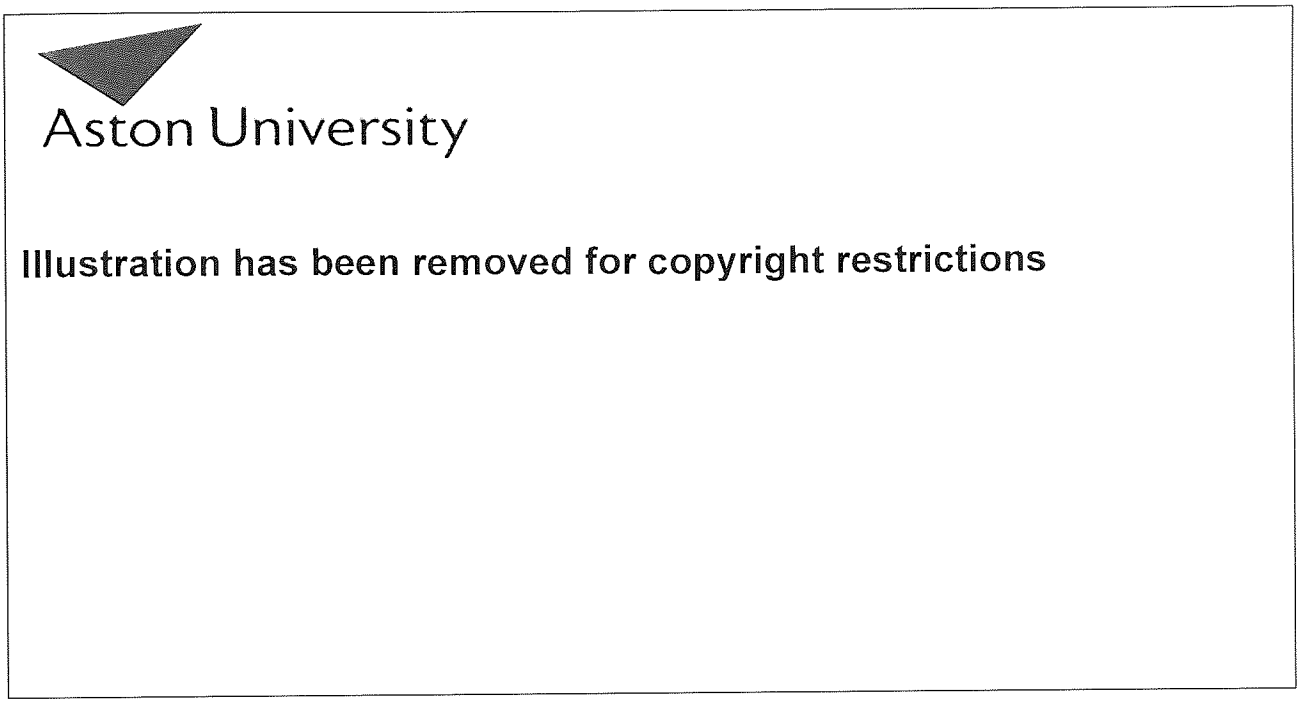
2.1.5.1 Striate cortex

The occipital region characterised by a thick band of dendrites and axons midway between the surface and depth of the cortex, visible to the naked eye and which is called the "stria of Gennari" (also known as the white line of Gennari after its discoverer) can be called the striate cortex, Brodman's area 17, or visual area 1. In humans, the striate cortex is located on both sides of the calcarine fissure on the medial aspect of the occipital lobe, extending for a short distance onto the occipital pole (Brindley 1970). The retinal projection can be represented by one half of the retina spread over the surface of one striate area, with the macular region being placed posteriorly and the periphery anteriorly, the upper margin along its upper edge and the lower on its inferior border. Much more of the cortical area is devoted to the foveal representation than the rest of the retina (fig. 2.5).

Cells of a given geniculate layer project to specific targets, thus afferent fibres arriving in the striate cortex terminate in specific layers (fig. 2.6). These geniculate fibres end mainly in, but not exclusively, in layer IV of area 17 (Ferster and LeVay 1978; Gilbert and Wiesel 1979). In addition, the superficial layers receive inputs from the C layers of the dLGN (containing Y and W cells) in the cat, and from the pulvinar in the monkey. The monkey dLGN sending afferents terminating primarily in layer IV and sparsely in layer I, also receive reciprocal connections from the striate cortex from the pyramidal cells of layer VI (Wilson and Cragg 1967; Garey and Powell 1971; Hubel and Wiesel 1972; Lund 1973). Fibres from the parvocellular layers of the dLGN generally terminate in a narrow and less dense band just above the stria of Gennari in layer IVA and in a broad and dense band in layer IVCbeta which in turn projects to layers II and III in the blobs region and the upper part of layer VI (De Yoe and Van Essen1985; Shipp and Zeki 1985). Fibres from the two magnocellular layers terminate in the deeper part of layer VI and mainly to layer IVCalpha which in turn projects to layer IVB which projects to extrastriate regions in Brodman's area 18 and middle temporal visual area (MT), whose major cortical output is to the adjoining superior temporal region and then to the parietal cortex (Mishkin et al. 1983).

The distinction between the magnocellular and parvocellular layers is evident with the cortical cells in the upper part of layer VI projecting primarily to the magnocellular layers whereas the projection to the parvocellular layers arise mainly from the cells in the lower aspect of layer VI.

Figure 2.6 Summary of the connections from the lateral geniculate nucleus to the striate cortex and from the striate cortex to other regions. (From Hubel 1987).



Neurones in layer V of the striate cortex project back to the superior colliculi and the pulvinar (although the major connections of the pulvinar are with the extrastriate cortex). Axons from the pulvinar to the striate cortex terminate in layer I, among the dendrites of the layer V cells whose axons project back to the pulvinar and the superior colliculi. Hence the striate cortical cells projecting to the subcortical areas are located in the deep cortical layers V and VI. The striate cortex is also extensively interconnected with other cortical regions. Pyramidal cells of the striate cortex project to the extrastriate visual regions primarily in the superficial layers II and III. Cells projecting via the corpus callosum to the contralateral striate cortex also arise in layer III. Reciprocal connections from the extrastriate cortex and the contralateral striate cortex terminate in layers II and III of the striate cortex (Lund 1973).

Two main types of functional columns have been identified in the striate cortex, "ocular dominance" and "orientation" columns. Autoradiographical studies of Hubel and Wiesel (1972a) revealed a pattern of thalamic input to the visual cortex with a regular array. The cortical cells of layer IVC receive input from either one or the other eye via the separate layers of the dLGN and the output from layer IVC goes to the layers above and below it, where signals from the two eyes converge upon individual cells. These clusters of terminals relaying input from each eye to separate zones within layer IV form the anatomical basis of ocular dominance. The ocular dominance column is 250-500 μm wide (Hubel and Wiesel 1969, 1972, 1972a and 1974). The total width of an adjoining pair of right and left ocular dominance columns is similar to the width of an orientation column that encompasses a complete cycle of orientations through 180^{o}.

2.1.5.2 Extrastriate cortex

The striate cortex is surrounded by areas also concerned with vision and are known as the extrastriate regions (also known as peristriate and parastriate, Brodman's areas 18 and 19, secondary visual cortex, visual association areas, or visual areas II and III). The exact boundaries between the visual areas are based on structural and physiological criteria. In Brodman's area 18, for example, the striate appearance of layer IV disappears; large characteristic cells are found in layer III; and coarse, obliquely running, myelinated fibres are found in the deeper layers. There are more than just three cortical areas that exist in the occipital cortex of rats, guinea pigs, and monkeys, all concerned with vision, and each containing its own representation of its visual field projected in an orderly manner (Van Essen 1979).

The extrastriate areas receive visual input from two main sources; thalamic connections from the pulvinar and corticocortical connections from the striate cortex and other extrastriate visual regions (Pearlman 1981). The pulvinar in the rhesus monkey, projects extensively to the extrastriate cortex with sparse a projection to the striate cortex. In primates only the striate cortex receives direct input from the dLGN, which in turn then projects to the extrastriate cortex, which in turn is extensively interconnected with the striate cortex and with itself. However, a relatively small projection to the extrastriate regions in the monkey has been demonstrated from the dLGN. These neurones projecting from the dLGN to the extrastriate regions are different from those projecting to the striate cortex. The neurones projecting to area 18 are more scattered and mostly belong to the interlaminar regions which do not project to the striate area.

Hubel and Livingstone (1985) revealed that Brodman's area 18 received inputs from three regions in area 17, from layer IVB (the magnocellular output), and from the blobs and interblobs (the parvocellular layers). They also reported that the dark thick stripes which receives projection from the magnocellular dLGN, contained cells which were sensitive to binocular disparity (stereoscopic depth). The cells of the thin stripes received input from the blobs and were colour and brightness selective like the the blobs, i.e. yellow-blue, red-green, and achromatic, but with no orientation specificity. The cells in the pale stripes tend to be orientation selective and approximately 60% end-stopped.

Neurones from Brodman's area 18 also project to area 19 (the medial temporal cortex), to areas 20 and 21 (the inferotemporal cortex, and to area 7 (the posterior parietal cortex). The inferotemporal cortex and area 18 both receive input from the pulvinar and the thalamus (Kandel and Schwartz 1985).

2.2 Neurophysiology of the visual pathway

Kuffler (1953) first described the centre-surround organisation of cat retinal ganglion cells. The retinal ganglion cells of the monkey also has almost circular receptive fields, divided into functionally distinct concentric centre with antagonistic surround regions (Hubel and Wiesel 1960; Dreher et al. 1976). Light falling in one of the regions, ("ON"- region in either the centre or surround) excites the ganglion cell by increasing the rate of discharge, whereas light falling in the other ("OFF-" region) inhibits by reducing the rate of discharge. Consequently light falling uniformly over the whole receptive field has little effect. Under scotopic conditions, the behaviour of the retinal ganglion cell alters such that the surround mechanism becomes less effective in antagonising the centre region, which may be accompanied by an increase in the diameter of the central region of the receptive field and a change in the spectral sensitivity of the central mechanism such that a ganglion cell with spectral sensitivity typical of cones under photopic conditions may change to have one typical of rods (possible since ganglion cells are connected to both rods and cones). The size of the receptive field of the ganglion cells vary, being smaller in the central retina than in the periphery. Also, in each retinal area, the ganglion cells have overlapping receptive fields with a range of sizes.

The bipolar cells also have a concentric receptive fields organisation, with an antagonistic centre-surround arrangement (Kaneko 1970). The ON-centre cell is inhibited by a transmitter (probably glutamate), thereby a decrease in the amount of transmitter released, disinhibits and excites the cell. The OFF-centre cell is however,

excited by the transmitter so that decrease in neurotransmitter levels, inhibits the cell. Glutamate is thought to hyperpolarise and inhibit the ON-centre cell by closing sodium ion channels. Each cone photoreceptor can synapse on both ON- and OFF-centre bipolar cells. An ON-centre cell is depolarised by direct illumination of the cone whilst the OFF-centre is hyperpolarised by direct illumination of the same cone. Each type of bipolar cell is further connected with a parallel set of ganglion cells, correspondingly called the ON- and OFF-centre ganglion cells (Kuffler et al. 1984).

The geniculate cells behave in a similar manner to the retinal ganglion cells, with concentrically organised receptive fields of various sizes. However, the surround regions are more diffuse than those of the retinal ganglion cells. Generally, the parvocellular layers have mainly colour-opponent cells (except for maybe 15-20%) and the magnocellular layers broad-band cells (Wiesel and Hubel 1966; Dreher et al. 1976; Schiller and Malpeli 1978; Kaplan and Shapley 1986). Leventhal et al. (1981) showed that in the monkey, these layers received input from retinal colour-opponent and retinal broad-band ganglion cells respectively. In the monkey, the ratio of colour-opponent and broad band geniculate cells to retinal cells is almost 1:1 (Lee et al. 1989), while in the cat Y cells comprise about 4% in the retina but almost 50% in the LGN. Other functional properties of parvocellular cells include responding in a sustained manner, having small receptive fields, linear spatial summation properties, receiving input from axons with medium conduction velocities, and conducting at medium velocities to the striate cortex (Schiller 1986). The magnocellular cells tend to respond in a transient manner, have larger receptive fields, be less linear in their spatial summation properties, receive input from axons conducting at high velocity, and send high-velocity signals to the cortex, and have greater contrast sensitivity than do parvocellular cells.

McIlwain (1964) showed that the responses of ganglion cells and LGN cells could be influenced by stimuli appearing up to 90^{o} from the receptive field centre. This effect was termed the "periphery effect" and thought to be attributable to retinal amacrine cells, and was more prominent in the magnocellular layers of the monkey LGN.

In primates, the geniculate cells respond quickly to moving stimuli, but do not show any directional effects (Kuffler et al. 1984). In addition, individual geniculate cells can only be stimulated by one eye, with a majority of cells capable of being inhibited by stimulating corresponding retinal regions of the other eye. The geniculate cells can also be influenced by stimulation of the vestibular and other sensory systems.

In the cat, layer V cortical cells have been shown to project monosynaptically to the tectum (Chalupa 1984). The receptive field properties of these neurones in Brodman's

area 17 were found to be directionally sensitive binocular complex cells (see below) with large receptive fields. In the monkey, the cortico-tectal cells were very similar to those of the cat, but more broadly tuned for orientation.

Physiology of the striate cortex is more complex. The cortical cells are also grouped on the basis of their receptive field properties. They include concentric, simple, complex, and hypercomplex, with the cell types connecting not only to each other but in a hierarchical order (Hubel and Wiesel 1968).

The concentric cells recorded from layer IV of the striate cortex have receptive field characteristics similar to those of the geniculate cells (Hubel and Wiesel 1968). The fields are arranged in the concentric centre-surround fashion and receive input only from one eye. They receive direct geniculate input since they are located in layer IV where most of the geniculate afferents terminate.

The receptive fields of simple cells are not arranged concentrically but rather in parallel bands with a central on or off band with parallel antagonistic flanking regions on either side (Hubel and Wiesel 1968). These flanking regions may not be equal in size and there may be only one sometimes. These cells respond best to a bar rather than a spot of light, moving in a particular orientation. In primates the majority of simple cells are located in layer IV, but also occur in the superficial and deeper layers. Those of layer IV are monocular with smaller receptive fields than those in other layers.

Complex cells do not have distinct antagonistic on and off regions in their receptive fields (Hubel and Wiesel 1968). These cells will give the same response to a line stimulus anywhere in the receptive field, and respond to a moving stimulus throughout the field. Complex cells respond best to light or dark bars of a specific orientation. They also receive input from both eyes and are thus binocular. The receptive fields of any given binocular complex cell are on corresponding parts of the two retinas with identical receptive field properties. Complex cells are located in the cortical layers above and below layer IV of the striate cortex, and rarely in layer IV itself.

Hypercomplex cells have all the properties of complex cells with the added feature of requiring the line stimulus to be of specific length (Hubel and Wiesel 1968). A correctly orientated stimulus in the activating region of the receptive field produces a vigorous response, which diminishes if the stimulus extends outside the activating region. These antagonistic regions either side of the activating regions, only reduce the response from the activating region; they are unable to produce a response when stimulated alone.

Some cells of the striate cortex are colour specific, especially in those areas representing the fovea (Livingstone and Hubel 1988). These have the same spatial organisation as non-colour-coded concentric, simple, complex, and hypercomplex cells, but respond preferentially to particular colours rather than white light. Colour-coded concentric and simple cells are located in layer IV, while the majority of colour-coded complex and hypercomplex cells are located in layers II, III, V, and VI. Layers II and III in addition, have double opponent cells which have a concentric centre-surround receptive fields, with the centre and surround having opponent colours.

2.3 Parallel pathways

Parallel pathways, channels, or streams of visual processing exists throughout the visual pathway. Comprehensive reviews of parallel processing of visual information have been provided by Bassi and Lehmkuhle (1990), Lennie (1980), Sherman (1985) and Stone (1983).

Parallel processing is attributed to the existence of different sub-classes of retinal ganglion cells differentiated according to their anatomical, physiological and psychophysical properties, which are preserved throughout the pathway from the retina, through the diencephalon, to the striate and extrastriate cortex. The functional characteristics are not entirely exclusive of each other, as there is some overlap and even mixing at the cortical level.

2.3.1 Anatomical properties

Physiological studies of the cat optic nerve reported the initial evidence that there are different ganglion cells, which were described as X, Y (Enroth-Cugell and Robson 1966) and later the W cells, (Stone and Fabian 1966; Fukuda 1971; Stone and Fukuda 1974; Stone and Hoffmann 1972). The morphological correlates of these cells in the cat retina are the alpha, beta and gamma cells for the Y, X and W cells respectively, with a further variation in the gamma group which were termed epsilon cells (Boycott and Wässle 1974). The X and Y cells project to layers A, A1, and C of the cat dLGN, with a few X cells projecting to the midbrain but probably not to the superior colliculus, and many of the Y cells projecting to the superior colliculus and medial interlaminar nucleus (Cleland et al. 1971; Fukuda and Stone 1974). The W cells tend to projects mainly to the superior colliculus, as well as projecting to layer C of the dLGN, the vLGN, the medial interlaminar nucleus, and pulvinar, (Hoffmann 1973; Fukuda and Stone 1974). In the cat, W-cells comprise about 40% and X-cells about

55-60% of the total ganglion cell population at all eccentricities. Y-cells comprise about 1% at the area centralis to 9% towards the periphery (Fukuda and Stone 1974).

Leventhal et al. (1981) described four morphological types of ganglion cells in the monkey. These were termed A, B, C and E (in addition to some unclassified cells), due to their close relationship to the cat alpha, beta, gamma and epsilon cells of Boycott and Wässle (1974). Generally, the A-cells have large bodies, extensive dendritic trees, the thickest axons, and the only cell type to project to the magnocellular layers of the dLGN, they do not project to the parvocellular dLGN but a few project to the superior colliculus. These A-cells have similar properties to the cat Y-cells. The B-cells have small bodies, compact dendritic trees and are the the only cell type to project to the parvocellular dLGN. They do not project to the magnocellular dLGN or the midbrain, and their properties are similar to the cat colour opponent X-cells. The C-cells have small bodies, sparsely branched dendritic trees and fine axons, and their properties are similar to the cat W-cells. The C cells project to the superior colliculus and to the pretectum. The E-cells have medium to large bodies, fine to medium axons, and extensive dendritic trees and project to the pretectum.

The primate retina is generally divided in having two major types of ganglion cells, which were observed initially by Gouras (Gouras 1968 and 1969), with X- and Y-like properties. There also exists a third rarer type of cell in the primate retina, termed the W-like cell. However, the W, X and Y terminology was derived from the cat visual system and although they display many similar qualities, there are differences that exist between the cat and the primate retina (Shapley and Perry 1986). Therefore in the primate, the retinal ganglion cells are more commonly termed M, P and W-like cells.

The magnocellular or M-cell pathway generally refers to retinal ganglion cells projecting to the magnocellular layers of the dLGN, and parvocellular or P-cell pathway to those projecting to the parvocellular layers of the dLGN. In the primate retina, ganglion cells of the M-cell pathway can also be termed parasol cells, Primate P alpha, Pa, or A, and account for 10% of the total retinal ganglion cell population (Perry et al. 1984). The P-cell pathway may also be termed midget cells, Primate P beta, Pb, or B and account for nearly 80% of the retinal ganglion cells (Perry et al. 1984). The remaining 10% are C and E (Leventhal et al. 1981) or P gamma and P epsilon (Perry and Cowey 1984), which project to the superior colliculus and pretectum and whose physiological properties differ from both the M and P populations (Schiller and Malpeli 1977).

The major features of these cells are summarised in Table 2.1. Generally, retinal cells of the M-cell pathway have larger cell bodies, thicker axons, and dendritic field arbors approximately three times greater than the diameter of cells in the P-cell pathway at equivalent retinal loci. The P cells tend to be distributed over the whole retina with a relatively high density in the fovea (De Monasterio 1978). The M cells display a more even distribution across the retina, being present even in the foveal pit although they are buried deep in the pile of ganglion cells (Kaplan et al. 1991). The ratio of M/P cells is almost constant at all eccentricities (Livingstone and Hubel 1988a).

However the foveal projection of M cells to the dLGN is weaker than that of the P cells, with almost 35 times more parvocellular neurones (Connolly and Van Essen 1984). Kaplan et al. (1991), suggested that the greater P cell projection compared to M cells is probably due to the chromatic selectivity of P cells. That is, P cells must limit their synaptic contacts to the fewest number of cones as possible. As a consequence the receptive fields of P cells are smaller and many more are needed to ensure adequate coverage of the retina.

Table 2.1. The major anatomical features of the retinal ganglion cells. (Adapted from Bassi & Lehmkhule (1990).

	P-cell	**M-cell**	**W-like cell**
Homologues	X cells; beta cells	Y cell; alpha cell	none
Relative soma size	small	large	small
Relative dendritic field size	small	medium	large
Relative axon size	medium	large	small
Diencephalic projections	parvocellular dLGN	magnocellular dLGN	interlaminar dLGN & superior colliculi

The size of the retinal ganglion cells not only vary according to cell type but also with retinal eccentricity, such that both M and P cells increase in size away from the fovea. However, a P cell that is in the periphery can be the same size as an M cell which is near the fovea (fig. 2.7).

The diameter of dendritic trees of the M cells tend to increase with eccentricity, while those of the P-cells tend to remain constant within the central 10^{o} (Rodieck et al. 1985; Perry et al. 1984). However, for eccentricities greater than 10^{o}, the diameter of the P-cell dendritic tree also increases and becomes variable (Shapley and Perry 1986). The

Figure 2.7. Schematic representation of the relationship of M and P cells with retinal eccentricity. (From Bassi and Lehmkhule 1990).

larger dendritic trees of M cells means they have a larger summation area which should result in greater sensitivity, while the greater number of P cells means greater spatial sampling suggesting that they should display a higher spatial resolution (Merigan 1989).

These pathways are segregated up to the striate cortex, where they display divergence, i.e. there are more cortical cells than retinal or geniculate cells, with the divergence being greater for the M-cell pathway (Schein and De Monasterio 1987). Retinal P-cells project initially to the four anterior layers of the dLGN (fig. 2.8) and subsequently to layer IVCbeta in the striate cortex, which in turn projects diffusely to the blob and interblob regions of layer III. The outer layers 5 and 6 of the dLGN are dominated by ON-centre red-green opponent cells and the middle layers 3 and 4 contain mainly OFF-centre broad band and blue-yellow type II cells (Schiller and Malpeli 1978; Michael 1988). Projections from the striate cortex include Brodman's area 18 of the extrastriate region whose major output is to dorsal lateral cortical area and to the caudal portion of the inferior temporal cortex.

The M-cells in dLGN tend to be broad-band cells (Michael 1988) and project to layer IVCalpha, to layer IVB and to the blobs of layer III. From layer IVB, there are direct connections to the middle temporal cortical areas and indirectly via area 18. The major output of the middle temporal (MT) area is to adjacent superior temporal and middle

superior temporal areas, which in turn send projections to the posterior parietal cortex. Mishkin et al. (1983), suggested that these higher visual areas can be subdivided into two pathways; one important for recognition of objects, and the other for determining their spatial relationships. The spatial discrimination learning requires the posterior parietal cortex and object discrimination requires the temporal cortex. Hence anatomically, the object recognition pathway represents a further continuation of the P-cell pathway, while the object localisation corresponds to the a continuation of the M-cell pathway (Livingstone 1990).

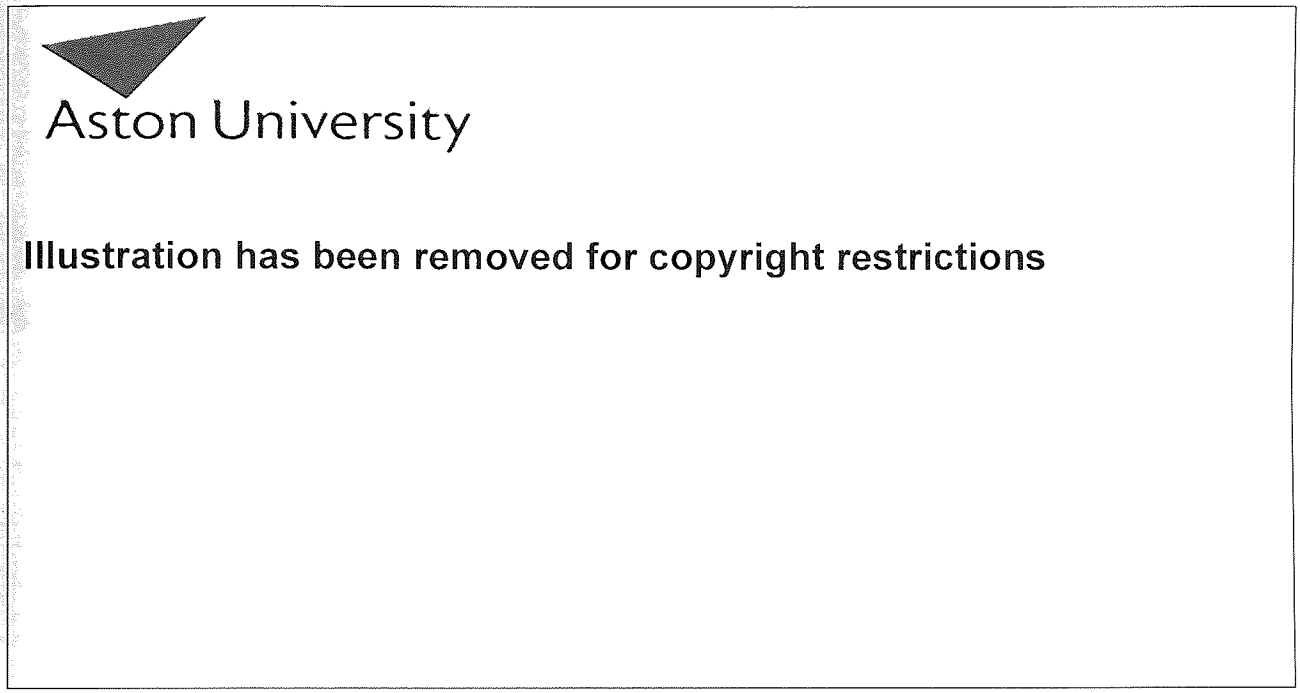
In the cat, W and Y cells are thought to innervate the tectum, whereas X cells do so rarely (Chalupa 1984). The bulk of the direct retinal input is from slow-conducting W cells, whereas the Y cell projection is largely from those associated with vision on the peripheral field. Schiller et al. (1979) reported that reversible blockade of the magnocellular layers of the monkey dLGN disrupted the visual responses of those cells in the superior colliculus that receive input from the visual cortex. In contrast, inactivation of the parvocellular layers had no effect on visual activity in the tectum. Since the magnocellular layers of the dLGN receive input from broad-band retinal cells, this suggested that the cortico-tectal pathway in the monkey is composed primarily of magnocellular broad-band input.

2.3.2 Physiological properties

The initial experiments by Enroth-Cugell and Robson (1966), involved measuring the contrast sensitivity functions of single cat retinal cells. They found a position in cells (later termed X cells) with a concentrically organised receptive fields which elicited no response on introduction of a grating stimulus, which was termed the "null position". However, there were other cells (Y cells) for which a null position could not be found. The X cells were thought to display a linear response where they summate the excitatory and inhibitory signals from different regions of their receptive fields, whereas the Y cells were nonlinear. In addition, when stimulated with a moving sinusoidal grating, the mean discharge frequency of the Y cells but not the X cells was greatly increased.

Cleland et al (1971), distinguished the ganglion cells according to the time-course of their response. They observed that both types of cells showed a sharp increase in discharge rate on initial exposure to a stimulus, which was maintained for the X cells but rapidly returned to the resting levels for the Y cells. These were subsequently referred to as "sustained" and "transient" cells respectively. Cleland and Levick (1974) further found that the X and Y cells displayed a "brisk" response compared to

Figure 2.8. Schematic illustration of the functional segregation of the M- and P-cell pathways in the primate visual system. (From Livingstone 1990).



W cells which displayed low responsiveness and termed "sluggish". Altogether Rodieck (1979) describes, in terms of receptive field properties, at least twelve distinct types of cat retinal ganglion cells.

2.3.2.1 Receptive fields

Both M and P cells have roughly circular, concentrically arranged receptive fields, (Wiesel and Hubel 1966). In general, M-cells have larger receptive field centres by a factor of 2/3 and thus respond more effectively to luminance changes extending across larger retinal areas (Kaplan et al. 1991). The P-cell have smaller receptive fields, concentrated in the fovea, and are more responsive to luminance changes extending across smaller retinal areas. Therefore, M-cells respond better to large spots or lower spatial frequencies whereas P-cells respond better to small spots or high spatial

frequencies. The difference in sensitivity of the M and P cells to stimuli of different sizes is further reflected in their contrast sensitivity functions.

2.3.2.2 Contrast sensitivity

The sensitivity to luminance contrast of the M-cells is on average 8-10 times greater than that of the P-cells (Hicks et al. 1983; Derrington and Lennie 1984; Kaplan and Shapley 1986; Shapley and Perry 1986; Crook et al. 1988). Furthermore, the contrast gain, i.e. the rate of elevation of response unit increase in contrast, of the M-cells is greater than for the P-cells. In addition, both the X- and Y-cells in the magnocellular layer of the dLGN have equally high contrast sensitivities. Consistent with these dLGN findings, the contrast sensitivity is higher for cortical cells in layer IVCalpha which receives projections from the magnocellular LGN than for cells in layer IVCbeta which receives projections from the parvocellular dLGN (Blasdel and Lund 1982). The M-cell responses also increase rapidly with increase in contrast and start to saturate at contrast levels as low as 10-15%, while the responses of the P-cells tend to be more linearly related and do not saturate at high contrast levels (fig. 2.9).

Figure 2.9. Graph illustrating the response vs contrast function for M and P cells in the primate retina. The responses of the ganglion cells were monitored at the LGN as S potentials. The stimuli was a sinusoidal grating of optimal spatial frequency for each cell, drifting at 4 Hz. The error bars are ±1 SD. The curves were derived from the Michaelis-Menton equation $y = ax / (b + x)$. The half-saturation value (b) was 0.13 for the M cells and 1.74 for the P cells. (From Kaplan and Shapley 1986).

The difference in the contrast gains thus provides a means of selectively stimulating one cell population without perturbing the other. Hence when trying to explore the M-cell contribution to cortical function, the use of low-contrast stimuli would strongly bias the input to the cortex in favour of the M-cell pathway, and minimizing the contribution of the P-cell pathway.

Purpura et al. (1988) examined the effects of luminance levels on the contrast gain of the M and P cells, since light adaptation is a major factor in determining the contrast sensitivity of visual neurones (Kaplan et al. 1991). The contrast gain of M and P cells when measured at various levels of mean retinal luminance, from photopic to scotopic levels (4000 to 0.005 monkey photopic trolands respectively), was found to decrease as the retinal illumination was reduced. However, due to the contrast gain of the M cells being 8-10 times that of P cells, the M cells continued to respond to patterns at scotopic retinal illumination levels of 1 photopic troland and below, whereas the P cells were not able to detect visual patterns under the scotopic conditions. In addition, the response from the M cells became more linear at lower light levels resembling the responses of the P cells at higher luminance levels. Kaplan et al. (1991) suggested that this result does not imply that P cells cannot be driven at all at scotopic levels, since Purpura et al. (1988) were able to elicit responses from P cells under such conditions, but using full-field stimulation, or stimuli of very low spatial frequency and very high contrast. Therefore, at scotopic levels, the P cells become blind to fine spatial patterns.

2.3.2.3 Spatial properties

Although P-cells have much smaller receptive fields they are still more responsive to high spatial frequencies (greater than 10 cpd) than M cells (Kaplan and Shapley 1982; Derrington and Lennie 1984).

The cat and primate parallel visual pathways are both differentially sensitive to low and high spatial frequencies. The M-cell pathway of the primate and the Y-cell pathway of the cat are both more responsive to low spatial frequencies, whilst the P-cell and X-cell pathways are more responsive to high spatial frequency. The M-cell pathway in general is about ten times more sensitive than P-cell pathway to spatial frequencies less than 1 cycle/degree and display high contrast sensitivity to many frequencies. The P-cell pathway however, displays lower contrast sensitivity and due to their smaller receptive fields they are still more responsive than M-cells at higher frequencies above 10 cycles/degree. Due to their high foveal concentration, they have higher spatial resolution and thought to subserve visual acuity responses. The X-cells of the cat are also more sensitive and respond better to spatial frequencies above 1-2 cycles/degree

(high for a cat), and the Y-cells are more sensitive and respond best to spatial frequencies below 0.5 cycle per degree (Lehmkhule et al. 1980; Troy 1983).

14C-2-deoxyglucose (2-DG), is a labelled form of a glucose analogue taken up into metabolically active cells and cannot be altered by enzymes, used to measure cells that are active during a task. Tootell et al.(1988) measured 2-DG activity in monkeys presented with different spatial frequencies. They found high spatial frequency stimuli (5-7 cpd) resulted in the greatest uptake of 2-DG in layer IVCbeta and the interblob regions of CO stained striate cortex. The low spatial frequency stimuli (1-1.5 cpd) caused the greatest uptake in layer IVCalpha and the blob region of layers II and III. These findings are consistent with the P-cell pathway being involved in high spatial frequency processing and the M-cell pathway in low spatial frequency processing.

2.3.2.4 Chromatic sensitivity

The majority of the retinal ganglion and geniculate cells are reported to be colour sensitive (De Monasterio and Gouras 1975; Dreher et al. 1976; Schiller and Malpeli 1977). The "colour-opponent" cells give an "ON" response to a colour in their receptive field centre and an "OFF" response to another colour in their periphery, or vice-versa. However, cells with broad band receptive fields do not have the centre surround spectral opponency and respond to a wider range of wavelengths. The parvocellular geniculate cells have mostly concentric colour-opponent receptive field organisation, whereas the magnocellular cells have broad band receptive field organisation (Schiller and Malpeli 1978).

Wiesel and Hubel (1966) further divided the monkey geniculate cells into four types: Type I cells have concentric centre-surround receptive fields with a colour opponent organisation, type II cells have colour but not spatially opponent receptive fields, type III cells have spatially but not colour opponent receptive fields, and finally type IV cells have "ON-centre" colour opponent receptive fields. The majority of the parvocellular cells tend to be type I, but some are type II and III, whereas the magnocellular cells tend to be type III and a few type IV.

Kaplan et al.(1989) reported the P-cell system responded better to colour-contrast stimuli when compared to luminance-contrast stimuli. They also found that the responses to coloured gratings were higher than that for luminance gratings at any given spatial frequency, without altering the linear relationship of the response on stimulus contrast and never reaching the saturating response to contrast function as does the M-cell system.

In addition, chromatic contrast sensitivity tends to be reduced only after lesions or damage to the P-cell pathway (Merigan 1989a; Schiller et al. 1990; Merigan et al. 1991). However, although the P-cell pathway shows clear colour-opponent responses the M-cell pathway is not totally colour-blind. At photopic luminance levels, the M-cell pathway receives inputs from the cones with the relative weights of different cones varying from one M-cell to another (Derrington et al. 1984). However, the M-cell pathway is not specialised for extracting colour information like the P-cell pathway, due to its variability in colour direction and overall weak responses, but it is capable of signalling a border between two isoluminant patches of high chromatic contrast.

2.3.2.5 Temporal properties

The M and P pathways are also differentially sensitive to temporal rates as measured by conduction time, temporal resolution, visual latency, and response duration (Dreher et al. 1976; Marrocco 1976; Schiller and Malpeli 1977; Kaplan and Shapley 1982). Gouras (1968) described one of the first distinctions between the M and P cells based on their temporal characteristics, describing them as phasic and tonic respectively.

M and P cells differ in their capacity to respond to fast moving stimuli or resolve stimuli modulated at high temporal rates. M-cells have a higher temporal resolution or critical flicker fusion rates than P-cells (Marrocco 1976; Derrington and Lennie 1984). The visual latencies (a physiological response to a visual stimuli), of the M-cells is shorter than for P-cell (Marrocco 1976), probably due to the shorter conduction velocity and higher temporal resolving capacities of the M-cell pathway under certain stimulus conditions. The response duration classified as either sustained (continued response throughout the duration of the stimulus) or transient (only an initial burst of activity at stimulus onset), suggest that the M-cells are transient and the P-cells are sustained cells (Marrocco 1976). However, the response duration of retinal ganglion cells tend to be more sustained overall than geniculate cells.

The differences in temporal resolution and visual latency of the two pathways tend to be under the influence of the spatial parameters of the stimulus (Derrington and Lennie 1984). At low spatial frequencies, the temporal resolution of the M-cell pathway is better than the P-cell pathway. But for the higher spatial frequencies the difference in temporal resolution diminishes.

This differential sensitivity to temporal rates is thought to reflect to the difference in axon diameters of the M- and P-cells (Marrocco 1976). The more thickly myelinated M-cell fibres conduct faster than the more thinly myelinated P-cell fibres, but this is

only in the order of about 1-2 ms. Thus overall, M-cells respond with a short latency, in a brisk, phasic way, whereas P-cells are more sluggish and give sustained (or tonic) responses. This differential sensitivity of M- and P-cells to stimuli of different sizes is reflected in their spatial contrast sensitivity functions.

2.3.2.6 Selective lesions

Selective lesioning of the P-cell pathway can be achieved by administration of acrylamide monomer (Merigan 1989). Behavioural test using monkeys show a reduction in achromatic contrast sensitivity at low temporal frequencies, and a moderate reduction in visual resolution. However, the chromatic discrimination is severely impaired, although the sensitivity to flickering stimuli at higher temporal frequencies is unaffected. Merigan and Eskin (1986) conclude that the P cells must be subserving sensitivity not only to chromatic differences, but also to high spatial and low temporal frequencies, and that the M cells are sensitive to the higher temporal frequencies. The reduction in visual resolution even at high temporal frequencies of grating reversal may be due to both M and P cells contributing to some central channel which is degraded when the P cell input is disrupted (Kaplan et al. 1991).

Injection of ibotenic acid into the parvocellular layers of the dLGN of monkeys, showed that destruction of parvocellular layers responsible for a particular portion of the visual field, devastated the visual performance of the monkey in that portion of the field for tasks involving colour, and pattern discrimination, but had no significant effects on detection of coarse stimuli, stationary or moving (Schiller et al. 1988 and 1988a). Lesions in the magnocellular regions produce deficits in tasks involving movement perception, but not really affecting stereopsis or form perception. These results were interpreted as evidence for the M system being involved in movement perception, and the P system in most other visual tasks. This is not completely compatible with physiological evidence and the lesions are only confined to the cell bodies and do not impair signal flow in fibres of passage.

In primates, selective ablation using ibotenic acid (Merigan et al. 1991), of layers in the dLGN demonstrate that lesions in the parvocellular layers produce reductions in visual acuity, whereas the magnocellular lesions do not affect visual acuity. The parvocellular (but not the magnocellular) lesions also reduce luminance and chromatic contrast sensitivity, tested with stationary gratings of 2 cpd. But luminance contrast sensitivity to gratings of 1cpd counterphase modulated at 10 Hz was reduced by both parvocellular and magnocellular lesions. Hence leading to the conclusion that the parvocellular pathway dominates chromatic vision, acuity and contrast detection at low

temporal and high spatial frequencies, while the magnocellular pathway may mediate contrast detection at higher temporal and low spatial frequencies.

2.3.3 Psychophysical properties

Kulikowski and Tolhurst (1973) provided psychophysical evidences for parallel processing in the human visual system. They found evidence for two pathways, one responsible for discrimination of spatial structures, and sensitive to high spatial frequencies and the other mediating flicker and movement detection and is more sensitive to low spatial frequencies. Lehmkhule et al. (1980) measured spatial and temporal contrast sensitivity in the cat. They reported that the spatial contrast sensitivity function had an inverted U-shape for the X-cells, with a peak around 0.5-1.0 cpd. Whereas the spatial contrast sensitivity for the Y-cell was highest at low spatial frequencies and systematically declined at higher spatial frequencies. The spatial resolution for the X-cells was slightly greater than for the Y-cells at most eccentricities and declined for both with eccentricity. These workers also found that the temporal contrast sensitivity function was similar for both the X- and Y-cells. These cells exhibited highest sensitivity at low temporal frequencies with a systematic decrease at higher temporal frequencies. However, the temporal resolution was higher for the Y-cell than for the X-cell. These observations provide further evidence for the X-cells mediating fine spatial detail and position information, and the Y-cell mediating basic spatial pattern vision. In primates, the M-cell pathway has a higher sensitivity to low spatial frequencies temporally modulated at 5-10 Hz (Derrington and Lennie 1984).

2.3.4 Visual disorders

Selective impairment of any one of the pathway would result in the impairment of visual processing. Patients with glaucoma and Alzheimer's disease suggest the possible impairment of the M-cell pathway, by a reduction in sensitivity to the lower spatial frequencies and a reduction in temporal resolution.

In the case of glaucoma, it has been suggested that the M-cell pathway is affected before the P-cell pathway (Quigley et al. 1982). There is a loss of nerves with the larger diameter in the optic nerve, as well as a loss of the larger diameter retinal ganglion cells, suggesting the loss of M-cells.

2.3.5 Summary

The physiological studies find that the M-cell pathway responds quickly to low-middle spatial frequencies modulated at higher temporal rates and the P-cell pathway responds

more slowly to high spatial frequencies modulated at slower rates. Therefore, they are serving different visual functions.

Table 2.2 summarises some of the properties of the M and P cells.

Zeki (1980) suggests that in the macaque, area MT is important in motion perception, as the cells are very sensitive to moving stimuli and are often direction selective too, while cells in area V4 seems to convey information about colour. Subsequently, Livingstone and Hubel (1988) combining the anatomy and physiology, suggested the following three subdivisions of the geniculocortical visual pathway:

1. The M-cells → thick stripes → area MT pathway, is characterised by cells selective for movement and stereoscopic depth.

2. The P-cells → the interblobs → pale stripes pathway, seems to be responsible for high resolution static form perception cells These cells respond to both luminance contrast and colour contrast borders but are not concerned with colour perception as most of the cells are not wavelength or contrast-sign selective. This system is responsible for form and shape detection using either colour- or brightness- contrast edges.

3. The blobs → thin stripe → area V4 pathway, is responsible for colour but not movement, shape discrimination, or stereopsis. The system has lower acuity than the interblob regions (by a factor of almost 3/4).

In general, the M-cell pathway provides a quick view of the visual information with coarse information about the type and location of the stimuli. Information about fine spatial detail is provided by the P-cell pathway, generating positional information for foveation.

Table 2.2. Summary of properties of M and P cells in the monkey retina. (Adapted from Kaplan 1989 and Kaplan et al. 1991).

Property	P cell	M cell
Luminance contrast gain	Low	High
Receptive field size	Small	Large
Colour selectivity	90% Achromatic (Pi) 10% Chromatic (Pii)	Absent
Conduction velocity	Low	High
Response to light steps	Tonic	Phasic
Function at scotopic levels	Absent	Present
Linearity of spatial summation	Linear (X)	75% Mx, 25% My
Spatial resolution	Similar	Similar
Pattern vision at scotopic levels	Absent	Present
Number of cells (millions)	1	0.1

2.4 Neurotransmitters in the visual pathway

Communication between neurones is achieved at the synapse by either electrical coupling (e.g. in the retina) or chemically. Communication via a chemical synapse is mediated by the movement of chemical substances -the neurotransmitters. Before considering the neurotransmitters involved in the visual pathway a definition of "neurotransmitters" is required. Iuvone (1986) provides a concise definition, suggesting that a neurotransmitter may be defined "as a chemical substance that is synthesised and released from the presynaptic terminals of neurones. This substance must be able to diffuse across the synaptic gap and bind to specific receptors located on the postsynaptic membrane. Binding of the neurotransmitter to the receptor should then elicit an electrophysiological response of rapid onset and short duration. The neurotransmitter action must be terminated by enzymatic degradation or by its re-uptake into the presynaptic terminal. Identification of a substance as neurotransmitter in experimental studies should fulfil certain criteria such as demonstration of its synthesis and release, its postsynaptic action, presence of a mechanism for the rapid termination of its postsynaptic action and finally, synaptic mimicry and antagonism pharmacologically by selective receptor agonists and antagonists, respectively".

Substances classified as "neuromodulators" are generally defined as endogenous substances released by either neurones or glia that produce effects on neural cells that are different from the rapid and short duration effects of the neurotransmitters. Neuromodulators may act postsynaptically to alter the electrophysiological response to a neurotransmitter while having no electrophysiological effect of its own. They may also act presynaptically to modulate the release, or re-uptake of a neurotransmitter or produce long-term changes in cellular metabolism. Neuromodulators can act either locally on adjacent cells or diffusely through the extracellular space on distant neurones in the tissue. Finally neuromodulators may affect its cell of origin, modulating that cell's response to other neurotransmitters.

2.4.1 Retina

The effects of the neurotransmitters and neuromodulators generally involve changes in the electrical activity of single cells, via the opening or closing of selective ion channels in the neuronal plasma membrane. However in the retina, they may also elicit morphological changes in the neurones that influence their function.

There are a wide variety of substances identified in retinal preparations, but very few have been associated with any one retinal cell population. Most investigations have

been carried out in many animal species leading to conflicting findings. It is believed that identification of the retinal neurotransmitter systems will help to predict drug effects and a better understanding of the underlying mechanisms of some diseases associated with the eye and hence provide a basis for developing the appropriate drug treatment (Regan 1989).

2.4.1.1 Photoreceptors

Glutamate and aspartate are probably the best candidates for neurotransmitters associated with photoreceptors (Slaughter and Miller 1985; Iuvone 1986). There are at least four types of receptors which mediate responses to these dicarboxylic acids in terms of agonist specificity, preferring either kainic acid (KA), quisqualic acid (QQ), N-methyl-D-aspartic acid (NMDA), or 2-amino-4-phosphorous butyrals (APB). Evidence exists for the presence of high concentrations of aspartate aminotransferase (an aspartate and glutamate synthesising enzyme) localised in photoreceptor cells (Altschuler et al. 1982). Aspartate and glutamate released from toad retinal photoreceptors has been observed to occur under high concentrations of K^+ and aspartate released in rabbit is reduced in light conditions, a response expected for a photoreceptor transmitter (Miller and Schwartz 1983; Iuvone 1986). The presence of high-affinity uptake systems in rods and cones of several species (Bruun and Ehinger 1974; Marc and Lam 1981), presence of postsynaptic receptors on second-order retinal neurones (horizontal and bipolar) (Slaughter and Miller 1981), and the relatively selective antagonists blocking of responses to the natural transmitter, all indicate that aspartate and glutamate as possible candidates for photoreceptor transmitter substances.

Taurine (2-aminoethanesulfonic acid) appears to play a role in photoreceptor function possibly as a neuromodulator, but its exact function is unclear (Iuvone 1986). Taurine is the most abundant amino acid in the vertebrate retina and seems to be vital for retinal function (Pasantes-Morales 1985). Cats and rats fed on taurine-free diets exhibit disruptions of their photoreceptor outer segment, photoreceptor cell death, and eventually complete loss of the electroretinogram (Hayes et al. 1975). Several lines of evidence to suggest taurine as a neurotransmitter, including presence of specific low- and high-affinity uptake systems, accumulation by photoreceptor cells, pigment epithelium and Müller cells, as well as some evidence of taurine synaptic sites (Brecha 1983). Although taurine is involved in the maintenance of photoreceptor function and viability, its mechanism of action is unknown. Therefore, data thus far can also be interpreted to suggest that taurine is critical for the normal maintenance of the retina and that it is not a retinal transmitter substance.

Melatonin (5-methoxy-N-acetyltryptamine) has also been suggested to function as a neuromodulator that is secreted from photoreceptor cells (Iuvone 1986). Biochemical studies indicate the presence of endogenous melatonin, existence of metabolic enzymes and synthesis from its precursor serotonin or 5-hydroxytryptamine (5-HT), strongly suggest that melatonin is synthesised in the retina itself (Brecha 1983). Pharmacological application of melatonin produces effects on rods, cones, and inner retinal neurones suggesting a neuromodulatory function (Iuvone 1986). In addition, melatonin levels exhibit a circadian rhythm that can be entrained by light and in the guinea-pig, with its release being greater in darkness than in light (Brecha 1983).

Serotonin or 5-Hydroxytryptamine (5-HT), has reported to be accumulated by photoreceptor terminals in rats, the accumulation being greater in light than darkness. Almost 90% of 5-HT is found in the pineal gland where it acts as a precursor for melatonin synthesis, and the retina contains very low levels of 5-HT (Redburn 1985). Therefore 5-HT may only be present in photoreceptors as a precursor to melatonin.

Autoradiography has localised a high-affinity choline uptake mechanism in the photoreceptors, but the choline is converted mainly to choline-containing phospholipids, hence acetylcholine (ACh) is probably not a neurotransmitter of the photoreceptors in mammalian or avian retina. However, in lower vertebrates like the mudpuppy and turtle, certain photoreceptors may synthesise acetylcholine (Iuvone 1985; Puro 1985).

2.4.1.2 Horizontal cells

γ-Aminobutyric acid (GABA) may be a neurotransmitter of some H1 horizontal cells of fish and possibly in other nonmammalian vertebrates (Morgan 1985). GABA released from horizontal cells seems to be controlled by an excitatory input from cones via a glutamatergic pathway and modulated by an inhibitory input from dopamine interplexiform cells (Kamp 1985). However, GABA is not a neurotransmitter of horizontal cells in mammalian retinas as they neither accumulate nor possess any GABA synthesising enzymes (Bruun and Ehinger 1974).

In the goldfish, dopamine has been found to depolarise cone L-type horizontal cells and reduce the amplitude of their light response (Kamp 1985). In the teleost retina, the overall action of dopamine on the horizontal cells, is to reduce its lateral inhibitory effects in the outer plexiform layer (Dowling 1986). Dopamine actions on horizontal cells include, reduction in the light responsiveness of the cell, reduction in its receptive field size, and reduction in the release of neurotransmitter (Dowling 1990). The action of dopamine is mediated via activation of the enzyme adenylate cyclase that increases

the cyclic AMP levels, which enhances the sensitivity of the horizontal cell to transmitters released by the photoreceptors, rendering the light-induced reduction of transmitter release from receptors less effective. Since horizontal cells form the antagonistic surround response of the bipolar and photoreceptor cells, a decrease in their surround responses occurs upon dopamine application (Dowling 1986). The horizontal cell in turn is also under the influence of interplexiform cells which modulates the inhibitory interactions mediated by the horizontal cells.

Cholecystokinin-like immunoreactivity has been reported in cat horizontal cells, but this has not been characterised biochemically (Thier and Bolz 1985). There is no evidence for ACh as a transmitter substance in horizontal cells of any species, although in mudpuppy and turtle, horizontal cells may be affected by cholinergic agents (Puro 1985).

2.4.1.3 Bipolar cells

The transmitter systems involved in retinal bipolar cells are generally unknown, but electrophysiological studies suggest that they are excitatory rather than inhibitory. Aspartate in the cat retina is released from both ON and OFF bipolar cells onto sustained ganglion cells (Ikeda and Sheardown 1982; Iuvone 1986). Glutamate has shown no consistent effects, with no effect displayed on the transient ganglion cells. In contrast, glutamate may be a neurotransmitter of both the ON and OFF bipolar cells of the mudpuppy retina (Slaughter and Miller 1983 and 1985). Most of the bipolar receptors in the mudpuppy retina, have been reported to be not of the aspartate but of the glutamate-preferring type. In contrast, there is little evidence histochemically of aspartate- or glutamate-accumulating bipolar cells. Autoradiographic studies indicate these dicarboxylic acids are accumulated by photoreceptors cells, glia, and some amacrine but not by bipolar cells. Therefore, related compounds acting on aspartate and glutamate receptors may be the natural bipolar cell neurotransmitter (Iuvone 1986).

Glycine may be a neurotransmitter candidate in some retinal bipolar cells. In mammalian retinas, these tend to be cone bipolars, although their connections in the inner plexiform layer (IPL) are unknown. Generally, glycine tends to be an inhibitory transmitter yet bipolar cells are usually considered to be excitatory (Marc 1985). Radioactively labelled glycine (^{3}H-glycine) and GABA (^{3}H-GABA) have been observed to be taken up by bipolar cells of the mudpuppy (Pourcho et al. 1983). Cholinergic activity has not been reported in bipolar cells of most species, yet certain bipolar cells in chick and turtle retina may be cholinergic (Puro 1985). Overall, the exact neurotransmitter(s) of bipolar cells remains unclear.

2.4.1.4 Amacrine cells

Amacrine cells consist of a very heterogeneous population and thus are associated with a large number of transmitters, identified using high-affinity uptake and ^{3}H-labelled binding studies.

Considerable biochemical, morphological, and physiological evidence suggests that ACh may be the neurotransmitter in approximately 5% of amacrine cells in nearly all vertebrate retinas (Brecha 1983; Puro 1985; Masland and Tauchi 1986). In fact, ACh is sometimes thought to be a retinal transmitter only in the amacrine cells. Histochemical evidence indicates that ACh is concentrated in the IPL and inner nuclear layers (INL), within amacrine cells. There is evidence of ACh-containing cells in the ganglion cell layer (GCL), however these have been attributed to a subclass of amacrines known as the displaced amacrine cells (Hayden et al. 1980). The amacrine and displaced amacrine cells are further thought to belong to a subclass known as the "starburst" amacrine cells, so-called due to their dendritic appearance (Famiglietti 1983). These also seem to be identical with the so-called "coronate" amacrine cells of Vaney (1984). The starburst amacrine probably serve cone pathways and function to modulate directly the activity of the retinal ganglion cells.

Both light and K^+ evoke a Ca^{2+}-dependent release of ACh from retinal amacrine cells, suggesting that it is modulated by light. Ligand binding studies indicate the presence of both muscarinic and nicotinic receptor sites (Iuvone 1986). GABAergic and glycinergic neurones tonically inhibit cholinergic amacrine cells, by reducing the light-evoked release of ACh in the rabbit retina, but is unaffected by dopamine or the neuropeptides (Cunningham and Neal 1983).

Dopamine has been established as a classical retinal neurotransmitter and may also have a neuromodulatory function, since it activates an intracellular second messenger system (Masland and Tauchi 1986). Dopamine-containing cells have been found not to contact bipolar cells or ganglion cells. In most species dopamine has been localised to a subclass of amacrine cells which only contact other amacrine cells, forming a subset known as interamacrine cells, which makes it unlikely that dopamine neurones have anything to do directly with pattern vision but rather some basic, general controlling function (Ehinger 1983). Dopamine release was observed to be enhanced by photic and electrical stimulation and by K^+-induced depolarisation (Kramer 1971). The light-evoked activation of dopaminergic amacrine cells is mediated transsynaptically by photoreceptors, bipolar cells, and other amacrine cells which probably operate with GABA or glycine as neurotransmitters (Iuvone 1986). Both dopamine and ACh may also interact to regulate adenylate cyclase activity in those cell

that are postsynaptic to both ACh- and dopamine-containing amacrine cells. In addition, dopaminergic amacrine cells have been shown to be activated by α-MSH and quisqualate (a glutamate analogue), and inhibited by dopamine itself, GABA, α-adrenergic agonists, opiate receptor agonists, and melatonin.

There is biochemical and electrophysiological evidence suggesting that dopamine influences retinal transmission to the brain, through dopamine receptors which increase the metabolic activity in postsynaptic cells in areas such as the superior colliculus (McCulloch et al. 1980). Dopamine antagonists have been shown to increase the latency of early peaks in flash evoked potentials recorded from the visual cortex, lateral geniculate nucleus, and optic tract of the rat, but had no effect on the evoked potentials caused by electrical stimulation of optic tract (Dyer et al. 1981). Dopamine is also suggested to be involved in visual processing via its influence ganglion cell activity. This is probably indirect since dopamine-containing amacrine cells only seem to synapse with other amacrine cells but not with ganglion cells (Dowling 1990). Thus, dopamine seems to be involved in an interamacrine cell network.

In addition, dopamine may also be a neuromodulator influencing the response of cells to other putative neurotransmitters, but work in this area is confined generally to developing synaptic systems and not in mature synapses (Iuvone 1986).

Catecholeamines other than dopamine, such adrenaline and noradrenaline, have been detected in bovine and rat retina (Nesselhut and Osborne 1982; Hadjiconstantinou et al. 1983). The majority of the noradrenaline in the rat retina, is associated with sympathetic nerve terminals, while adrenaline is apparently contained in intrinsic retinal cells (Hadjiconstantinou et al. 1983). However, there is strong evidence suggesting the presence of adrenaline-containing amacrine cells in the rat retina. Thus adrenaline may be a neurotransmitter or neuromodulator in a subclass of amacrine cells in rat and may be in bovine retina, but its presence in other vertebrates awaits further investigation (Iuvone 1986).

Serotonin or 5-HT and related indoleamines are accumulated in retinal amacrine cells of most species (Brecha 1983). Evidence exists for 5-HT synthesis, release, high-affinity uptake systems and receptor sites (mainly 5-HT1 and 5-HT2) but very little exists for the neurotransmitter role for 5-HT itself. However, 5-HT in the rabbit retina increases the spontaneous and light-evoked activity of ganglion cells, while infusion into the carotid artery of the cat retina inhibits the ganglion cell activity (Iuvone 1986). In monkeys, cats, and rabbits, indoleamine-accumulating amacrine cells are contacted

by bipolar cells, other amacrine cells and make synapses on other amacrine and ganglion cells (Ehinger 1983).

GABA is a neurotransmitter candidate of a subpopulation of retinal amacrine cells in most vertebrates. Histochemical studies provide the localisation of GABA in not only horizontal cells, but amacrine cells of rabbit and rat, and Müller cells of most mammalian species (Brecha 1983). In rat, GABAergic amacrine cells receive synaptic input primarily from bipolar cells and synapse upon other amacrine, and ganglion cells (Vaughan et al. 1981). Rod amacrine cells which are interposed between rod bipolar and ganglion cells are usually GABAergic and may be the primary relay cell for both on- and off-rod pathways from a single type of rod bipolar cell to two types of ganglion cells (Famiglietti 1983). GABA synthesising enzymes, high-affinity uptake systems, and light activated release have been demonstrated (Iuvone 1986). Electrophysiological and pharmacological studies provide further evidence for functional GABA receptors (Enna and Snyder 1976). In addition, GABA may inhibit the ON-centre ganglion cells and it may also bestow the properties of directional selectivity to directionally selective ganglion cells.

GABA also has a modulatory role, it inhibits the light-evoked release of ACh from amacrine cells. GABA antagonists (bicuculline and picrotoxin), increase the light-evoked and spontaneous release of ACh, suggesting that GABA tonically inhibits ACh-containing amacrine cells (Brecha 1983). Dopamine-containing amacrine cells are also only inhibited by GABA in the dark and not in the light. GABA binding sites may also be associated with benzodiazepine binding sites in the retina in a similar manner to that usually found in the brain (Brecha 1983).

The highest concentration of glycine in the vertebrate retina is found in the amacrine cells, the IPL, and GCLs (Chin and Lam 1980). Glycine is accumulated and released by specific amacrines, and has physiological effects. Autoradiographical studies indicate that it is not only concentrated mainly in amacrine cells, but in interplexiform cells of some species (Brecha 1983). There appears to be five types of amacrine glycinergic neurones probably of a common cellular origin such that they are homologues recruited for different functions in different contexts (Marc 1985). Light-stimulated release, and uptake of glycine has been observed. Influence of glycine on ganglion cell may be mediated via glycine receptor mechanisms. Glycine inhibits the spontaneous and light-evoked activity of ganglion cells, while glycine antagonists (strychnine), increases ganglion cell activity (Ames and Pollen 1969). Glycine may also have an inhibitory input to OFF-centre ganglion cells. However, taurine also

inhibits ganglion cells in a strychnine-sensitive manner (Cunningham and Miller 1976).

Other amino acids have also been implicated to a subpopulation of amacrine cells. Aspartate and/or glutamate may be present in retinal amacrine cells in the monkey and guinea-pig (Altschuler et al. 1982; Mosinger 1983). However, there is little evidence in other species.

Taurine is accumulated by processes in the IPL and by cell bodies in the amacrine cell layer (Pourcho 1981). Thus, taurine may be present in amacrine cells as well as photoreceptor cells, although its role is unknown. Taurine has been shown to inhibit the b wave of the ERG and the activity of ganglion cells (Pasantes-Morales 1985). This effect was blocked by strychnine, suggesting the effects were receptor mediated, but it is not clear if taurine acted on specific taurine receptors or if its actions were mediated by glycine receptors.

A subpopulation of amacrine cells indicate the presence of several neuropeptides including, enkephalin, vasoactive intestinal peptide (VIP), luteinising hormone-releasing hormone (LHRH), substance P, glucagon, somatostatin, neurotensin, and cholecystokinin (CCK) (Brecha and Karten 1985; Brecha 1983). Several peptides appear to modulate retinal adenylate cyclase activity. Glucagon and VIP increase cyclic adenosine monophosphate (cAMP) in the retinal neuronal cells, as well as in Müller cells and retinal pigment epithelium cells (Iuvone 1986). Thus some of the peptides may have a neuromodulatory effect on metabolism instead of or as well as to neurotransmitter roles. Each neuropeptide-containing cell has different morphology and histochemistry from other neuropeptide-containing cells, implying a distinct functional role for each of the cell populations in retinal processing (Iuvone 1986).

2.4.1.5 Interplexiform cells

In species such as the teleost fish and some New World monkeys, dopamine is regarded as the neurotransmitter of some interplexiform cells (Ehinger 1983). The teleost retina indicates the presence of dopamine receptors and dopamine-sensitive adenylate cyclase activity. Dopamine modulates the receptive field properties of cells in the outer nuclear layer (ONL) of the teleost (Dowling 1986). It augments the central response to light of the bipolar while depressing the surround response. These cells also modulate the inhibitory actions of horizontal cells on bipolar and photoreceptor cells. Autoradiographical evidence exists for dopamine-containing interplexiform cells in the rat, mouse, and human retina (Iuvone 1986). However, these are out-numbered by dopamine-containing amacrine cells and there is no electrophysiological evidence

supporting dopamine as a neurotransmitter in the OPLs. Serotonin, substance P, and ACh may in turn influence the activity of dopamine-containing interplexiform cells of fish retina (Iuvone 1986).

Glycine has been shown to be accumulated in the interplexiform cells of the goldfish and frog retinas (Chin and Lam 1980). These cells are presynaptic to horizontal cells, and in the goldfish they are also postsynaptic to H1 horizontal cells. Application of glycine hyperpolarised some goldfish horizontal cells and depressed their response to red light, suggesting a possible role for glycine in the OPL (Marc 1985).

In cat retina, a subtype of interplexiform cell accumulates GABA which synapse on bipolar processes in the ONL. In the IPL, these cells also appear to be presynaptic to amacrine cells (Ehinger 1983). Interplexiform cells have also been observed for substance P and somatostatin, but their cell contacts have not been analysed.

2.4.1.6 Ganglion cells

Glutamate and/or aspartate may be neurotransmitters in some ganglion cells (Iuvone 1986). High concentrations have been found in the GCL in the monkey retina, but had low concentrations in the optic nerve. These neurotransmitters may possibly be localised in displaced amacrine cells or Müller cells, which have a high proportion of their cytoplasm in the GCL. However there is evidence in the pigeon retina, from work on the retinotectal projections, for aspartate and/or glutamate ganglion neurotransmitter systems. 5-10% of cells of the GCL have shown to accumulate aspartate following intravitreal administration, which was transported to layers 1-7 of the contralateral optic tectum, confirming its accumulation in ganglion cells (Ehinger 1983). Similar ganglion cells may exist in other species, aspartate-accumulating cells have also been observed in the GCL of guinea-pig, rabbit and monkey retinas (Altschuler et al. 1982; Ehinger 1983; Mosinger 1983).

ACh may be a ganglion cell transmitter in the toad (Oswald et al. 1979). The toad optic nerve and tectum (the chief target of ganglion cells of lower vertebrates) contain significant amounts of ACh and choline acetyltransferase (the ACh synthesising enzyme) activity. There is further evidence that the receptors present are nicotinic, but muscarinic effects have also been found in the carp, rabbit and cat retina (Puro 1985). In mammalian retinas, ACh is probably not a transmitter of the ganglion cells but may be for certain ganglion cells in fish and amphibians (Puro 1985; Iuvone 1986). Cholinergic systems have been found to differ both quantitatively and qualitatively in retinas of different species. However, in all vertebrate retinas, cholinergic neurones are predominantly amacrine cells which although only make up less than 1% of all

retinal neurones, they still influence the activity of a majority of ganglion cells (see section 2.4.1.4). The action of cholinergic input to ganglion cells is generally to increase the rate of spontaneous activity of the number of action potentials in light-evoked potentials. ACh may excite ON-centre ganglion cells or those with directionally selective receptive fields but the OFF-centre ganglion cells tend to be insensitive (Masland 1980).

There is some evidence for neuropeptide-containing ganglion cells (Brecha and Karten 1985). Enkephalin has been shown to increase the spontaneous activity and light-evoked response of ON-centre ganglion cells and inhibit the spontaneous activity and light-evoked response of OFF-centre ganglion cells in the teleost retina. Substance P seems to excite most ON- and OFF-centre ganglion cells in the carp (Brecha 1983).

Dopamine seems to have some influence on the ganglion cells. In the cat, dopamine agonists were reported to reduce the light evoked response in ganglion cell axons of the optic nerve. In the rabbit retina, dopamine increases the activity of OFF-type ganglion cells while reducing the activity of ON-type ganglion cells (Kamp 1985).

In the cat X- and Y-ganglion cells, GABA antagonists preferentially reduces the contribution of the surround-driven component to the discharge of Y-ganglion cells as compared to the centre-driven component (Famiglietti 1983; Morgan 1985). GABA antagonists also produce a shift from surround to centre dominance on Y-cell discharge, but they have no effect on the centre-surround balance of X-cells in the cat retina. Therefore, X- and Y-cells are pharmacologically different and GABA may be important in mediating the surround influence on Y- but not X-cell discharge. Thus, the surround for X-cells may be mediated via horizontal cells which have not been shown to be GABAergic in mammals, while the surround of Y-cells may be mediated by amacrine cells (Davson 1990).

In contrast, Ikeda and Robbins (1989) found that the excitation of sustained-X cells was mediated by aspartate, released by bipolar cells on NMDA receptors, whilst transient-Y cell excitation was mediated by ACh released by cholinergic amacrines on nicotinic receptors. The physiological segregation of the neurones into the ON- and OFF-centre types in the retina also correlate with a pharmacological separation of inhibitory neurotransmitter inputs. GABA is probably released to suppress ON-centre cells when OFF-centre cells are activated by dark objects at the visual axis, whilst glycine is released to suppress OFF-centre cells when ON-centre cells are firing in response to bright objects, this effect being strongest for cells present in the visual axis and poor in the peripheral retinal cells (Priest et al. 1985). Dopamine inhibits all types of retinal ganglion cells indiscriminately in the peripheral retina where the selectivity of

GABA-ON and glycine-OFF is poor, whilst it does not affect cells at the visual axis (Ikeda et al. 1986). This pharmacological separation does not seem to be inborn but develops as the eye matures, from studies carried out on the kitten retina (Ikeda and Robbins 1985).

2.4.1.7 Summary

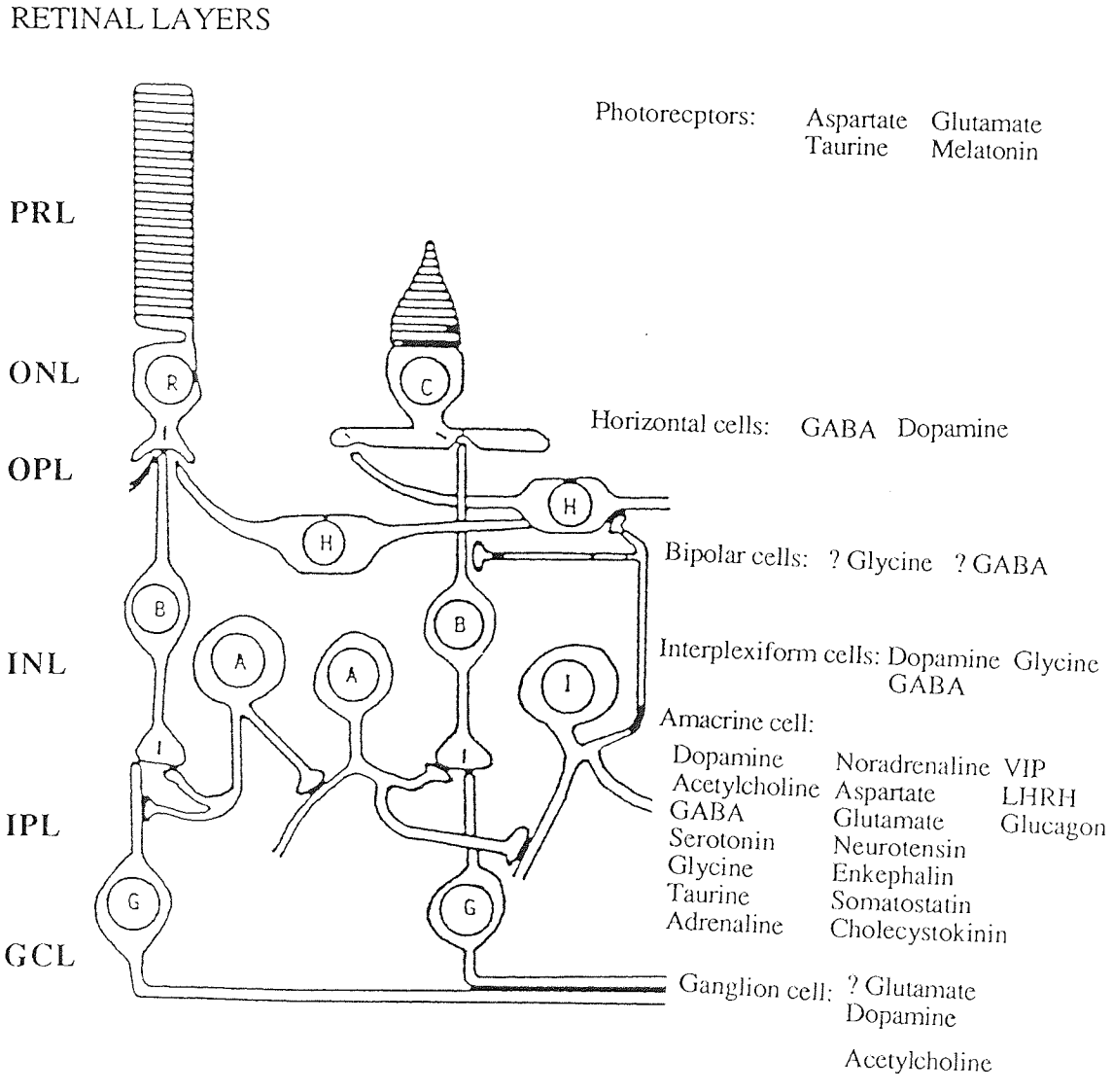
In summary therefore (fig.2.10), glutamate and/or aspartate seem to be good candidates for neurotransmitters in photoreceptors, although there is wide species variability. In nonmammalian vertebrates, a subtype of horizontal cell seems to use GABA, while the neurotransmitter of the other types of horizontal cells of mammalian and nonmammalian retinas have not yet been identified. Bipolar cells probably utilise excitatory amino acids such as aspartate and glutamate. Amacrine cells have a very large number of neurotransmitters identified. Overall, there seems to be some sort of direct pathways from photoreceptor to visual areas in the brain, while lateral processing of visual information within the retina is subserved by a wide variety of excitatory and inhibitory transmitter systems. Visual function may also be influenced by neuromodulators, such as melatonin, and some chemical substances may have both neurotransmitter-like and neuromodulator-like functions (Iuvone 1986).

2.4.2 Dorsal lateral geniculate nucleus

The dLGN receives input not only from the retina, but also from other visual and nonvisual areas such as the visual cortex, the pretectal nuclei, the superior colliculus, and some nonvisual structures from the brainstem. The chemical substances involved at this stage of the visual pathway are generally unclear. Many likely chemical transmitters have been identified together with their possible sources whether it is retinal or cortical. Pasik et al. (1990) provides a comprehensive review of possible transmitters and their sources. It is generally believed that the transmitters from areas such as the brainstem exert a state-dependent modulation of the dLGN transmission.

In the cat dLGN, ACh occurs in appreciable amounts, particularly in the C laminae (Hoover et al. 1978; De Lima and Singer 1987). However, studies show that ACh is not involved in either the retinogeniculate nor the corticogeniculate transmission (Miller et al. 1969; Bigl and Schober 1977). The source of cholinergic input to the dLGN seems to be from the brainstem, mostly from the pedunculopontine tegmental nucleus (PPTN) and the rostral portion of the dorsolateral tegmental nucleus (DLTN) in the rat and monkey (Woolf and Butcher 1986; De Lima and Singer 1987; Hallanger et al. 1987). The PPTN subsystem is an important component of the ascending

Figure 2.10. Summary of putative neurotransmitters and neuromodulators of the retina. The letters in the cell nuclei are abbreviations of the cell type as **A**, amacrine cell; **B**, bipolar cell; **C**, cone photoreceptor cell; **G**, ganglion cell; **H**, horizontal cell; **I**, interplexiform cell; and **R**, rod photoreceptor cell. Retinal layers are abbreviated as **PRL**, photoreceptor layer; **ONL**, outer nuclear layer; **OPL**, outer plexiform layer; **INL**, inner nuclear layer; **IPL**, inner plexiform layer; **GCL**, ganglion cell layer. (Adapted from Ehinger 1983; Iuvone 1986).



reticular activating system which influences the geniculate cells directly, including the geniculate interneurones, which in turn inhibit the geniculate neurones, thereby contributing to a sharpening of the tuning characteristics of geniculate neurones. In addition, ACh may also disinhibits geniculate cells via an inhibitory action on the perigeniculate nucleus (PGN), and the thalamic reticular nucleus (TRN). ACh generally causes an increase in dLGN excitability during waking conditions. Cholinergic neurones of the PGN are directly excitatory of dLGN neurones, but inhibitory in cat, leading to a dual action of ACh on the dLGN (Pasik et al. 1990). In the cat dLGN, ACh directly excites relay neurones (both X and Y) via a nicotinic receptor-mediated increase in cation conductance, followed by a muscarinic receptor-mediated decrease in K^+ conductance (McCormick 1988). Local application of ACh results in an increase in responsiveness of dLGN neurones to visual stimuli. Stimulation of brainstem cholinergic neurones projecting to the dLGN also causes an increase in relay cell responsiveness (McCormick 1988).

5-HT in the dLGN mainly originates from the midbrain dorsal raphe nuclei (Pasquier and Villar 1982; De Lima and Singer 1987). In the cat, 5-HT innervation of the C lamina is greater than the A lamina, suggesting that it may mainly be targeting neurones from the W-type retinal ganglion cells (Mize and Payne 1987). The proposed role of 5-HT in dLGN released primarily from nonsynaptic varicosities, act on membranes of geniculate neurones through 5-HT1 receptors resulting in a decrease in excitability of these neurones. In addition, 5-HT released synaptically would excite the geniculate interneurones via 5-HT2 receptors which in turn would inhibit the geniculate neurones through a GABA-mediated mechanism (Pasik et al. 1990).

The concentrations of noradrenaline in the dLGN are very low and thought to originate from the locus coeruleus (Kromer and Moore 1980; De Lima and Singer 1987). Noradrenaline exerts a powerful modulatory influence on dLGN transmission, with an effect similar to that of ACh but opposite to that of 5-HT. Both noradrenaline and ACh are thought to be involved in blocking the spike after-hyperpolarisation of geniculate neurones and thereby reducing the adaptation inputs of long duration. Also, by moving the membrane potential away from the bursting firing range, and so favouring single-spike activity, they would suppress thalamocortical rhythms and facilitate transfer of sensory information to the cortex (McCormick and Prince 1986). In the rat, stimulation of the locus coeruleus causes an increase in the spontaneous firing rate of dLGN relay neurones while decreasing the excitability of intrageniculate interneurones (McCormick 1988).

GABAergic neurones provide inhibitory inputs to modulate the dLGN, projecting from the visual part of the TRN (Jones 1983). The GABAergic neurones of the TRN exert a recurrent inhibition of dLGN neurones which in turn are regulated by cortical and brainstem inputs on the TRN via systems using glutamate, ACh, noradrenaline and 5-HT as transmitters (Pasik et al. 1990). Intrageniculate interneurones are also thought to use GABA as their transmitter which directly control retinal ganglion cell axons in the dLGN (McCormick 1988).

Glutamate and aspartate may be involved in the corticogeniculate pathway. Destruction of the visual cortex in rats causes a reduction in the uptake of the substances in the ipsilateral dLGN and superior colliculus (Jones 1983). The dLGN also receives afferents from the superior colliculus and certain pretectal nuclei, but the nature of the neuroactive substances in these pathways is largely unknown (Pasik et al. 1990).

2.4.2.1 Summary

The main transmitter substances thought to be involved in the main retinogeniculate and geniculocortical pathways are glutamate and aspartate. However, the dLGN is under modulatory influence from the major neurotransmitters such as ACh, 5-HT, noradrenaline and GABA, which can all modify the signal before it is processed in the cortex.

2.4.3. Visual cortex

The transmitter systems associated with the mammalian visual cortex have not been studied in as much detail as the retina and dLGN. The majority of the work seems to be based on the rat. The main transmitters associated with the visual system seem to be ACh and GABA. However, others such as 5-HT, noradrenaline, several neuropeptides such as VIP, avian pancreatic polypeptide, CCK and somatostatin may also be involved.

A laminar and regional distribution of GABAa-receptor binding sites has been found in the cat visual cortex (Needler et al. 1984). GABA displays an increase in binding corresponding to increased levels of darkness. The highest concentration of binding sites in the cat and the monkey visual cortex, occur in layers IV and the lowest in layers V and VI the deepest layers, but there is a fairly even distribution in the rat visual cortex with higher levels in layer IV (Parnavelas and McDonald 1983). In the rat visual cortex, antibodies to the GABA synthesising enzyme glutamic acid decarboxylase (GAD), have been shown to bind to both smooth and sparsely spinous

nonpyramidal cells (Ribak et al. 1981) and also to chandelier cells (Peters et al. 1982). Since GABA is a well known inhibitory transmitter in the cortex, these neurones are likely to be inhibitory. For example in the cat visual cortex, GABA antagonist bicuculline, causes a decrease in the orientation and direction specificity of simple and complex cells and affects the inhibitory end zones of hypercomplex neurones (Parnavelas and McDonald 1983). In addition, GABA may also be involved in the ocular dominance shift that occurs in the cat visual cortex following monocular eyelid suture.

Muscarinic ACh receptors in the visual cortex, have been found to be densest in the outermost layers I through III with a conspicuous gap in layers V and VI (Dykes 1990; Shaw et al. 1984). Cholinergic excitation is mainly muscarinic in character and most clearly observed in cells below layer III and especially by pyramidal cells. Nicotinic binding sites tend to be specific to layer IV (Prusky et al. 1987). In contrast, some cells in the superficial layers are suppressed by ACh. Nonpyradmidal bipolar cells and occasional multipolar cells have been shown to be positive to antibodies to the ACh synthesising enzyme CAT in the rat visual cortex (Houser et al. 1983). Layer V showed the highest level of CAT activity followed by layers I, II and III, IV and VI respectively.

The cholinergic innervation of the striate cortex comes from the basal forebrain which comprise the septum, the vertical and horizontal limb of the diagonal band of Broca, the basal nucleus of Meynert, and the substantia innominata (Mesulam 1990; Singer 1990). In the adult cat visual cortex, 92% of responses to visual stimuli have been found to altered by ACh administration (response being enhanced in 61% and depressed in 31%) eventhough only 20% of fibres were actually driven by ACh (Sillito and Kamp 1983). There may be an interaction between ACh and noradrenaline, but this needs clarifying. Hence the role of ACh in the visual cortex may be neuromodulatory.

The noradrenergic innervation of the visual cortex is lower compared to other primary sensory areas (Parnavelas and McDonald 1983). In the rat, the locus coeruleus provides the noradrenergic innervation (Peters 1985). The outer superficial layers of the visual cortex tend to be more densely innervated than the deeper layers. There are only a few obliquely orientated fibres occurring in layer V, but a distinct band of tangentially oriented fibres exist in layer I. In the cat, the effects of noradrenaline on single cells is either an increase, a decrease or no change in their visual responsiveness (Parnavelas and McDonald 1983), with the simple cells being more sensitive than complex cells. Overall the role of noradrenaline is thought to be modulatory and/or

inhibitory, by suppressing background spontaneous activity. Noradrenaline may also facilitate ACh transmission and enhance inhibitory GABA synaptic mechanisms (Parnavelas and McDonald 1983).

Serotonin in the rat visual cortex, has the greatest density in layer I, although serotoninergic fibres form a plexus through all layers (Peters 1985). 5-HT- containing fibres arise from the medial and dorsal raphe nuclei and cell groups in the mesencephalon. In the primate visual cortex, 5-HT projection is sparse in layers V and VI but very dense in layer IV, a pattern complementary to that exhibited by the noradrenergic innervation (Parnavelas and McDonald 1983). The role of 5-HT has been suggested to modulate the response of neurones to other neurotransmitters.

Glutamate/ aspartate have also been suggested as neurotransmitter substances in the visual cortex (Baughman and Gilbert 1981) with their receptors identified as NMDA, kainate, quisqualate and L-AP4. However, direct evidence for pyramidal cells of the visual cortex using these substances is very limited. 3H-Glutamate and 3H-aspartate injected in the cat dLGN produce accumulation in layer VI pyramidal neurones, suggesting a role in the corticofugal pathway (Baughman and Gilbert 1981), but no accumulation was observed in the superior colliculus. Further evidence for a role in corticofugal pathways comes from observations of a unilateral ablation of the rat visual cortex resulted in a reduction in aspartate and glutamate uptake in the ipsilateral dLGN and superior colliculus, but the uptake in the contralateral visual cortex remained unaffected. The excitatory effects of glutamate in the cat visual cortex include an increase in diameter of the cells' excitatory receptive fields and enhancement of the response amplitudes within the excitatory receptive fields.

VIP-containing neurones have been visualised using antibodies (Connor and Peters 1984). Approximately 3% of neurones contained in the mature rat visual cortex have been found to be VIP-positive (McDonald et al. 1982). All have cell bodies in layers II-VI, but mainly contained in layers II and III. All are nonpyramidal, the majority being bipolar cells (85-90%) and the remaining being multipolar neurones. Although the action of VIP in the cortex has been suggested to be excitatory and thought to affect synaptic transmission, its precise role in the cortex remains unclear.

In the rat visual cortex, cholecystokinin (CCK) comprise approximately 1% of the neuronal population. Some cortical bipolar cells which are positive to CCK antibodies, show the strongest reactivity in layers II and III (McDonald et al. 1982; Peters et al. 1983). Some CCK-positive neurones also occur in layer I, as well as some multipolar and bitufted cells in layers II-V. The role of CCK in the visual cortex

is unclear, although iontophoretically applied CCK can excite some cortical neurones and inhibit others (Rehfeld 1980).

Avian pancreatic polypeptide (APP) immunoreactivity comprise about 1-2% of neurones in the rat visual cortex. They are mainly in bipolar cells, multipolar, bitufted cells, and a very small number of pyramidal cells (McDonald et al. 1982). These neurones tend to be concentrated in layers V and VI. In addition, some pyramidal cells also seemed to react to APP antibodies. Again the role of APP is unclear.

Somatostatin-like immunoreactivity has been found in multipolar neurones of layers II-VI of rat visual cortex (McDonald et al. 1982). Although the precise effect of somatostatin in the visual cortex remains unclear, it has been shown to have both excitatory as well as inhibitory effects on cortical neurones.

Taurine is found in high concentrations in the cerebral cortex as a whole and can be released by the visual cortex on electrical stimulation or potassium depolarisation. However, there are findings contradictory to this (Parnavelas and McDonald 1983). A suggested role of taurine is that it may play a role in development. The monkey visual cortex reveals presence of taurine in large amounts in early life but gradually declines later in development.

2.4.3.1 Summary

The visual cortex receives afferents from many subcortical areas, accounting for the presence of numerous neurotransmitter substances. However, most are involved in modulating this afferent visual information before it is processed by higher visual areas.

2.5 Chapter summary

In general, the visual signal from the retina can be transmitted via at least two pathways, either the geniculostriate and the nongeniculate (tectal) pathway directly to the visual cortex. The properties of the visual signal can determine which cell-type will transmit the signal, e.g. signals of high spatial frequency, high contrast levels and low temporal frequency are likely to be transmitted via the parvocellular pathway, whereas signals of low spatial frequency, low contrast levels and high temporal frequency are likely to be transmitted via the magnocellular pathway. These parallel pathways are not entirely exclusive of each other as a certain degree of overlap exists between them.

The neurotransmitters involved in the transmission of the visual signal are numerous. The retina has provided evidence for a great variety of neurotransmitters and neuromodulators which can influence the signal transmitted along the optic nerve. Glutamate and/or aspartate are thought to be responsible for transmitting the signal from the retina to the relay centres (such as the dLGN) and subsequently to the visual cortex. However, the visual signal may be modulated by a wide variety of neurotransmitters. For instance, both the dLGN and the visual cortex receive inputs from numerous regions within the CNS, hence the visual signal can be influenced by the neurotransmitters associated with these areas.

CHAPTER 3

Visual Evoked Potentials

3.1 Introduction

Visual evoked potentials (VEPs) can be defined as electrical potentials recorded from the scalp, over the occipital cortex in response to a visual stimuli. Adrian and Matthews (1934) observed responses which followed regularly repeated flashes of light that could be recorded from the occipital cortex. This provided one of the earliest published report of the visually evoked response (VER). The nomenclature of the VEP can vary according to the investigators, e.g. cortical evoked potentials, evoked occipital potentials, photic evoked potentials, visual occipitogram, and visual evoked response. The VEP consists of a sequence of waves or deflections, characterised by the polarity, latency and amplitude of the waves. Other sensory stimulation used in clinical testing includes auditory or the electrical stimulation of sensory nerves (somatosensory), but only visual stimulation will be considered.

3.1.1 Recording the VEP

Evoked responses tend to be of small amplitude when compared to the ongoing background EEG activity. Hence advances in evoked potentials were limited, until Dawson (1947) developed a superimposition technique to enhance the signal-to noise ratio. This technique resulted in the features that commonly occur at the same moment in time after the stimulus presentation, to become more clearly defined compared to the background noise. The superimposition technique allowed the investigator to see reliability of a response, but was useful only when the signals were not too noisy, and was ineffective in retrieving very small signals with precise quantitative measurement of the responses being impossible. Later, Dawson (1951), introduced the automatic signal averager which discriminated against irregular noise by defining signals that were time-locked to the stimuli. Briefly, the averaging technique was based on the assumption that the evoked response was time-locked to the stimulus and slowly added together to produce a clearer evoked response, whereas the background noise of the EEG would be random containing both positive and negative waves which slowly approximated to zero. A more detailed technical account of both the superimposition and automatic averaging techniques can be found in Regan (1989).

Electrical signals are usually recorded from the surface of the scalp using disc electrodes. The electrode consists of a silver metal cup coated with silver chloride,

with a central hole and a flat rim with an outer diameter of 4-10 mm. Other electrodes include the clip electrode where the electrode is mounted in a clip and used for recording from the ear lobe. Needle electrodes are sharp steel or platinum wires which can be inserted into the superficial layers of the skin or scalp. Needle electrodes are used only under restricted conditions due the high risk of infection, discomfort and their high impedance for low frequencies. Recording from the exposed cortex during neurosurgery are carried out with electrodes consisting of spring-mounted metal balls or saline-soaked cotton wicks. Finally, intracerebral electrodes are multicontact wire electrodes which are inserted stereotactically into the brain for acute recordings during surgery or for subsequent chronic recordings. However, experiments contained in this thesis only uses the EEG scalp electrodes.

The placement of electrodes is important for replication and interpretation of signals. There are various systems of electrode placement, but the most frequently used is the "Ten-Twenty system" introduced by Jasper et al. (Jasper 1958) (fig. 3.1). This system uses easily detectable landmarks such as the nasion (the dip at the top of the nose), the inion (the lowest point of protuberance above the base of the skull) and the pre-auricular depressions. Electrodes are placed at points 10 or 20% of the distance between the above landmarks. Each position is identified by a letter according to the cortical area beneath the electrode, i.e. Fp (frontal pole), F (frontal), C (central), P (parietal), O (occipital) and T (temporal). In addition, the midline positions are indicated by the letter "z", those on the left hand side of the head by odd numbers and those on the right hand side by even numbers (fig. 3.1). This system was designed especially for EEG recording and VEP recordings based on detailed analysis on the occipital activity therefore require a montage encompassing many electrodes over the area to be studied. These positions tend to be defined by distances from the inion or positions derived from the 10/20 system, usually in cm. One of the biggest drawbacks of the 10/20 system is that it assumes that the transcentral line is equidistant from the nasion to the inion, and that the head is symmetrical. However, Binnie (1987) reported that most normal heads were asymmetrical in circumference as well as plagiocephalic, i.e. the frontal or occipital region being larger on one side than the other in about 40% of normal subjects. However, the 10/20 system remains the most widely accepted method of electrode placement. Electrodes are connected to an amplifier as defined by EEG convention. Two electrodes tend to be used, one is connected to grid I (also called the black lead or active electrode) of the amplifier and becomes electronegative in relation to the other electrode which is connected to grid II (white lead or reference electrode), resulting in an upward deflection of the negative potential. The convention of using

Figure 3.1. The ten/twenty system of electrode placement showing **A** lateral, **B** frontal and **C** superior views of the skull and the percentage distances between electrode positions. (From Jasper 1958).

arrows going from the grid I to grid II electrode is usually used in figures and diagrams.

The measurement of potential are relative, not absolute, and refers to potential difference between two input leads. The electrodes may be linked using either monopolar or bipolar recording. Monopolar recording is based on recording the potential difference between an active electrode site and a reference electrode. The reference site should ideally be inactive but no truly inactive area exists. A common reference point is the Fz which is symmetrically placed with respect to occipital O1 and O2 positions and is almost diametrically opposite the occipital area on the scalp (Halliday et al. 1977; Drasdo 1980; Halliday 1980). However, the frontal reference is susceptible to blink artifacts especially with the flash stimuli (Harding 1980; Hobley and Harding 1989). Hobley and Harding (1989) also reported a frontal negative component occurring around the same latency as the occipital P2 hence, the use of the Fz as the reference position could lead to the enhancement of the P2. Alternatively, if this frontal negativity and occipital P2 were asynchronous, the Fz reference could result in an apparent shift in P2 latency (Hobley and Harding 1989). The central references C3 and C4 also has similar limitations, but the negativity over this site is of smaller amplitude and therefore preferred to Fz when recording flash VEPs.

Bipolar recordings regard both electrodes as active and involves measuring the potential difference between both of them. The system is arranged as chains of electrodes e.g. if electrode "a" is connected to grid I of channel 1, with electrode "b" connected to grid II of channel 1 as well as grid I of channel 2, then each channel will have common electrode. Since the potential differences between electrodes is measured, a negative potential under one electrode will be displayed as a downward deflection on one channel but an upward deflection on another channel to which the electrode is common, resulting in a phase reversal. A potential which arises midway between two electrodes will result in no potential difference and no deflection will occur, whereas adjacent channels will display phase reversal (Harding 1974; Binnie 1987). This technique is particularly useful in localising the source of EEG and evoked potential activity, but it is difficult to tell whether a particular deflection is due to a positivity under one electrode or negativity under another. Recorded signals are amplified, filtered and averaged using predetermined parameters.

3.1.2 The stimulus

VEPs are distinguishable by the visual content, the rate of presentation, and the mode of presentation of the stimulus. The visual content of the stimulus tends to be

either diffuse or patterned. The diffuse stimulus consists of flashes of light and the resultant VEP tends to result from changes in luminance. The most commonly used patterned stimuli consists of a geometric pattern such as checkerboard or bar gratings.

A square-wave grating pattern consists of dark and light (usually black and white) elements with sharp borders, while a sine-wave grating pattern consists of dark and light stripes with a gradual transition of brightness between the stripes. The VEPs to these patterned stimuli tend to be due to the visual content of the pattern, i.e. they relate to the contours and contrast present in the pattern. Chromatic pattern stimuli such as red-green checkerboard or grating pattern have also been used frequently.

The depth of contrast between the light and dark elements of a pattern stimulus (fig. 3.2A) is defined as the difference between the luminance of the light (Lmax) and the dark (Lmin) elements divided by their sum:

$$\text{Contrast} = (L_{max} - L_{min}) / (L_{max} + L_{min}).$$

The contrast is usually expressed as a percentage with the maximum contrast of 100% and minimum of 0%.

The size of the pattern elements can affect the VER and hence always described. The light and dark elements are normally of equal size unless stated otherwise. The size of the pattern elements are described by the visual angle of subtense and the spatial frequency. Visual angle describes the size of the image of one light or dark element at the retina and is derived from

$$\text{Tan } A = a / b$$

where "a" is the length of the element and "b" is its distance from the eye, (fig. 3.2B). The spatial frequency describes the number of repetitions of the light and dark elements per degree of visual angle. The visual angle of an element of 1 minute of arc (abbreviated as 1'), at the eye will have a spatial frequency of 30 cycles per degree (cpd). Spatial frequency is more commonly used to describe gratings and visual angles to describe checkerboards.

VEPs are divided into either the transient or steady-state type, depending on the rate of stimulus presentation. A relatively low, e.g. 1-2 Hz, stimulus presentation rate is used for transient VEPs, allowing the visual system to return to its resting state before the next stimulus presentation (Spehlmann 1985; Regan 1989). Increasing the stimulus rate to between 4-6 Hz, results in the interaction of individual VEPs

Figure 3.2 A. Luminance profiles of the pattern. B. Size of the pattern elements defined by (i) the visual angle of subtense of a single light or dark element at the retina as tan A =a/b and (ii) spatial frequency as the number of recurring pattern elements, or cycles, per one degree of visual angle. (From Spehlmann 1985).

while increasing the rate further, e.g. above 10 Hz, results in the steady-state VEP. Stimulus rates above 50 Hz for the flash VEP have also been used in the determination of the critical frequency of photic driving (Halliday 1982).

Transient VEPs consist of a polyphasic waveform which can be defined by polarity, latency and amplitude measurements, whereas the steady-state VEP consists of a series of regular quasi-sinusoidal waveform defined by the peak-to-trough amplitude and phase. With steady-state presentation, the individual components are no longer visible, simplifying the measurement (Spehlmann 1985). The response has to be analysed using band-pass filters and Fourier techniques. The latency however is determined indirectly in terms of changes in phase. The steady-state technique is useful when studying stimulus related properties of the VEP (Campbell and Maffei 1970; Kulikowski 1977), but the number of variables measurable in one run tend to be small. Whereas, in studies where the physiological significance of individual response components is to be investigated, transient VEPs are more useful (Mackay and Jeffreys 1973). Overall, the two techniques are complementary in their ranges of usefulness.

VEPs to the flash stimulus were the earliest and the most frequently recorded VEP until the 1970s. However, in the late 1960's, a wider range of more subtle (patterned) stimuli were introduced, which allowed a more detailed examination of the visual sensitivities and processing of the visual pathway (Regan and Spekreijse 1986). The mode of presentation of the patterned stimuli can either be a reversing pattern where the black elements become white while the white elements become black, pattern onset-offset (or appearance-disappearance) with a pattern stimuli alternating with a diffuse screen which is usually of the mean contrast level of the black and white checks, or flashed patterned where the pattern is briefly presented. The VEPs to the flash and pattern reversal stimuli are are the subject of the present study and only they will be dealt with in detail.

3.2 The flash VEP

The stimuli of diffuse light flashes tend to presented as brief flashes, longer light pulses are not commonly used for clinical studies. The normal transient VEP to a diffuse flash has generally been described as having up to seven peaks or components. However, the polarity, latency and amplitude of the peaks can vary greatly due to different laboratory conditions (Cobb and Dawson 1960; Ciganék 1961; Kooi and Bagchi 1964; Perry and Childers 1969).

Cobb and Dawson (1960) published the first averaged transient VEP using a very bright flash stimuli at low frequencies (1-2 Hz). They reported that the first wave, after the flash presentation, was a positive wave occurring 20-25 ms followed by a negative wave at about 40-50 ms. They also reported that these early waves were of cortical origin and not due to possible volume conductance from the electroretinogram (ERG). These early waves have been confirmed and investigated by many authors (Cracco and Cracco 1978; Harding and Rubinstein 1980 and 1981; Pratt et al. 1982; Whittaker and Siegfried 1983), and thought to be generated in subcortical areas but only seen with very bright flashes.

The waveform of the normal VEP to a flash stimulus is illustrated in fig.3.3. The primary response according to Ciganék (1961), includes waves I-III (occurring in the 0-90 ms time window); the secondary response includes waves III onwards (occurring in the 90-240 time window) and finally, the response ends with a rhythmic after-discharge (occurring between 130-350 ms). The after-discharge occurring on averaged records, is absent in the raw EEG, and consists of a phase-locked sinusoidal potential with a frequency in the alpha range or higher (Regan 1989). The occurrence, duration and regularity of the after-discharge is variable (Ciganék 1975).

Figure 3.3 The normal flash VEP. Positivity is represented as a downward deflection. (From Ciganék 1961).

Nomenclature of the flash VEP components vary according to the investigators, (table 3.1). The system of labelling to be used will be that recommended by Harding (1974), denoting the component with either a P or N according to its polarity followed by sequential numbering. The most prominent component of the flash VEP is a positive wave occurring around 95-120 ms which is known as the P2 and will be discussed in more detail.

Table 3.1 Alternative nomenclatures for the components of the flash VEP.

Polarity of peaks	**P**	**N**	**P**	**N**	**P**	**N**	**P**	**N**
Ciganék (1961)	-	I	II	III	IV	V	VI	VII
Gastaut & Regis (1965)	1	2	3	4	5a	5b	5c	6
Rietveld, Tordoir & Duyff (1965)	-	-	A	B	Γ	E	Z	H
Dustman &Beck (1969)	-	A	B	C	D	E	F	G
Korol & Stangos (1972)	-	-	A	B	C	-	-	-
Harding (1974)	P0	N1	P1	N2	P2	N3	P3	N4

3.2.1 Properties of the flash VEP

The flash VEP has been regarded as a mixture of an "on" and "off" responses. Clynes et al. (1964) separated these on and off responses using a light stimulus of prolonged duration. They found that the after-discharge at the alpha frequency was associated with the off response.

The flash VEP has good repeatability from run to run in any one subject and is stable over long periods of time (Kooi and Bagchi 1964). Dustman and Beck (1963) reported correlation coefficients (Pearson product moment) of 0.72-0.99 (M=0.88), for components occurring in the first 300 ms. This was in agreement with Contamin and Cathala (1961) who only found a ±15% variation in latency of the major components.

Ciganék (1969) investigating the variability of the VEP in 20 subjects, found the amplitude variability was high and attributed to background activity of the EEG, whereas the variability of the evoked potential itself was negligible. The inter-individual variability was high with amplitude variability being greater than latency variability. About half of the subjects showed a reduction in variability 80 ms after the stimulus due to blocking of the background activity.

Ciganék (1961 and 1975) reported that the primary response (waves 0-III) displayed the least interindividual variability, the secondary response (waves IV-VII) was more variable, and the rhythmic after-discharge showed the greatest variability both within and among subjects. Thus there was a general increase of interindividual variability with longer latencies with peak latencies displaying less variability than amplitudes. Aunon and Cantor (1977) confirmed this finding but also reported the interlaboratory standard deviation to be twice as that of the intralaboratory standard deviation, which was due to the differences in recording techniques. Within a laboratory, the intrasession variability was significantly less than than intersession variability.

Kooi and Bagchi (1964) investigated the flash VEP in 100 adult subjects. No relationship between the VEP and and factors such as eye colour, colour blindness, type of refractive error, pupil size, subjective estimate of light intensity, hours of sleep, alpha frequency, alpha amplitude and alpha persistence index was found. Increasing the pupillary size acts like increasing the stimulus intensity, but generally did not have a significant effect on the VEP (Spehlmann 1985).

The flash VEP has been reported to be of foveal origin (Rietveld et al. 1965). The amplitude has been found to decrease as the stimulus is moved peripherally, away from the fovea, in accordance with the photoreceptor distribution of the retina (Copenhaver and Perry 1964). Eason et al. (1967 and 1968) reported that stimulation of the temporal part of the visual field evoked larger responses over the ipsilateral occipital lobe than stimulation of the nasal visual field. These workers also found that stimulation of the lower half field resulted in responses of greater amplitudes and shorter latencies than with stimulation of the upper half field (Eason et al. 1967a and 1970).

3.2.2 Effect of age on the flash VEP

The morphology of the flash VEP has been reported to change with age. Ellingson (1966) reported that in premature infants, (after 24 weeks gestation), the flash VEP consisted of an occipital negative peak occurring around 200-300 ms. After 32-35

weeks gestation, a positive peak occurring before 200 ms appeared before the negative peak. With further development, the amplitude of the positive peak increased while that of the negative peak decreased. With increasing gestational age, the VEP expanded from the occipital to frontotemporal areas. At full term, later additional peaks appeared and the latency decreased. Dustman and Beck (1966 and 1969) found that the overall amplitudes reached a peak at 5-6 years of age. The early peaks reached adult values in early childhood while later peaks did not completely mature until puberty. The latency and amplitude were more variable in children than adults. After adulthood, the latency tended to increase with increasing age, but the amplitude was increased or decreased (Dustman and Beck 1966 and 1969). These changes were attributed to the intracerebral anatomical and electrophysiological variations that occur with age (Dustman and Beck 1969). Morrell and Morrell (1965) related the development of the VEP to the gradual myelination process of thalamo-cortical radiation fibres. The maturation of the VEP was reported to reflect the slower myelination of nonspecific projection and association fibres compared with specific sensory fibres (Creutzfeldt and Kuhnt 1967; Umezaki and Morrell 1970).

Wright et al. (1985) also investigated the effect of age on the flash, pattern reversal and pattern onset-offset VEPs. The flash VEP displayed the P2 component to be extremely consistent, being present in 69 out of 70 subjects and was the only component that significantly varied with age. The P2 latency was increased progressively from a mean latency of 114.4 ms in the youngest age group of 10-19 year olds, to 134.25 ms in the 70-79 year olds, the oldest age group studied. The occurrence of the early positive P1 component was found to be related to age by not occurring in the 10-19 year group, but occurring increasingly with the increasing age groups. In place of a P1, the younger groups displayed a negative component with a latency intermediate to N1 and N2. The N2 component was apparently earlier in the younger age groups which was thought to be due to the merging of N1 and N2 in those without a P1 component. The later N3, P3 and N4 components were found to be more variable and did not show any changes in incidence with age. The amplitudes were also found to be higher in the young 10-19 year group compared to the other groups.

Hobley and Harding (1989) reported a frontal negativity (referred to as N120) occurring around the same time as the occipital P2. The frontal N120 and occipital P2 were found to be of the same latency throughout the age range. Therefore, using Fz as reference would lead to enhancement of the P2 and when the N120 and P2 are asynchronous, the use of Fz would result in an apparent shift of the P2. In middle

age, the use of Fz was thought to result in an artificial appearance of P1 which was really the reflection of the frontal negativity (N75). Hence with increase in age, there can be an enhancement of the P1 with the Fz reference site, and in asynchronous conditions, cause an apparent shift in latency.

Wright et al. (1985) proposed two hypotheses for the relationship of the P1 and P2 components: Firstly, in middle age, the P1 may be hidden by the P2 component, such that in later life when the latency of the P2 increases, the P1 is revealed. Alternatively, the P1 and P2 arise independently. Hobley (1988) confirmed the age related changes in the P2 component and appearance of the P1 in later life. However, Hobley (1988) found that in the young age-group, the effect of eye closure when viewing the stimulus results in an increase in the P2 without the appearance of the P1. In addition, when a red filter is used over the stimulus, the P2 latency again increases without revealing an underlying hidden P1 component. Therefore the P1 was thought to be a result of a new event.

3.2.3 Effect of stimulus intensity

Increasing the stimulus intensity has been reported to decrease the P2 latency (Cobb and Dawson 1960; Contamin and Cathala 1961; Spehlmann 1985). In contrast, an increase in latency with increase in flash intensity has been found to occur in components occurring up to 600 ms after the stimulus (Wicke et al. 1964; Shipley et al. 1966). The effect on amplitude was more complex. Shipley et al. (1966) found an increase in amplitude (measured as peak to trough) of the flash P2 component with increasing intensity up to a saturation level and then a decrease at higher intensity levels, however the amplitudes of later components increased monotonically with intensity.

The flash VEP has been described as photopic, i.e. it is predominantly a cone response and can be elicited in light adaptation with very bright stimuli (Ciganék 1975; Spehlmann 1985). The signals from the rod cells which converge upon the same ganglion cells as the cone signals, are thought to be prevented from reaching supraretinal levels by their slower refractoriness compared to the faster cone signals. The P2 is thought to reflect both photopic and scotopic function (Harding 1974). The P2 was subdivided into P2a (as labelled by Gastaut et al. 1966) which reflected photopic and P2c which reflected scotopic function (Harding 1974).

The flash VEP, due to its large interindividual variability in waveform, can be considered as unreliable for clinical testing. However, Wright et al. (1985) illustrated that the flash VEP was reliable, with the major P2 component being

identified in 69 out of 70 normal subjects. Therefore, although the interindividual variation is high in the flash VEP, it is not sufficient to be impractical.

3.3 The pattern VEP

A patterned stimulus has been regarded as a more spatially structured and hence more effective for stimulating cortical cells than a diffuse flash. This was based on the neurophysiological findings of Hubel and Wiesel (Wiesel and Hubel 1966; Hubel and Wiesel 1968) that for cats and monkeys, the majority of neurones in the visual cortex were sensitive to contoured visual stimuli and were relatively insensitive to overall luminance changes. Periodical patterns such as gratings and checks were thought to be more appropriate for stimulating retinal ganglion cells who are primarily contrast detectors rather than luminance detectors (Maffei 1982).

Pattern VEPs studies initially used checkerboards (Spehlmann 1965; Rietveld et al. 1967). This stimulus was thought to be the closest approximation to the radial arrangement of the retinal receptive fields while maintaining a simple enough shape for pattern reversal (Spekreijse et al. 1977). Polygons of a higher order than checks approximated more closely to the circular shape of the receptive fields, but these polygons contained more than four neighbours so not feasible for pattern reversal at constant luminance levels (Spekreijse 1980).

Square- and sine-wave gratings have also been used by workers who consider these to to be optimal stimuli for the receptive fields of the visual cortex, consisting only of a single spatial frequency (Campbell and Kulikowski 1972; Bodis-Wollner and Hendley 1977; Kulikowski 1977; Maffei 1982). Gratings were also thought to have an advantage over checkerboards in activating neurones which are orientation-specific (Arden et al. 1977). However, the morphology and topography of VEPs using gratings has not been as extensive as checkerboards.

The use of sinusoidal gratings has been based on the hypothesis that the visual system analyses the visual image into narrow spatial bands by means of the spatial frequency channels. For instance the spatial square waves representing the light and dark elements of a checkerboard consist of a mixture of frequencies. The major Fourier component of the spatial frequencies of a checkerboard, are orientated diagonally to the squares with a wavelength 1.4 (square root of 2) times the side length of the squares, hence vertically orientated patterns were more effective than obliquely orientated ones (Kelly 1976; Spehlmann 1985). There are several major advantages of using sinusoidal gratings including the comparability of the VEP with psychophysical and single-unit data, the simple description in terms of spatial

frequency spectrum, and the absence of the effect of stimulus element size and edge sharpness considerations (Regan 1989).

3.3.1 Pattern reversal VEP

The technique of pattern reversal stimulation was first described by Cobb et al. (1967), and Cobb and Morton (1970), but has been extensively investigated by Halliday and co-workers (Halliday and Michael 1970; Michael and Halliday 1971; Halliday 1982). Pattern reversal stimulus (also referred to as contrast reversal, counterphase modulation and pattern shift), involves the rhythmic interchange of the light and dark elements. A single pattern reversal stimulus cycle involves two reversals per second, which corresponds to the temporal modulation frequency of 1 Hz (or 1 cycle per second). The pattern reversal response is thought to contain both motion and contrast constituents (Kulikowski 1977a).

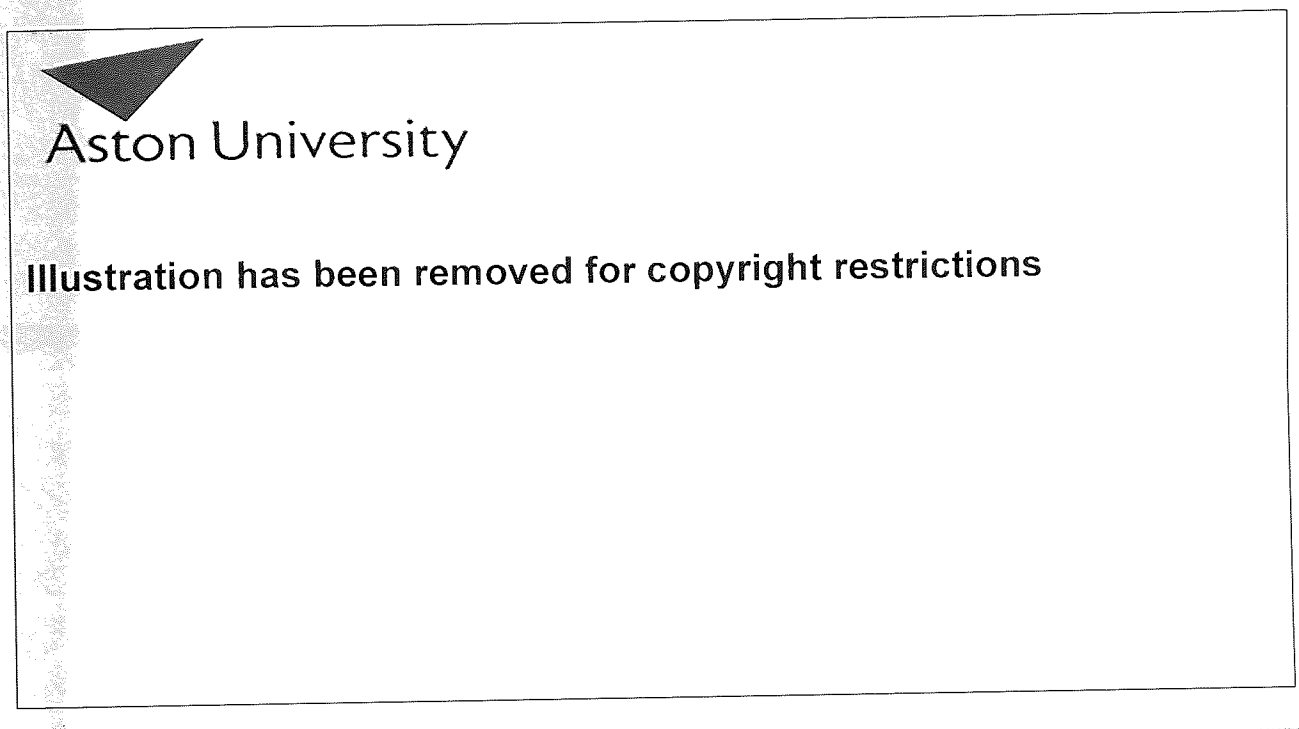
A reversing black and white checkerboard pattern is a commonly used patterned VEP. It is characterised by a positive peak at around 90-110 ms termed the P100, preceded and followed by the negative peaks N75 and N145 respectively (fig. 3.4). The P100 is the major component of the pattern reversal VEP and the most clinically useful (Halliday and Michael 1970; Halliday 1982). The preceding and following negativities, and following positivity, are more variable in appearance and latency and do not appear to be more sensitive than the P100 to disease (Allison 1987).

3.3.1.1 Effect of age and sex

The latencies of the pattern reversal VEP components appear to be shorter and the amplitudes larger in females than males (Shaw and Cant 1981; Allison et al. 1983; Allison et al. 1984; La Marche et al. 1986; Celesia et al. 1987; Hammond et al. 1987), but does not reach statistical significance. Halliday et al. (1982), reported that the mean latency of the P100 in females was 3.5-4 ms shorter and 4-5 μV larger than the male. This difference was attributed to the smaller average head size and skull thickness of females. The VEP latency in the female group was also subject to a more pronounced and earlier increase related with age, which was attributed to structural, metabolic or temperature changes associated with menopause.

The effect of aging on the pattern reversal P100 component has included an increase of 10-15 ms between the ages of 20-70 years, with small checks (Celesia and Daly 1977; Sokol et al. 1981a; Celesia et al. 1987), and an increase of 5-7 ms in older age groups (older than 60 years of age) with larger checks (Asselman et al. 1975; Halliday et al. 1982; Allison et al. 1983; Allison et al. 1984). Wright et al. (1985)

Figure 3.4 The normal transient pattern reversal VEP to a checkerboard pattern. Electrode montage was Oz-Fz. Negativity is represented as an upward deflection. (From Kriss and Halliday 1980).



reported an overall latency increase of 6 ms, 2.5 ms and 11 ms for 56', 19' and a foveally viewed 13' checkerboard respectively. Therefore confirming the suggestion that the effect of age on the pattern reversal VEP was dependent on the check size used (Sokol et al. 1981a; Celesia et al. 1987). These workers found that the increase in the P100 latency occurred for both the 12' and 48' check, but that the rate of increase was twice as fast for the smaller 12' check.

Numerous factors associated with age and visual function have been proposed to account for the changes in the VEP with increasing age. Although changes occurring at the level of the eyeball include aging of the cornea, this does not appreciably affect the eye optics (Celesia et al. 1987). However, aging of the crystalline lens and pupil may reduce the amount of light reaching the retina. The pupil diameter can decrease linearly from 20-60 years of age (Loewenfeld 1979), with the small pupil in the elderly being referred to as senile miosis. There is also a decrease in the pupil diameter under low photopic conditions with age. The reduction in pupil size may be related to a reduction in the retinal luminance levels which could in turn increase the P100 latency. However, Sokol et al. (1981a) found that the reduction in retinal illumination due to the reduction in pupil size was not enough to account for their increase in the P100 latency. Hence other factors were

proposed to be the major contributing factors, e.g. degeneration of retinal ganglion cells (Vrabec 1965) and alterations in the outer nuclear layer around the macula (Gartner and Henkind 1980) in elderly individuals.

The changes occurring beyond the retinal level include a diminution of axons in the optic nerve especially after the seventh decade (Dolman et al. 1980; Balazsi et al. 1984) and various biochemical and cytoarchitectural changes that are thought to occur in the LGN and the occipital cortex (Ordy and Brizzee 1979). In addition, neuronal drop-out and loss of dendritic spines and synaptic contacts in the visual cortex with aging has been reported (Scheibel et al. 1975; Wisniewski and Terry 1976; Devaney and Johnson 1980). There can be up to a 50% reduction in neuronal density from the age of 20 to 80 years in the visual cortex (Devaney and Johnson 1980). There are also changes in the neurotransmitter function and increased synaptic delay in the neurones in the visual cortex (McGreer and McGreer 1976; Samorajaski 1977).

The effect of age on amplitude is varied among investigators, for example, some have reported that amplitude decreased rapidly from childhood to adolescence (Shaw and Cant 1981; Snyder et al. 1981); some reported that amplitude decreased until the age of 30 years followed by a small increase and then another decline after 50 years of age (Shaw and Cant 1981); some found no significant change in amplitude with age (Celesia and Daly, 1977; Halliday et al. 1982; Wright et al. 1985) and Asselman et al. (1975) thought that amplitude was too variable to draw any conclusions!

3.3.2 Effect of stimulus parameters on the pattern VEP

3.3.2.1 Spatial content

Pattern VEPs using a checkerboard stimuli tend to be more defined with greater amplitudes than those to a grating (Armington et al. 1971; Mackay and Jeffreys 1973; Spekreijse et al. 1973; Van der Tweel 1979; Ristanovic and Hajdukovic 1981) due to the presence of corners in the checkerboard (Drasdo 1980). Spekreijse et al. (1973) demonstrated that responses to either vertical or horizontal gratings were "sluggish", (rising and falling slowly) but superimposing these gratings resulted in a brisker response resembling a checkerboard VEP. This has been explained by checkerboard pattern containing a greater number of constituent frequencies according to Fourier theory (fig. 3.5). The spatial frequency spectrum of the sine-wave grating is composed one fundamental frequency, while that of the checkerboard is composed of two oblique fundamentals at 45^{o} and 135^{o} with higher harmonics forming the edge of the checks (Kelly 1976; Bodis-Wollner and Onofrj

Figure 3.5 A sinusoidal grating and a checkerboard pattern showing the Fourier spectrum of each. The numbers indicate spatial frequency and the radial dimensions and orientation of the spatial frequency components of each pattern. The sine wave grating only contains energy at the fundemental frequency (3 cpd), while the checkerboard contains energy at a number of frequencies. The relative contribution of each harmonic to the total amplitude of the pattern is indicated by the size of the dot at each spatial frequency. (From Bodis-Wollner and Onofrj 1982).

1982). In addition, a non-linear mechanism exists in the human visual system which can only be best fitted by sharp edges (Regan 1989).

3.3.2.2 Spatial frequency

The pattern VEP amplitude has been reported to display spatial tuning effects. The general trend being central small field stimulation result in maximal amplitudes for small check sizes. This has been displayed for both the transient pattern reversal VEP (Ristanovic and Hajdukovic 1981; Plant et al. 1983) and steady-state pattern reversal VEP (Regan and Richards 1973).

Ristanovic and Hajdukovic (1981) using pattern reversal stimulus showed that the central foveal area of 4^{o} displayed greatest amplitude to small checks of 7.5'-30' subtense, whereas the stimulation at 4.5^{o}-7.5^{o} displayed optimal responses to checks of 30'-60' subtense (also reported by Armington et al. 1971) and gratings of 7'-15' arc. In addition, latency of the major P100 component decreased with decreasing spatial frequency. Kurita-Tashima et al. (1991) using an 8^{o} field found that the latency and amplitude of the N75 component displayed an inverse linear relationship to logarithmic increases in check size, whereas the P100 and N145 displayed a curvilinear relationship with minimal latencies at 35' checks.

Plant et al. (1983) investigating the pattern reversal VEP to sinusoidal gratings, reported increased amplitude of the N1-P1 complex with the spatial frequency, reaching a maximum at 4 cpd and declining thereafter. There was an appearance of a later positive component (P2 in their terminology), with a latency around 200 ms but only at intermediate spatial frequencies. The P100 (P1 in their terminology) was split into an a-b-c- type or "W" morphology at low spatial frequencies (below 1cpd). The earlier part of the split P100 was attributed to peripheral retinal stimulation since it was enhanced at lower spatial frequencies (0.25 cpd) and larger field size (20^{o}). Torok et al. (1992) showed similar findings with both the pattern reversal and pattern onset-offset VEPs displaying maximal amplitudes between checks of 2.1-2.8 cpd and a field subtending 20^{o} x 20^{o}.

Steady-state checkerboard pattern reversal VEPs have shown maximum amplitude for check sizes between 10'-20' side length for a foveally viewed field of about 2^{o}-6^{o} diameter (Regan and Richards 1971). These authors later reported (Regan and Richards 1973) that this amplitude to check size function was not affected by contrast levels between 4-100%.

Pociatti and Von Berger (1981) investigated the effect of check size (1.2' to 111') on both the transient (1 Hz temporal frequency) and steady state (8-16 Hz temporal frequency) high contrast pattern reversal VEPs. They reported that with check sizes from 20' to 111', the P100 latency remained fairly constant at 108 ms then gradually increased with decreasing spatial frequency reaching 147 ms for a 2.3' check. The steady state VEP showed that the phase of the response remained unaltered across the full range of spatial frequencies. The apparent latency of the steady state response (as evaluated by the slope of phase versus temporal frequency function) was about 110 ms. The increase in latency with spatial frequency was attributed to two possibilities: Firstly the possible selection at high spatial frequencies, of retinal ganglion cells with smaller receptive fields and lower conduction velocities.

Secondly, the increased latency may be related to the gradual transition from the fast Y system to the slower X system. But the two systems are thought to have overlapping conduction velocities so that variations in latency could occur in either system. In the transient VEP, both systems probably contribute to the response with the X system contributing mainly at the higher spatial frequencies due to its smaller receptive fields. This explained the constant latency at 20'-111' checks which could be related to the low sensitivity of the Y system to the spatial structure of the stimulus. The steady state response was also thought to be mainly attributable to the transient Y system because of the fairly constant P100 latency and the higher spatial discrimination threshold obtained compared to the transient VEP. These findings were supported by comparing the responses in two patients with retrobulbar neuritis, where one patient was affected mainly axially and the second mainly peripherally. The patient with mainly axial effects showed a delayed response in the affected eye, only to the transient VEP at high spatial frequencies. Whereas the other patient showed a delay at both low and high spatial frequencies being more evident at the lower spatial frequencies to the transient VEP, while with the steady state VEP, the delay was only detectable a low spatial frequencies.

Armington et al. (1971) suggested that the check size function was related to the size of the antagonistic centre-surround receptive fields in the retina. This implies that a maximal response will be obtained by the check size which produces the maximum excitatory and minimum inhibitory response and hence, larger or smaller checks stimulating both areas result in a smaller response (Harter 1970; Ristanovic and Hajdukovic 1981). Therefore, the ratio between check size and receptive field will determine the strength of a signal. But in addition, there will be signals from any antagonistic cells with smaller receptive fields which have coincided with the edges of the checks and any luminance detectors which have been stimulated by a bright check. These signals provide the input to the visual cortex based on spatial frequency and orientation selective cells, which respond to the fundamental and harmonic frequencies of the stimulus. The peak amplitude obtained for the peak spatial frequency hence depends on a combination of all of these factors.

3.3.2.3 Field size

The central 3^{o}-5^{o} of the retina has been thought to be responsible for the pattern VEP with checks between 10'-30'. This has been demonstrated for all the major types of pattern stimulation and VEP recording studies where the stimulus field diameter was increased until no further increases in amplitude was obtained (Rietveld et al. 1967; Behrman et al. 1972), in studies where the diameter of an

occluded portion in the centre of the patterned stimulus (central scotoma) was increased until the VEP is abolished (Rietveld et al. 1967; Behrman et al. 1972), and in experiments where a reduction in the amplitude of the VEP was recorded as a stimulus of constant size was moved further away from the fixation (Armington and Brigell 1981; Meredith and Celesia 1982). In addition, Ristanovic and Hajdukovic (1981) suggested that the majority of the VEP signal was derived from stimulation of the central 10^{o}, but that the VEP signal overall was a balance between responses to both central and peripheral stimulation.

The predominance of the central response has been attributed to the proximity of the cortical central field representation to the scalp electrodes, and also to the higher receptive field density and consequent cortical magnification of this area (Regan 1972; Armington and Brigell 1981). The contribution of the extrafoveal retina increases as the check size increases, (Behrman et al. 1972; Regan 1972; Van der Tweel 1979), predominating when the checks of greater than 60' in fields greater than 8^{o} are used (Meredith and Celesia 1982).

Celesia and Meredith (1982) investigating the effect of field size on the transient pattern reversal VEP reported that the P100-N145 amplitude was reduced with increasing distance from the fixation point, along the horizontal, vertical and oblique meridians. Field sizes greater than 14^{o} eccentricity required increasingly larger check sizes and fields to evoke similar maximum responses. The findings correlated with the decreasing cone density in relation to retinal eccentricity; the density distribution of the human ganglion cell population along the horizontal axis and the reduction in visual acuity in relation to eccentricity, confirming earlier suggestions of Ristanovic and Hajdukovic (1981).

3.3.2.4 Effect of luminance and retinal illuminance

Halliday et al. (1973) found that for a ten-fold reduction in luminance, the pattern reversal VEP latency increased by about 15-20 ms while the amplitude was only reduced by about 18%. The report did not state if artificial pupils were used as a reduction in luminance may have caused a dilation of the pupil, partially overcoming the changes and accounting for the different results (Van der Tweel et al. 1979). Steady-state pattern reversal VEPs also show a delay in latency with a reduction in luminance but amplitude was not affected (Behrman et al. 1972).

Penne and Fonda (1981) reported that a decrease in pupil diameter from 8 mm to 2 mm resulted in a ten-fold reduction in retinal illumination. This was used to explain latency increases of the pattern reversal VEP in studies where the pupil diameter has

been reduced by miotic drugs (Hawkes and Stow 1981; Penne and Fonda 1981), or artificial pupils (Penne and Fonda 1981; Sokol et al. 1981). Mydriatic drugs can produce a decrease in latency of the order of 20 ms for a 8 mm change in pupil diameter, and therefore cannot be overlooked when comparing sizes due to pathology, drug therapy or age (Hawkes and Stow 1981; Penne and Fonda 1981; Sokol et al. 1981).

3.3.2.5 Effect of contrast

The effect of progressively increasing the contrast of a checkerboard pattern results in an initial progressive increase followed by saturation in the VEP amplitude. The exact saturation contrast level varies between 10% and 50% depending on the stimulus parameters (Spekreijse and Estevez 1972; Padmos et al. 1973; Spekreijse et al. 1973; Estevez 1976; Jones and Keck 1978). Spekreijse et al. (1973) also found that the saturation point decreases with increasing luminance and check size. The relationship between the amplitude (at subsaturation levels) and the stimulus contrast has been shown to be logarithmic when using gratings of 3 cpd or higher with steady-state pattern reversal conditions (Campbell and Maffei 1970; Campbell and Kulikowski 1972).

Van der Tweel et al. (1979) found that the shape of the VEP remained constant although the latency was increased when the contrast of the stimulus at each luminance level was a constant multiple of the threshold. This suggested that the decreasing luminance levels only affected the speed of signal transmission along the optic nerve but not the generation of the signal itself. Contrast also seemed to have a greater effect than luminance such that a two-fold change in contrast resulted in having the equivalent effect of a ten-fold change in retinal illumination.

The rate of change of contrast has been reported to change the waveform of the pattern reversal VEP (Van der Tweel et al. 1969). The pattern reversal response displayed a monophasic waveform at slow rates of change, whereas higher rates of change resulted in a diphasic waveform. Estevez and Spekreijse (1974) reported that the latency of the pattern reversal also increases with slow rates of contrast change, with the peak latency corresponding to the point at which maximum contrast is reached.

Bobak et al (1984) investigated steady-state pattern onset-offset VEP to a 6 cpd sine wave grating modulated at 7.5 Hz. The resulting VEP contained both the fundemental and second harmonic frequency components. A plot of amplitude against these components revealed a two-branched plot which underwent a transition

around 10-15% contrast, which was interpreted as being due to the two parallel processing systems, i.e. the low-contrast limb of the plot was attributed to the magnocellular system and the high-contrast limb to the parvocellular system. In addition, Nakayama and Mackenben (1982) who investigated the steady-state pattern reversal VEP in alert monkeys at five spatial frequencies (0.55; 1.9; 4.5; 6.7 and 10.3 cpd) also found a two-branch relationship between amplitude and contrast. The contrast levels at which point a transition occurred increased with spatial frequency, further implying a magnocellular system being responsible at low contrast levels spatial frequencies and the parvocellular system at the higher contrast levels and spatial frequencies.

3.3.2.6 Effect of blur

Blurring does not affect the flash VEP (Wright 1983), but has been reported to attenuate the pattern VEP, especially for foveally viewed small checks less than 20' (Regan and Richards 1971; Spekreijse et al 1973; Wright 1983). The pattern reversal VEP to a 13' check only required a 0.5 dioptre (D) error in accommodation to attenuate the VEP (Regan and Richards 1971; Wright 1983), whereas larger checks required increased dioptre for blur. However, some subjects displayed an increase in amplitude with blur (Regan and Richards 1971). Increasing refractive blur also increases the VEP latency (Sokol and Moskowitz-Cook 1981; Wright 1983). The effect of blur is greater on smaller checks than larger checks. For instance, 12' arc checks gave a 4-6 ms increase per dioptre of blur whereas 48' of arc checks gave about 2.5 ms increase per dioptre of blur (Sokol and Moskowitz-Cook 1981). The blurring on checkerboards and square wave gratings affects the spatial frequency content and reduces the contrast, whereas on sine wave gratings it only reduces the contrast levels (Regan 1989).

3.3.2.7 Temporal characteristics

Regan (1977) reported that the temporal tuning of pattern reversal VEP to small checks differed dramatically from that of the flicker VEP to an unpatterned field. Comparison of a checkerboard reversal and flicker VEPs from a 3^{o} diameter foveal area revealed that the response to small checks (12') peaked at 6-7 Hz being weak around 10 Hz, whereas the blank-field flicker response was concentrated near 10 Hz. In addition, the response to large checks (40') were an intermediate case, with a peak response around 3-7 Hz and at 10 Hz. This led to the conclusion that pattern VEPs to large checks contained a mixture of pattern-specific and local flicker responses, and that they depend differently on temporal frequency.

Generally, stimuli of low spatial frequencies give the largest response predominantly at high temporal frequencies and high spatial frequency stimuli to low temporal frequencies. For example, Regan (1989) found that a 8 cpd grating gave a maximum response in two frequency regions at 5-8 Hz and 12-13 Hz with a minimum at 10 Hz, but the 1 cpd grating response resembled the flicker response with a maximum response at 16-18 Hz and minimal around 5-8 Hz. This correlates with the parvocellular system operating at the higher spatial and low temporal frequencies and the magnocellular system at the low spatial and high temporal frequencies.

3.4 Physiological characteristics of VEPs

Harter (1970) reported the effects of the check size on the amplitude of the flashed patterned VEP was related to the retinal structure and physiology, i.e. the amplitude was a function the receptive field centre size. This was based upon the findings of Wiesel and Hubel (1966), who demonstrated that the LGN cells of the rhesus monkey were sensitive to the size of the black and white spots of light falling on the retina. These cells have concentrically arranged receptive fields with either an excitatory centre-inhibitory surround or inhibitory centre-excitatory surround (type I and II respectively). The effect of stimulus size was measured and found that when entire receptive field was stimulated the sensitivity of the cell was low, but increased to a maximum when the spot diameter was reduced to the size of the receptive field centre (30'), with further reductions in spot diameter resulting in a decrease in sensitivity.

Spekreijse et al. (1972) provided evidence that the pattern VEP was a pure pattern response of the visual system, from studies where the appearance of the checkerboard could be produced by either decreasing or increasing the luminance of one set of checks, while that of the other check remains constant. In both cases, the VEP was due to the appearance of the pattern, regardless of the increase or decrease in the mean luminance. If the pattern VEP was due only to local changes in the luminance then symmetrical responses to the pattern onset and offset would be obtained since in both cases half the checks increase and the other half decrease in luminance (Padmos et al. 1973; Kulikowski 1977a; Van der Tweel 1979), but this is not the case as both the onset and offset are asymmetric. In addition, a pure luminance response would also predict that the amplitude of the response would saturate once the check size exceeds the receptive field size (Padmos et al. 1973). However, a plot of VEP amplitude against check size indicates a peak at intermediate check sizes. This can be explained by a centre-surround receptive field response to local luminance changes- the peak corresponding to the check size which

elicits a maximum excitatory and minimum inhibitory response (Armington et al. 1971).

The pattern VEP components are thought to reflect different aspects of the pattern stimulus. For instance, the CII (and CIII) components of the pattern onset-offset VEP, reflects details of the pattern i.e. contours and clarity, whereas the CI, offset and the pattern reversal P100 components reflect mechanisms responding to transient changes in contrast and movement (Spekreijse et al. 1973; Jeffreys 1977; Kulikowski 1977a). For small checks (less than 15'), the contour responses predominate over the contrast response and vice versa for larger checks (Regan 1972; De Vries-Khoe and Spekreijse 1982).

Kulikowski (1977a) used sinusoidal gratings at low spatial frequencies and temporal frequency of 1.6 Hz (which is believed to equally stimulate the pattern and movement detectors), to investigate the pattern onset-offset and pattern reversal VEPs. The pattern onset, offset and pattern reversal were found to be almost identical under these conditions. However, at higher spatial frequencies (2cpd and above), the differences between pattern onset and reversal VEPs were more prominent, suggesting that the increasing contribution of the pattern response to the pattern onset VEP and the movement detectors for the pattern offset and pattern reversal VEPs.

3.5 Origins and generator sites of the VEP

Corletto et al. (1967) provided convincing evidence that the VEP was of cortical origin by demonstrating its disappearance after surgical removal of the occipital lobe. The flash and pattern VEPs are generally believed to reflect different visual processes. VEPs to pattern reversal stimulus have larger amplitudes than those evoked by "in phase" (homogenous field) modulation of the checks by the same amount, indicating a different origin for the two types of responses (Spekreijse et al. 1977).

The cortical VEP is generally thought to be of two sources: firstly from activity of the retinal receptors which reach the occipital cortex via the LGN, and secondly from activity which initially arises from the retina but then reaches the cortex via other pathways such as the reticular formation and the diffuse thalamic projective system (Ciganék 1961; Sokol 1976).

3.5.1 Electrogenesis of the VEP

Fox and O'Brien (1965) demonstrated a close relationship between an intracortically recorded VEP and the activation cycles of some cells recorded with the same electrode. Their studies concluded two possible interpretations of VEP electrogenesis: Firstly, that the VEP consisted of both summated and asynchronous discharges from cell bodies in any localised area possessing the same probability of firing. Secondly, the VEP was a result of a number of sources both local or distant such as apical, basal or somatic dendritic post-synaptic potentials associated with electronic spread from dendrites.

Creutzfeldt and Kuhnt (1967) also proposed that VEPs were composed of compound excitatory and inhibitory postsynaptic potentials of not only pyramidal cortical cells but also of synchronous afferent and efferent neurone activity. Slow surface potentials occurring after 15-20 ms rise time were assumed to reflect the post-synaptic potentials of the so-called "average" cortical neurone. They proposed that the 7 waves of the flash VEP (Ciganék 1961), were due to a sequence of interactions between the excitatory and inhibitory post-synaptic potentials. The waves N1, N2, N3 and N4 (waves I, III, V and VIII in their terminology) represented a post-synaptic excitation or depolarisation of most cortical cells and that P2 and P3 represented a post-synaptic inhibitory response or repolarisation.

Babel et al. (1977) further proposed that the visual signal enters layer IV of the primary visual cortex and is amplified at the synaptic relays with type II Golgi cells present in the granular layer. The type II Golgi cells have short axons and transmit their signals in a limited area, hence their function is to amplify and reinforce the information received and to activate the pyradmidal calls and the large stellate cells in the same layer. These stellate cells depolarise the large pyramidal cells of layers V and VI. Activation of the large pyramidal cells results in a post-synaptic depolarisation current originating in the cell body, which is recorded on the cortical surface as a positive wave. On reaching the apical dendrites in the superficial layers, the electrical potential is reversed to become a negative wave on the cortical surface as repolarisation occurs.

Thus the VEP signal seems to originate from the massed activity of large numbers of cortical neurones. The individual impulse or spike potential cannot appear on the scalp electrodes due to the severe attenuation by the intervening dura, bone and skin which have a high electrical impedence. It is the result of pre- and postsynaptic graded potentials of cortical neurones with the participation of glial cells that are likely to generate the complex series of waves of the VEP.

3.5.2 The dipole theory of cortical generators

Vaughan (1982) using intracranial recordings, found that the fields within the active cortex changed with time as the distribution of inward and outward transmembrane current flowed, due to synaptic activity. As the microelectrode passed through the striate cortex from the surface to the deeper layers, the early negative components underwent an inversion in polarity. He suggested that this related to an initial superficial excitatory post-synaptic activation with a subsequent return of the current in deeper layers. Vaughan (1982) proposed that the origin of the varying positive and negative changes could be thought of as an intracortical dipole generator which varies in strength over time as the internal currents change in strength and location.

Wood (1982) reviewed the theoretical dipole model in localisation studies and showed that the mathematical analysis of the potential distributed over the surface of a homogenous spherical volume conductor arising from a dipole generator inside could be applied to the distribution of the VEP over the scalp. The cells generating the VEP are thought to be arranged in parallel with each other, perpendicular to the surface of the cortex and thus considered as a dipole sheet of generators. This sheet can be considered in terms of a single equivalent dipole located near the centre of the sheet. The simplest representation of the visual cortex is to consider the foveal representations on areas 17, 18 and 19 as represented by a single dipole radially arranged to the scalp, and the medial surface of the cortex (corresponding to the projections of the vertical octants of the field) as represented by an equivalent dipole arranged tangentially to the scalp (Biersdorf 1977).

The schematic dipole models of the visual cortex present a very simplified picture. The VEP amplitude is attenuated and the distribution made more diffuse by the layers of membrane, skull and scalp (Jeffreys and Axford 1972; Young 1981; Wood 1982). Babel et al. (1977) suggested that the scalp response is 1000 times less than that on the cortex. The distribution and the amplitude of the scalp recorded VEP are also dependent on the proximity of the generator site - the distribution over the scalp increasing and the amplitude decreasing with increasing distance of the generator with respect to the recording electrodes (Young 1981; Wood 1982). In addition,the cortex is very convoluted and different signals from different areas summate and partially cancel in a linear relationship (Vaughan 1982; Wood 1982). The individual variations in cortical architecture also cause variations in the scalp distribution of VEPs (Drasdo 1982; Vaughan 1982). The changes in the scalp distribution of apparent dipole sources during the course of the VEP can be interpreted as changes

in the location or orientation of a single source, or the combined effect of many sources with asynchronous activation.

3.5.3 Flash VEP to sinusoidal and Gaussian-noise modulated light

Spekreijse et al. (1977) proposed that the flash VEP was composed of several mechanisms distinguishable on the basis of different temporal frequencies. Studies were carried out using sinusoidally modulated light with the high frequency region being maximal around 45-60 Hz, the medium frequency around 12-25 Hz and the low frequency at around 10 Hz. Gaussian-noise modulated light can be presented at all frequencies simultaneously and analysed using cross-spectra or cross-correlation functions. Three parallel subsystems in the temporal frequency domain were proposed which seemed to deviate at the pre-ganglion cell level. These three subsystems were thought to reflect in man the X, Y and W systems studied in the cat (Spekreijse 1980). The three channels were suggested to be responsible for Ciganék's flash responses (Ciganék 1961), i.e. the high-frequency was thought to underlie the primary response originating in the striate cortex; the medium-frequency was associated with the secondary response originating in area 18 and possibly area 19; and finally the low-frequency after-discharge could not be attributed to a specific cortical region.

Theoretically since these flash responses differ in their frequency content, i.e. the primary response containing the highest and the after-discharge the lowest frequencies, they could be isolated in the frequency domain and studied separately. However, as Spekreijse (1980) pointed out, the relative strengths of the channels varies substantially among subjects and their frequency content (dynamics) also varies with stimulus parameters. Hence there is substantial scatter in the range of normal latency values.

3.5.4 Topographic distributions

Topographic VEP studies record from multiple arrays of electrodes such that individual variations in the localisation of the VEP can be observed and the relationship between various components. Numerous workers have employed this technique to identify the generators lying in different cortical regions reflecting the activity of the VEP components (Jeffreys 1971; Michael and Halliday 1971; Jeffreys and Axford 1972 and 1972a; Lesevre 1976 and 1982; Biersdorf 1977).

Numerous VEP stimulation techniques have been employed to reduce signal overlap and interactions. Half-field stimulation (of the vertical meridian) for instance,

confines the signal to one hemisphere and minimises cancellation of signals from the medial surfaces. Quadrant and octant stimulation minimises cancellations within the calcarine fissure (Halliday and Michael 1970; Jeffreys and Axford 1972). Foveal stimulation may be used to confine the signal to the posterior, relatively flat cortical surface (Drasdo 1980). Alternatively, the VEP stimulation can be modified to isolate components known to be enhanced and inhibit others (Drasdo 1981 and 1982). Jeffreys (1977) suggests another method of artificial enhancement involving bipolar recording from electrode positions corresponding to the maximum and minimum amplitudes of the component under investigation, or at positions where the component appears to reverse in polarity.

3.5.4.1 Flash VEP

Topographic studies of the flash VEP find that the P2 is maximal over the occipital electrodes (Groves and Eason 1967; Allison et al. 1977; Hobley 1988). The maximum amplitude of the P1 and P2 components evoked by a 10^{o} flash was localised over the occipital pole, whereas the N1, N2 and N3 components were more widespread over the posterior half of the head (Allison et al. 1977). However, Hobley (1988) reported the flash P1 had a more anterior distribution than the P2. In addition, Hobley (1988) reported a frontal negative which occurred around the same latency as the occipital P2.

Creutzfeldt and Kuhnt (1967) reported that the scalp distribution in neonates was confined to the occipital area, spreading across the scalp in later years. They suggested that this reflected a gradual maturation of non-specific projection and association fibres which are known to be myelinated later than specific sensory afferent fibres.

Bourne et al. (1971) investigated the flash VEP to foveal and parafoveal presentation. The response occurring between 10-120 ms was largest over the parietal area whereas that occurring between 190-310 ms was maximum around Oz. The group of potentials occurring over a large area of the occipital area, rotated suggesting a synchronous firing of neural populations. The positive potential, for foveal stimulation, was initially a gradual upward slope from the inion to anterior positions and rotated until a maximum was reached above the inion. Parafoveal stimulation demonstrated a large peak at the inion 20 ms later than that obtained from foveal presentation.

Nakamura and Biersdorf (1971) also reported that the flash VEP spread in a clockwise rotation. These workers also reported that the N1, P1 and N2 components

were maximal over the parietal region whereas the P2 and N3 were larger over the occipital region and showed more consistent localisation than the later components. A study of these early components evoked by half-field red light stimulation under light adapted conditions showed a maximum amplitude over the contralateral hemisphere to the stimulated field with a zero or reversed polarity potential over the ipsilateral hemisphere. The amplitude around the occipital region was zero, explaining the absence of the early components in the flash VEP when recorded from the midline Oz electrode. There was a reversal in polarity below the inion but this was not investigated further due to contamination of the signals from the neck muscles. Two possible dipole models were put forward to explain the results. Firstly a single dipole below the occipital pole, tangential to the surface or secondly, a surface positive in the contralateral hemisphere and surface negative in the ipsilateral hemisphere on half-field stimulation. The former would predict an origin in the primary visual cortex and the latter in the secondary visual cortex. The latter was the favoured model.

Biersdorf (1977) proposed a theoretical model of an equivalent dipole in a homogenous volume conductor, such as a sphere. A tangential dipole in relation to the back of the head, would produce a surface positive potential on one side of the head with a negative on the other side. A radial dipole would display a single potential maximal on the surface over the dipole with a reduction in amplitude to zero elsewhere. The former was thought to be responsible for the P1, N1 and N2 components of the flash VEP and the latter for the P2 and N3 components.

Remond (1969) showed that the early flash VEP components displayed maximum amplitudes just anterior to the inion, while the components occurring between 50-100 ms were largest a little farther forward and the after-discharge was maximal about 8 cm anterior to the inion. Harding Smith and Smith (1980) reported that the most prominent flash VEP components the P2 and N3 showed a maximum near the midline but no localising properties were found with half-field stimulation. They also found a conflicting localisation of the P2 component, occurring ipsilateral to the stimulating half-field in normal subjects but in the contralateral hemisphere in patients with homonymous hemianiopia.

Ciganék (1961) proposed that the primary response to the flash stimulus, was specifically evoked in the primary visual cortex (Brodman's area 17) based on its short latency and being relatively unaffected by increasing the presentation rate (up to 35 Hz) or whether the subject is alert or in various states of sleep. The secondary response was proposed to be generated by non-specific and diffuse pathways due to

its longer latency, disappearance at increasing the rate of stimuli (up to 10 c/sec) and being affected by sleep. Spekreijse et al. (1977) calculated that the primary response would theoretically arrive at about 35 ms, which corresponded to the latency of the primary response. These workers not only confirmed the striate origin of the primary response, but also proposed that the secondary response was generated in area 18 and 19 (extrastriate regions).

3.5.4.2 Pattern reversal VEP

Using a transverse row of electrodes, full-field pattern reversal VEPs display the P100 component maximum over the midline with a fairly symmetrical distribution over the two hemispheres (Leuders et al. 1980; Sphelmann 1985). There is a widely distributed negativity situated anteriorly with a maximum at Cz or Fz, which can be of the same or sometimes higher amplitude than the P100 recorded over the occiput. Leuders et al. (1980) consider this negativity to represent a far-field effect of a sagittally oriented dipole in the occipital cortex.

Halliday and Michael (Halliday and Michael 1970; Michael and Halliday 1971) observed that the polarity of the P100 was of maximum amplitude 5-7.5 cm above the inion over the contralateral hemisphere with half-field stimulation, with responses from the lower half-fields being bigger than from the upper half-field. They also demonstrated a polarity reversal, obtaining an N100 for the upper and a P100 for the lower half-field stimulation, but not with the horizontal left and right half-field stimulation. They concluded that the P100 originated from a surface positive dipole sheet in the extrastriate region of the visual cortex located inside the calcarine sulcus, where the neurones for the upper and lower half-fields would be inverted in relation to each other. These findings were later supported by Lehmann et al. (1982).

A striate origin for the P100 was considered unlikely since the octant stimulation did not show a distribution consistent with an origin in the cruciform arrangement of the striate cortex, nor was the amplitude maximum at the electrode over the occipital pole, within 2.5 cm above the inion (Halliday and Michael 1970; Michael and Halliday 1971). They concluded that the polarity reversal was due to the folding of the surface of the visual cortex such that an electrode above the inion would be facing opposite sides of the cortex representing the upper and lower fields. This was confirmed by recordings with mid-frontal reference which showed that the upper field became positive when recorded from electrodes situated below the inion. Using an ear reference electrode, the traces from these lower electrodes were relatively flat, indicating that the ear reference was also picking up the signal from

the upper field generator on the under surface of the cortex. An upper field VEP is expected to be of substantially smaller amplitude than the lower field VEP due to the larger distance between the generators and the recording electrodes. But this was not the case, indicating that the orientation of the generator sheet with respect to the recording electrode is more important than the actual distance between them. However, the lower field response predominates in the full field VEP recorded from electrodes above the inion as the prominent pattern reversal component is positive.

Barrett et al. (1976) investigating left and right half field stimulation of radius 7.9° found a clear positive component over the contralateral hemisphere, as expected from anatomical considerations. However, using a larger field of 16° radius and 50' checks, a surprising lateralisation of the response over the ipsilateral hemisphere occurred. Two explanations were put forward for this paradoxical ipsilateral lateralisation, either the signal was actually generated in the ipsilateral hemisphere or that the recording electrodes over the ipsilateral hemisphere were in fact ideally placed to record potentials arising from the medial surface of the contralateral hemisphere (fig. 3.6). The latter explanation was proved more plausible by recordings from patients who had hemispherectomy (Blumhardt et al. 1977). The VEP could still be recorded over the ipsilateral side, despite the absence of an occiput under these electrodes.

A more foveal field stimulus of 2° radius, producing lateralisation over the contralateral hemisphere was consistent with the cortical generators. Barrett et al. (1976) attributed this to the different orientation of the central field representation on the posterior-medial surface of the scalp. Harding, Smith and Smith (1980) confirmed that the field size was a crucial factor in the lateralisation effect. Using a transverse row of electrodes 10% above the inion referred to a mid-frontal electrode they showed that the ipsilateral response to half-fields of 14° radius became slightly contralateral when the radius of the field was reduced to 2.5°. However, reduction of the check size in a 14° radius half-field did not affect the ipsilateral lateralisation of the VEP. This was confirmed by studies using multiple electrode arrays (Lehmann and Skrandies 1979; Lehmann et al. 1982).

Using a transverse row of electrodes, the pattern reversal VEP shows a maximum over the midline electrode with a gradual reduction in amplitude over the ipsilateral electrodes. Bipolar linkage of these electrodes therefore gives the appearance of a contralateral localisation of the response since this is the point of steepest potential gradient (Cobb and Morton 1970; Spekreijse and Estevez 1972; Barrett et al. 1976). There will be little activity over the ipsilateral electrodes as they are each picking up

Figure 3.6. Schematic representation of the relationship between the cortical generator area for the left half field response and the scalp electrodes. The electrodes at the midline and ipsilateral to the stimulated half field are best placed to record the response from the posteromedial surface of the contralateral visual cortex. (From Barrett et al. 1976).

an evoked response of approximately equal amplitude. Blumhardt et al. (1977) have also shown that the transverse distribution of the full field VEP was equivalent to the addition of the distribution of the two half fields, i.e. maximum at the midline with a gradual reduction in amplitude either side.

The full-field responses of the pattern reversal VEP to checkerboard has been shown to approximate to the algebraic summation of the left and right half-field responses obtained and each originating from the contralateral hemisphere (Halliday et al. 1979; Halliday 1982). Half-field responses were composed of a NPN (N75, P100, N145) complex over the ipsilateral hemisphere to the visual field stimulated and an PNP (P75, N105, P135) complex over the contralateral hemisphere. The ipsilateral P100 component was found to depend on foveal stimulation, whilst the contralateral N105 was dependent on parafoveal stimulation. Therefore, the distribution of the pattern reversal P100 to half-field stimulation can be summarised by assuming that it is likely to be generated in the primary visual cortex (area 17). Topographic studies by several workers agree on the striate origin of the pattern reversal P100 component (Barrett et al. 1976; Leuders et al. 1980; Halliday 1982; Edwards and Drasdo 1987).

The topographic distributions of the flash and pattern VEPs show a wide distribution for the flash VEP, suggesting diffuse neural connections, whereas the pattern VEP is less variable and highly localised (Kinney 1977; Drasdo 1982). The topographic distribution of the pattern reversal VEP also shows that it is consistent with the retinopic organisation of the visual cortex (Halliday and Michael 1970; Jeffreys 1971). However there is still some way to go before agreement is reached on the specific generator sites of the VEP. A fuller understanding of this question will greatly advance the uses of the VEP in both visual research and clinical diagnosis.

3.5.5 Intracortical recordings

Ducati et al. (1988) studied the neural generators of the flash and pattern reversal VEPs in awake humans using intracortical and surface electrodes. They reported that the generators of the flash and pattern reversal VEP were entirely cortical. They found that not all the activity recorded by the intracortical electrodes could be picked up by the surface electrodes hence demonstrating an attenuation of the signal at the cortical surface. The flash response was thought to activate the entire retina and consequently the whole extension of the striate cortex. The peripheral retinal areas stimulated by the flash were thought to activate nerve fibres with a higher conduction velocity compared to the macular and paramacular regions. They also suggest the Y system was responsible for generating the flash response, but that for the earlier components such as the P1, may be attributable to the X system being

recordable at the occipital pole (the foveal projection area). The pattern reversal P100 component was thought to be generated in at the same cortical level as the flash VEP components but the P100 disappeared at more superficial levels than the flash VEP. The pattern reversal response was attributed to the macular and paramacular regions and generated mainly in the striate but also to some extent to the extrastriate regions of the occipital cortex.

Kraut et al. (1985) investigated the intracortical generators of the flash VEP in monkeys and extrapolated their findings to the human flash VEP. They concluded that the P1 (their P40) component was of subcortical origin, the N2 (their N70) was generated by the initial excitatory response within the lower lamina IVC, the P2 (their P100) was a manifestation of inhibitory processes within the thalamorecipient lamina IV, the N3 (their N130) reflected the subsequent input from stellate cells to supragranular pyramidal cells located above layer IV and the P3 (their P170) represented extrastriate activity. Kraut et al. (1985) further suggested from physiological studies of single neurones identified as X- or Y-like, that the diffuse flash being a featureless stimulus with only transient luminance changes would predominantly lead to activation of the magnocellular cortical projection to lamina IVCalpha. However, their results indicated that the flash exclusively activated the parvocellular thalamorecipient lamina (IVA and IVCbeta), without eliciting a response in lamina IVCalpha. Ducati et al. (1988) explained that this was due to the recordings being limited to the opercular striate cortex where the fovea is represented and not in other areas where the more peripheral retinal fields may be represented.

Brazier (1964) recorded flash VEPs in patients undergoing surgery for temporal lobe epilepsy, using intracellular electrodes placed in the hippocampus, the hippocampus gyrus (entorhinal cortex) and the amygdala. She reported that responses were obtained from the hippocampus and the hippocampus gyrus but not from the amygdala. Three pathways or routes were proposed whereby visual information may be processed by these structures: firstly a route through specific thalamic relays to selected cortical areas informing them that a stimulus had been received and identifying its sensory modality, without serving perception or storage of the stimulus; secondly, a non-specific route through the mid-line thalamus to cortical areas not concerned with identification of the originating sense organ, only in the recognition and awareness of the incoming stimuli; and finally, centres in the hippocampus and related areas concerned with storage and the later retrieval of information coming to the brain. This third pathway may serve as a possible link

into the delayed flash response and Alzheimer's disease as this area is severely affected in the disease and has a substantial cholinergic input.

3.5.6 Neuroimaging studies

Positron emission tomography (PET) studies have been used to study regional blood flow and regional glucose cerebral metabolism of neuronal structures. There is a direct relationship between increased functional activity in brain regions and increased regional cerebral blood flow (rCBF) and glucose metabolism in that same area, hence these can be used to determine areas activated by VEPs. Studies by Celesia and co-workers (Celesia et al. 1982; Celesia et al. 1984; Celesia 1986) investigated the relationship between PET scans and VEPs to flash and pattern reversal (64' checks) stimuli in patients with homonymous hemianopia. The patients with normal VEPs also had normal rCBF in the occipital lobes. Patients with abnormal VEPs displayed abnormal PET scans, and macular splitting was demonstrated by perimetry in two cases. One subject who displayed no metabolic change in the right occipital cortex, showed that the flash response was distributed over the left hemisphere whereas the pattern reversal responses were "paradoxically" distributed over the right hemisphere. The workers concluded that the different characteristics and topography of the flash and pattern reversal VEPs indicated that they were generated in different although overlapping neuronal pools. They further suggested that VEPs were a result of complex interactions of electrical fields rather than the sole volume transmission of the striate dipoles.

3.6 Clinical applications of the VEP

The VEP serves as a valuable tool in research providing clues into the functioning of not only the visual system, but also on how the brain works. However, VEP studies play a greater role as a diagnostic aid in many clinical conditions. The technique can assess the integrity of the visual pathway from the retinal level through the optic nerves and tract to the visual cortex. The combined flash and pattern VEP is usually used since they provide complementary information.

Harding, Crews and Good (1980) provide some examples of the use of VEPs in neuro-ophthalmic diseases. These workers used the flash VEP in conjunction with the pattern reversal VEP. Patients with acute optic neuritis displayed a delayed flash P2 and pattern reversal P100 components, the latter being more marked especially with small checks. Patients with optic trauma generally displayed a reduced VEP recorded from the affected eye compared to that from the unaffected eye. Patients with ischaemic optic neuropathy, which usually affects the older generation, showed

a markedly reduced and delayed flash and pattern reversal VEPs from the affected eyes. A triphasic P-N-P type of response to the flash was usually seen in those with temporal arteritis. A similar finding was obtained in patients with hereditary optic atrophy, both the dominant form and Leber's type of inheritance.

A most common diagnostic use of the VEP has been with multiple sclerosis (Halliday et al. 1973; Asselman et al. 1975; Matthews et al. 1977; Adachi-Usami 1991; Holder 1991). The pattern reversal P100 component is usually delayed co-existing with a flash P2 of normal latency (Wright et al. 1984). The rate of this delayed P100 is high in patients with a history of retrobulbar neuritis, optic pallor, reduced visual acuity and visual field defects, but a delayed P100 also exists in those without and history or clinical findings suggesting the involvement of the visual system. The VEP can also distinguish between the spinal form of multiple sclerosis and transverse myelitis of other causes (Asselman et al. 1975; Adachi-Usami 1991; Holder 1991). The disease involves formation of plaques most frequently in the optic nerves, the spinal cord, the medulla and the periventricular white matter. This demyelination hence leads to reduced conduction velocity, conduction block, and an impaired ability to conduct fast trains of stimuli due to an increased refractory period (McDonald 1977).

The latency and amplitude of the flash P2 component can be used to predict the visual outcome of patients with major eye injuries (Crews et al. 1978) such as cataract (Thompson and Harding 1978). Thompson and Harding (1978) graded the response from the normal eye compared to the affected eye, such that a response of normal amplitude was called a Grade I response, a reduction in amplitude less than 50% was a Grade II response and that greater than 50% was a Grade III response. The Grade I response resulted in a post-operative acuity of 6/12 or better and Grade III resulted 6/24 or worse. Crews et al. (1978) graded the latency of the flash P2 component in patients with major eye injuries. A Grade I response was that of normal latency and amplitude, Grade II response was that with a reduction in amplitude less than 50% but no marked delay, a Grade III response was that with an amplitude reduction greater than 50% and a latency delay greater than 30 ms from normal limits and a Grade IV response was an absent or greatly reduced and delayed even at the highest intensity of the flash stimulus. These workers applied a similar system to grade ERGs, and improving the diagnostic value of the outcome when used in combination.

Obviously there are a great deal of clinical situations both ophthalmological and neurological where the VEP may be applied and a comprehensive account cannot be

given as it is beyond the scope of this thesis. However, Alzheimer's disease is of great importance to the investigations to be reported in following chapters, thus the next chapter will be devoted to the major findings of this condition.

3.7 Summary

The flash and pattern reversal VEP provide complementary information about the visual pathway. Both types of stimuli are greatly influenced by parameters such as age and sex, stimulus intensity, spatial content, etc., and thus used in numerous neurological and ophthalmological conditions. Studies on the generators of the flash and pattern reversal VEP suggest that they have different cortical generators. The flash P1 is thought to be generated in the striate cortex whereas the major P2 component is thought to be generated in extrastriate regions of the visual cortex. The pattern reversal VEP is thought to reflect the integrity of the geniculostriate pathway with the striate cortex being the generator of the P100 component.

CHAPTER 4

Alzheimer's Disease

4.1 Introduction

The proposed studies in this report include investigation of agents developed for use in the treatment of Alzheimer's disease (AD). Hence there is a necessity to describe the background of the disease. This chapter aims to provide a brief account of AD, emphasising areas such as the pathology occurring along visual pathway and the current treatment strategies.

Dementia is the primary symptom of AD. Dementia (meaning literally, "from the mind"- madness), has been defined by Cummings and Benson (1983) as "an acquired syndrome of intellectual impairment produced by brain dysfunction". Katzman (1986) referred to dementia as a syndrome characterised by intellectual deterioration occurring in an adult which is severe enough to interfere with occupational or social performance. However, progressive deterioration of intellectual activity leading to dementia is common with advancing years although not inevitable. Its occurrence however, is a major problem in modern societies with ageing populations, due to increased longevity and a low birth rate. There are over 70 causes of dementia and AD is but one of them, yet it alone can account for over 80% of all cases of dementia (particularly of the senile type), hence its importance (Katzman et al. 1988). The term Alzheimer's disease is applied generally to both presenile dementia, (with age of onset between 45-65 years), and an almost identical condition occurring in those aged over 65 years, commonly referred to as senile dementia of the Alzheimer type (SDAT), (Katzman 1986).

4.2 Incidence

In 1970, there were an estimated 291 million people over the age of 60, of these 26 million were over 80 years of age (Besson 1983). The corresponding figures for the end of the century are expected to be approximately 600 million and 58 million respectively, with 20% or more of the 80 year olds, being demented (Besson 1983). AD is the commonest of the dementias with almost 0.5 million people in Britain and over 2 million in the U.S.A. being affected (Wisniewski and Iqbal 1980; Corkin 1981). Other dementias in old age include those caused by vascular disease (multi-infarct dementia) which account for 15%, mixed forms which account for 22%, and a small group of rarer disorders some of which may be reversible e.g. normal pressure hydrocephalus, (Besson 1983).

4.3 Symptoms

AD begins insidiously, advances in an uneven manner and limits life expectancy (Corkin 1981; Katzman 1986; Ashton 1987). The disease is characterised by marked deficits in cognitive function including memory (which is more prominent in AD than in most dementias), language use, praxis, the ability to learn necessary skills, solve problems, think abstractly, make judgements, and other complex sensorimotor and perceptual capacities. The personality characteristics can be maintained or even intensified in some patients, but disturbed in others. A common feature tends to be social avoidance and fearfulness. However, in most patients, social behaviour remains appropriate until the late stages of the disease. Paranoia and delusions sometimes occur together with irritability, agitation, and even verbal and physical aggression towards family and friends, especially on awareness by the patients on the increasing loss of control of their environment. AD is a progressive disease which can be more distressing to the carer than the sufferer, (Corkin 1981; Katzman 1986; Ashton 1987).

4.4 Neuropathology

The pathophysiological aspects of the AD have been based on biochemical and morphologic studies carried out on brains at autopsy. The major morphological changes in the brain include cortical atrophy, loss of neurones, and the presence of neurofibrillary tangles (NFT) and neuritic or senile plaques (SP), (Mann 1985). These changes occur in the majority of AD patients, although the extent of the changes may vary in all cases.

4.4.1 Microscopic changes

The brain of the Alzheimer patient is characterised by the presence of characteristic cellular changes. NFTs are abnormal neurones with the cell body filled with masses of paired helical filaments. SPs are a focal collection of degenerating nerve endings, dendritic and axonal, surrounding a core of "amyloid" protein. Other pathological changes include the presence of cerebrovascular amyloid, which is found in blood vessels in the meninges, the cerebral cortex and the hippocampus in many cases (Vinters and Gilbert 1983); granulovacuolar bodies, which often occur as inclusions in the hippocampal neurones (Ball et al. 1983); and Hirano bodies, elongated eosinophilic structures that are occasionally found in the hippocampus (Price 1986).

These pathological changes are not exclusive to AD. For example, tangles (usually in a more diffuse pattern than in AD) exist in disorders such as dementia pugilistica (or

"punch drunk" syndrome), postencephalitic Parkinson's disease, and the Guam-Parkinson-dementia complex, a condition associated with abnormal mineral intake. In rare cases, elderly subjects with no apparent clinical cognitive impairment have shown considerable brain atrophy which exceeds that found in some demented patients (Tomlinson 1980). However, in the majority of apparently normal elderly cases, there is only a small number of plaques and tangles (Davison 1986). Most of the morphological changes occur in the outer cortical layers, in the depths of sulci and tend to be concentrated in the anterior temporal lobes, particularly the amygdaloid nucleus and overlying cortex and in the hippocampus and hippocampal gyrus, compared to other areas. Thus the difference between the elderly normal and AD subjects (especially of the presenile type) becomes largely quantitative (Tomlinson 1980).

However, there are two main underlying features of pathological changes in AD; firstly, they occur in a characteristic pattern and distribution, and secondly, the submicroscopic changes that occur (both intracellularly and extracellularly) involve altered fibrous proteins, some of which are components of the normal cytoskeleton (Katzman 1986).

4.4.2 Brain areas affected by pathology

The overall pattern of pathological changes in the AD brain is relatively specific, although variations between patients can occur (Katzman 1986a). There is an overall weight loss attributable to shrinkage of cerebral white matter in the gyri, widening of the sulci and ventricular dilatation which may be a compensatory effect to the cortical atrophy (Mann 1985). The loss occurs on both sides of the brain but is not necessarily symmetrical (Corkin 1981). Greatest atrophy occurs in the gyri of the cortical association areas with relative sparing of the primary motor, somatosensory and visual cortices (Foster et al. 1983; Terry and Katzman 1983; Pearson et al. 1985). Atrophy tends to be common in the cerebral cortex, with frontal and particularly the temporal lobes being most involved (Hubbard and Anderson 1981; Mann et al. 1987). Other affected areas include the hippocampus, amygdala, entorhinal cortex, locus coeruleus, nucleus basalis, and the olfactory nucleus (Perry 1984; Terry and Katzman 1985; Ashton 1987).

Cell loss seems to be confined to the large pyramidal cells with a cross-sectional area greater than 90 μm^2, with relative sparing of smaller cells (between 40-90 μm^2 in area) the most numerous cells in the cortex (Appel et al. 1988), although there may be an increase in glia in some affected areas (gliosis) and some astrocytes can be fibrous. For example, in the association cortex, there is almost 50% loss of large pyramidal

cells, with smaller neurones remaining relatively preserved. In the entorhinal cortex, the larger pyramidal neurones of layer II projecting to the hippocampus via the perforant path, are lost whereas the adjacent neurones remain relatively intact. In the hippocampus, pyramidal cells of the subiculum and CA1-3 areas are most affected (Brun 1983).

The evidence that the severest pathological changes occur in the hippocampal formation and the adjoining entorhinal cortex (Brun 1983), together with evidence that the disease may spread along cortical connecting fibres (Pearson et al. 1985), has led to suggestions that the degeneration of cortical areas rather than subcortical nuclei may be of primary importance in the disease process.

4.5 Diagnosis

An assured diagnosis of AD has proved to be difficult as there are over 70 possible causes of dementia (Katzman et al. 1988). Misdiagnosis of AD can lead to inclusion in AD research protocols of individuals with causes of dementia other than AD, resulting in inconclusive studies and erroneous research outcomes, as well as to mismanagement and inappropriate or inadequate treatment of the patient. Therefore, the accuracy of the diagnosis of AD has been greatly emphasised (Benson 1987; Katzman et al. 1988).

In 1987, the Medical Research Council (MRC 1987), published a report aimed at workers in Britain, recommending the minimum data to be collected to aid comparison between research studies and be helpful in the application of the accepted diagnostic schemes as the DSM-III (American Psychiatric Association 1980) and National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria (McKhann et al. 1984). However, there are several limitations associated with these criteria (Jorm and Henderson 1985; Katzman et al. 1988), but these still provide the best available, universally accepted guidelines on diagnosing AD.

Diagnosing AD maybe difficult, due to the heterogeneous nature of the disease (Mayeux et al. 1985; Neary et al. 1986). The disease is insidious in onset, therefore initial suspicion of presence of AD depends on the sensitivity of the family and friends of the patient and thus early cases are rare in research. AD is a progressive disease therefore the symptoms and signs change slowly but continuously and there is also a great deal of variation in presentation with different prime features. There can be approximately 7 stages (Cummings and Benson 1983) and sometimes the disorder cannot be defined by the DSM-III or NINCDS / ADRDA criteria alone (Jorm and

Henderson 1985; Katzman et al. 1988). AD is more common in the elderly, who almost always have some other medical disorder that may compromise mental efficiency so that a complex clinical picture is often present. Therefore, rigidly applied exclusion criterias would reject many cases of primary AD. Furthermore, end stages of AD is similar to those of many other types of dementia.

The diagnosis is made mainly through differential diagnosis, with a high error rate, especially during the early stages of the disease where the cognitive and neurological deficits are slight and pseudodementia is a problem (Katzman et al. 1988). Crude confirmation can be made from the many short tests in use e.g. Mini-Mental State Examination (Folstein et al. 1975), and the Blessed examination (Blessed et al. 1968). Each has significant limitations, but useful in confirming and monitoring progression of dementia. These psychometric tests are used in conjunction with numerous laboratory tests and brain imaging (CT, PET and MRI) techniques to exclude other causes of dementia. Brain biopsy is a means of positive confirmation, but it is a mutilating surgical procedure with a risk of significant residuals and high "inconclusive" rate and thus not generally used as an experimental procedure (Neary et al. 1986a). Although a diagnosis may be based upon clinical criteria, the definitive diagnosis has to be a pathologic one. Hence autopsy is regarded by most as the "gold standard" for confirmation of AD, but this is of no benefit to the patient. In addition, some patients can have a clinical picture without the pathological changes (Earnest et al. 1979; Hagnell et al. 1983), while others may have pathological changes without the dementia (Newton 1948; Jacoby and Levy 1980).

Early detection of the disease is vital if therapeutic gains are to be made. Potential therapies need to be introduced when the brain is relatively intact and the dysfunction capable of being reversed or even arrested. Quantitative assessment methods sensitive to small changes in brain function are needed to evaluate drug treatments. Harding and colleagues have reported a delay in the flash VEP P2 component co-existing with a pattern reversal VEP P100 component of normal latency (Doggett et al. 1981; Harding et al. 1981, 1984, 1985; Wright et al. 1984a, 1986). These workers proposed that this was a sufficiently unusual finding to be of diagnostic use. This will be discussed in detail in section 4.10.7.

4.6 Aetiology

The aetiology of the disease remains uncertain. Theories include a slow-virus, special infective proteins (prions), deficient trophic factors, abnormal oxidation and peroxidation, genetic links (defect on chromosome 21), trace elements (especially

aluminium) and currently the involvement of the olfactory bulb (for reviews see Mann 1985; Wurtman 1985; De Estable-Puig et al. 1986; Price 1986).

A genetic link is the most favoured and has been suggested by observations of increased incidence of AD among relatives of patients (Larsson et al. 1963; Cook et al. 1979; Goudsmit et al. 1981). However, studies of monozygotic twins who differ in the onset of the disease, suggest that although there may be a genetic predisposition, environmental or metabolic factors may have a role in determining the onset of the disorder (Cook et al. 1981). But in spite of a great number of studies, no consistent pattern of genetic transmission has emerged, probably due to the heterogeneity in the aetiology and manifestation of the disease. Thus it has been proposed that there may two forms of AD, a familial form accounting for about a third of all cases and a sporadic form constituting the rest (De Estable-Puig et al. 1986). These familial cases also tend to comprise individuals with an earlier onset, more rapid progression and more severe structural and chemical pathologies (Heston et al. 1981). Additional evidence of a genetic link comes from patients with Down's syndrome, who over the age of 40 years almost invariably have plaques and tangles and cholinergic deficits in the neocortex and hippocampus, similarly to patients with AD (Yates et al. 1980). Therefore, chromosomal abnormalities (especially on chromosome 21), have been suggested, but the findings tend to be inconsistent (Katzman 1984).

The role of an infective agent has been suggested, since AD shares some pathological characteristics with spongiform encephalopathies such as Creutzfeldt-Jakob disease and Kuru (Goudsmit et al. 1980). Such an agent is thought to reach the brain via the olfactory pathway and spread to the amygdala, hippocampus and associated cortical regions. However, there is no clear evidence for the transmission of AD between humans or between humans and animals.

In some studies there are elevated amounts of aluminium in the brains of AD patients as well as in dialysis patients presenting progressive encephalopathy (De Estable-Puig et al. 1986). Aluminosilicates have also been found in the centre of the cores of senile plaques and thus hypothesized that it may play a role in the formation of plaques. But it is not clear if the aluminium concentrates in neurones as a primary process or results because of the damaged cells accumulate aluminium (Perl and Brody 1980).

AD has been associated with a lack of a trophic factor normally manufactured by cells in hippocampal or other cortical tissues. Evidence comes from injection of nerve growth factor into the cortex being transported retrogradely to neurones in the basal forebrain and increasing choline acetyltranferase activity in that region (Ojika and Appel 1983). Other theories include involvement of a serum protein, presumed to be

manufactured outside the CNS, that damages cerebral endothelial cells, enters the brain and leads to the formation of NFT and SP (Price 1986).

Overall, it seems likely that dementia of the Alzheimer type could result from any one, or a combination of the causes mentioned. The lack of knowledge about the aetiology of the disease is reflected in the apparent failure of effective treatment strategies.

4.7 Neurotransmitter changes associated with AD

There are at least four ascending projection systems affected by the disease: the cholinergic system arising from the basal forebrain, the noradrenergic system from the locus coeruleus; the serotoninergic system from the dorsal tegmentum; and the reticular projection from the paramedian reticular nucleus (Jones 1986). In each of the ascending systems there is often loss of some neurones and tangles may be found in some of the remaining cell bodies. The neurochemical changes in AD are presumed to result from the alterations in morphology.

The major neurotransmitter change reported in AD is a 40-90% decrease in the acetylcholine (ACh) synthesising enzyme choline acetyltransferase (CAT) in the cerebral cortex and hippocampus (Wurtman 1985). This alteration appears to be restricted to presynaptic terminals, with the loss of muscarinic M2 (Mash et al. 1985) and nicotinic (Whitehouse et al. 1986) receptors while the postsynaptic M1 muscarinic receptors remain relatively intact. There is also a substantial degeneration of neurones in the nucleus basalis of Meynert, (fig. 4.1), which provides the major cholinergic innervation of the cortex (Whitehouse et al. 1981). In addition, tangles have been observed in these neurones and cholinergic neurites in senile plaques (Mann 1985). However, there are also reports that the cortical pathology can occur without either severe cell loss in the nucleus basalis (Pearson et al. 1983) or significantly reduced CAT activity in this region (Henke and Lang 1983).

The noradrenergic innervation, dopaminergic innervation from the ventral tegmentum, and serotonergic innervation are also compromised in AD (Katzman 1986; Appel et al. 1988). There is evidence of atrophy and neuronal loss from the locus coeruleus (especially in dorsal areas projecting to the cerebral cortex) and loss of pigmented neurones from the dorsal motor vagus. The surviving neurones in the locus coeruleus have been shown to accumulate NFTs (Mann 1985). Similarly to the cholinergic system, postsynaptic adrenergic receptors tend to be relatively stable in AD (Cowburn et al. 1989). For the dopaminergic system, the substantia nigra which innervates the basal ganglia is relatively spared, however, the ventral tegmentum which is biochemically and developmentally similar to the substantia nigra, but which

Figure 4.1 The major cholinergic pathways implicated in Alzheimer's disease. The cholinergic neuronal cell bodies of the basal forebrain located in the nucleus basalis of Meynert, the diagonal band of Broca, and the medial septal nucleus send axons that innervate the entire cortex including the frontal, parietal, and occipital cortex, as well as the hippocampal formation. (From Wurtman 1985).

innervates the cortex and amygdala, shows neuronal loss and NFT accumulation (Mann 1985).

There are reports that degeneration of the locus coeruleus occurs in presenile cases of AD, but it remains relatively intact in the later onset cases of AD (Coyle et al. 1983). These may represent a subset of AD, in which the disease seems to progress at a faster rate with greater pathology, compared to the late onset cases (Davison 1982; Roth 1986).

Reduced concentrations of serotonin and its metabolites have been demonstrated in cortical areas, the hippocampus and the basal ganglia (Price 1986). There is neuronal loss in the raphe nuclei and presence of NFTs in the surviving neurones, although the degree of pathology is not as severe as in the nucleus basalis or the locus coeruleus (Mann 1985). Both presynaptic and postsynaptic receptors are affected (Bowen et al. 1983) and thought to reflect denervation of intrinsic neurones while loss of some postsynaptic neurones may be due to degeneration of cholinergic terminals from the nucleus basalis, on which these receptors are thought to exist (Quirion et al. 1985).

GABA, a major inhibitory neurotransmitter of the cerebral cortex, may also be affected in AD (Cowburn et al. 1989). In general, GABA levels tend to remain relatively stable in cortical and subcortical areas in AD, but reduced levels occur in the temporal cortex (Rossor et al. 1982). However, the reductions of GABA levels seen in AD have not been related to the severity of the disease. Hardy et al. (1987) have demonstrated a considerable loss of cortical and hippocampal GABAergic terminals in AD with relative sparing in subcortical areas. There seems to be preferential loss in the $GABA_B$ receptor subtype which may reflect the degeneration of glutamatergic, noradrenergic and serotonergic terminals on which these receptors exist (Chu et al. 1987).

The large pyramidal neurones affected in AD are thought to utilize glutamate or a related excitatory amino acid/small peptide as their neurotransmitter (Fonnum 1984). There seems to be a loss in cortical and hippocampal glutaminergic terminals (Cowburn et al. 1989). Studies on the integrity of glutamate receptors have largely concentrated on the NMDA subtype and has been reported to be both unaffected (Cowburn et al. 1989) and reduced in cortical and hippocampal areas (Greenamyre et al. 1987). Studies on the kainate receptor subtype report unchanged levels in the caudate nucleus which is thought to reflect relative sparing of intrinsic neurones in this area (Cowburn et al. 1989). But there is an increased density of these receptors in the hippocampus (Geddes et al. 1985) representing axonal sprouting in the dentate gyrus

in response to a loss of the perforant path input. Smith et al. (1985) have also reported a correlation between glutamate and cognitive impairment.

Neuropeptides such as somatostatin, corticotrophin-releasing factor (CRF), substance P, VIP, neuropeptide Y, neurotensin, and CCK account for less than 10% of cortical interneurones (Jones 1986). However, of these somatostatin dysfunction is the most consistently reported. NFTs have been observed in somatostatin neurones and somatostatin-like immunoreactivity shown in plaques (Cowburn et al. 1989). The most affected areas tend to be the cortical regions with the temporal lobe being most severely affected, but the subcortical areas remaining relatively intact. Somatostatin receptors have been reported by Beal et al. (1985) to be reduced in the frontal and temporal cortices and hippocampus, but not by Whitford et al. (1988). CRF levels have been reported to be reduced, although the CRF receptor levels are increased in AD (De Souza et al. 1986). CCK-, neurotensin- and VIP- containing neurones are all thought to be generally preserved in AD (Cowburn et al. 1989).

4.8 The "cholinergic hypothesis" and therapeutic considerations

4.8.1 The cholinergic hypothesis

The cholinergic hypothesis of memory dysfunction proposes that the cognitive (in particular memory) deficits in ageing are directly attributable to a decline in the integrity of central cholinergic function and reaches its most pronounced degree in AD (Bartus et al. 1982; Coyle et al. 1983; Dillman 1990). There are three main lines of evidence to support this hypothesis. Firstly, a correlation exists between cognitive deficits and postmortem decline in cortical CAT levels (Perry et al. 1978; Bartus et al. 1982; Coyle et al. 1983). Secondly, the deficits observed in the elderly subjects can also been mimicked by anticholinergic agents such as scopolamine (Bartus et al. 1982; Coyle et al. 1983). Finally, there is sporadic evidence that the functional impairments in ageing or dementia may be susceptible to alleviation by cholinomimetic therapy (Bartus et al. 1982; Coyle et al. 1983; Sahakian et al. 1989).

Studies show that scopolamine produces a transient amnesic state which can be reversed by administration of physostigmine (an acetylcholinesterase inhibitor), or arecoline (a muscarinic agonist), but not by the catecholaminergic drug amphetamine, an attention-enhancing catecholamine agent (Drachman 1977). Therefore the effects of scopolamine are unlikely to be related to its more general effects on arousal, attention, or similar sedative-like properties. Similar analogous blockade of dopamine or ß-adrenergic receptors have also resulted in some cognitive dysfunction related to memory (Bartus et al. 1982). However, the anticholinergic effects tend to produce

deficits most closely mimicking the natural age-related memory impairments. Nonetheless, trials of L-dopa, a dopamine precursor (Adolfsson et al. 1982), memantine, a dopamine agonist (Fleischhacker et al. 1986) and bromocriptine, another dopamine agonist (Phuapradit et al. 1978) have been conducted in AD patients, but have failed to demonstrate beneficial effects on either memory or cognition.

The effect of cholinergic agents on memory also tends to be specific. The drugs influence the subject's ability to store new information in long-term memory, with minimal effects on retrieval of information from long-term memory and no effect on immediate memory processes. This learning deficit is a frequently present in AD and is sensitive to cholinergic manipulation (Drachman 1977; Sitaram et al. 1978).

In summary, although ACh is not the only neurotransmitter system altered in AD, it is however the one found to be consistently decreased (Rosenberg et al. 1983). Together with the effects of cholinomimetics in enhancing learning and anticholinergics in precipitating a transient AD-like dementia, provides a sound rationale for the use of cholinomimetics as therapeutic agents. Treatment strategies in AD for ACh restoration have been largely based on drug therapy and/or neural transplantation.

4.8.2 Neural transplantation

Studies involving neural transplantation have been limited to animals, especially rats. However, there is some evidence that cholinergic-rich grafts can reverse some of the deficits produced by lesions denervating the neocortex or hippocampus of their cholinergic inputs (Dunnett 1991). These experimental lesions tend to be unspecific, thus only incomplete recovery is produced. As a result, the functional deficits in AD is thought only in part be attributable to cholinergic function and cannot explain the profound deficits observed in the disease. There are also suggestions that memory is not directly mediated by the ascending cholinergic system, but that it plays a role in modulating normal activity in the neocortex and hippocampus where mnemonic function is subserved (Dunnett 1991; Fibiger 1991). Dunnett (1991) further suggests that since AD involves a profound degeneration of intrinsic neurones as well as atrophy of their subcortical inputs, consequently, although the cholinergic grafts may restore cholinergic dysfunction, this will be without effect if the functional impairments are essentially the consequence of cortical degeneration, which seems likely. In addition, the neuropathological changes in AD are widespread, making transplantation seem unlikely to be of therapeutic use.

4.8.3 Drug therapy

The deficit in the cholinergic system in AD and its involvement of cholinergic systems in learning and memory has led to the hypothesis that drugs which increase cholinergic transmission should be therapeutically useful in the treatment of memory disorders, (Corkin 1981). In order to appreciate how a drug may be developed to restore deficient cholinergic transmission, and to act only where such restoration is needed, the life cycle of ACh has been illustrated (fig. 4.2).

Four strategies, are available to improve function in the cholinergic system (Drachman 1983; Growdon and Wurtman 1983):

1. ACh precursors: Choline and lecithin (phosphoditylcholine) increase plasma choline levels in humans. However, orally administered choline is largely ineffective and produces undesirable side-effects, and lecithin (a natural dietary source of choline) has also failed to demonstrate a consistent benefit (Rosenberg et al. 1983; Mann 1985).

2. Acetylcholinesterase inhibitors: Physostigmine is a centrally active acetylcholin-esterase inhibitor (AChEi) which prevents the enzymatic hydrolysis of synaptically released ACh (fig. 4.2), thereby increasing the ACh concentrations at the cholinergic synapse. Young adults given a very restricted range of single doses, have shown moderate improvement on cognitive tests (Sitaram et al. 1978; Bartus et al. 1982; Thal et al. 1983). However, doses outside the range produce either no change in performance or marked impairment. In addition, this optimal acute dose varies dramatically among elderly subjects. Physostigmine unfortunately also has the disadvantage of a very short half-life and adverse side-effects.

Other AChEis include di-isofluorphosphate (DFP) and tetrahydro-aminoacridine (THA). DFP is a noncompetitive AChEi with a longer duration of action than physostigmine, but again has potentially toxic side-effects. THA has a half-life intermediate between physostigmine and DFP, but its effects have not been consistent (Rosenberg et al. 1983).

Combination of precursor-loading with an AChEi have also been studied in response to the poor results with the drugs alone. Generally, the use of physostigmine with lecithin have produced better results when compared to lecithin alone (Peters and Levin 1982). Oral lecithin in combination with THA have also produced better results than treatment with either drug alone (Kaye et al. 1982).

Figure 4.2 Schematic representation of a cholinergic synapse. Choline (1) delivered to the synaptic cleft by blood enters a presynaptic cholinergic terminal, where CAT combines it with acetyl coenzyme A (2) to form acetylcholine (ACh) and stored into vessicles in the nerve terminal (3). Arrival of a nerve impulse depolarises the presynaptic neurone and ACh is released (4). ACh can either diffuse across the synaptic cleft to activate muscarinic M2 receptors (the predominate receptor in the brain) on the postsynaptic neurone, thereby transmitting the signal generated by the nerve impulse (5),or it can interact with the presynaptic muscarinic M1 receptor (6), thereby modulating the further release of ACh via negative feedback loop. Alternatively ACh can be broken down by acetylcholinesterase (AChE), yielding choline (7), which is taken up by the terminal (8) or carried off in the blood (9). In addition, the breakdown of phosphotidylcholine (lecithin), a constituent of cell membranes, also contributes to the choline supply (10). (From Wurtman 1985).

3. ACh receptor agonists: Arecoline a muscarinic agonist, has been used experimentally in young subjects and demonstrated a significant improvement in ability to recall recently learned verbal material (Sitaram et al. 1978; Bartus et al. 1982). However, as with physostigmine, arecoline has adverse side-effects, a short half-life and narrow effective dose range.

4. General metabolic enhancers (Nootropics): Nootropil or Piracetam (2-oxo-pyrrolidine acetamide), a GABA derivative, enhances oxidative metabolism and increases the release of ACh, particularly in the hippocampus. Cognitive functioning has been enhanced in young normals, elderly normals, and moderately impaired elderly, but no positive effects have been seen in severely demented patients (Rosenberg et al. 1983; Schneck 1983). Piracetam in combination with choline, produces a sharp improvement of retention in animals and AD patients, but further work is needed (Schneck 1983).

Apart from enhancing cholinergic function in AD, other approaches to drug therapy have also been tested. For instance, a variety of neuropeptides have been shown to have cognitive effects in humans, probably through their modulatory effects on the other neurotransmitter systems (Rosenberg et al. 1983). However, there is only weak positive effects reported related to attention, visual memory, or reduction in fatigue (Ferris 1983).

A variety of vasodilators have also been tried (Yesavage 1983). These have been reported to improve alertness, depression, anxiety, confusion and general severity of dementia. However, the magnitude of these effects have been small and no objective evidence for improvement in cognitive functioning has been reported (Rosenberg et al. 1983; Yesavage 1983).

Psychostimulants increase motor activity, reduce fatigue, and increase levels of concentration and alertness. These compounds increase central adrenergic activity and have enhanced performance on memory or learning tasks in animal (Prien 1983). Psychostimulants have been effective in improving withdrawn and apathetic senile behaviour and with their "mood elevating" properties have weak antidepressive action, but they have not yet demonstrated consistent positive cognitive improvement (Prien 1983).

Table 4.1 provides some examples of clinical trials investigating the effects of various agents in the treatment of AD. The table illustrates the great variety in the types of drug treatment examined, yet there is still no effective therapy.

Table 4.1 Summary of the results of a selection of clinical trials of drugs for treatment of AD.

Author	Drug and doses	Duration	Procedure	Patients	Major findings
Adolfsson et al. (1982)	*L-DOPA Placebo, "low-" and "high-" doses	10 Weeks	Open	37 AD	No objective clinical improvement.
Ahlin et al. (1991)	*THA (oral) (tritated doses) 75-150 mg/day	9 Weeks	Double-blind crossover	15 AD	Increased levels of ACh and monoamine metabolites in the CSF following THA. Slight improvement in cognitive function, but only in those with elevated liver enzymes.
Alhainen et al. (1991)	*THA (oral) 100 mg/day	4 Weeks	Open	18 AD	Identified 6/18 as "responders" who showed significant improvement in MMSE scores, orientation, attention and language scores. Word (long- and short-term) recall improved. EEG showed an increase in absolute alpha power and alpha-theta ratio.
Bajada (1982)	Combined Choline 1 g/4 hrs Physostigmine 0.4 mg/4 hrs	5 Weeks	Double-blind	6 AD (Moderate to severe)	No improvement in cognitive tests.
Beller et al. (1985)	Physostigmine (oral) 0.5, 1.0 and 2.0 mg/ day	8 Days	Double-blind crossover	8 AD	Dose-related improvement in objective short-term verbal memory tests with better scores on 2.0 mg/day.
Beller et al. (1988)	Physostigmine (oral)	1-3/4 years	Open	5 AD	no overall improvemnet in short-term verbal performance. 1 Patient

					deteriorated and was excluded after 1 year.
Blass et al. (1988)	*Thiamine (oral) 3 g/day	3 Months	Double-blind crossover	11 AD	Global rating by MMSE was higher than placebo. No improvement in subjective behavioural rating and clinical state.
Chase et al. (1982)	Lysine-vasopressin (intranasal spray) 16 I.U/day	10 Days	Double-blind non-crossover	17 AD	NS effect on cognitive function, only improved attention and motor responsiveness.
Christie (1982)	Physostigmine (s.c infusion) 0.25, 0.375, 0.5 and 0.75 mg/day	6 Weeks	Double-blind	11 AD	Improved recent memory function, but optimal dose varied from 0.25-0.75 mg/d.
	Arecoline (s.c infusion) 2 and 4 mg/day	6 Weeks	Double blind	11 AD	Improvement in 7 patients at 4 mg/d.
Davidson et al. (1988)	*4-Aminopyridine 0, 5, 10 and 20 mg/day	1 Day per dose	Double-blind	13 AD	Overall, NS effect on any symptom.
Delwaide et al. (1986)	*Phosphotidylserine (BS-PS) 300 mg	6 Weeks	Double-blind	42 AD (Hospitalised) 21 Treated 21 Placebo	Significant improvement in behaviour. No cognitive tests carried out.
Domino et al. (1982)	Lecithin (oral) 100 g/day	4 Weeks	Double-blind	12 Mild dementia without AD	NS difference in memory test, pulse, blood pressure, pupil size, plasma and red blood cell choline levels.
Ferris et al. (1982)	Combined choline 12 g/day and L-DOPA 2 g/day	16 Weeks	Double-blind	112 AD	No cognitive improvement.

	Piracetam (Nootropic) 7.2 g/day	5 Weeks	Double-blind	20 Mild to moderate AD	No consistent effects.
Ferris et al. (1982) cont'd	Combined Choline 9 g/day and Piracetam 4.8 g/day	7 Days	Open	15 Mild to moderate	Small non-significant improvements.
Fleischhacker et al. (1986)	Memantine (i.v) (Dopaminergic) 20-30 mg	20 Days	Single-blind	20 AD 10 Treated 10 Placebo	NS difference between placebo and treated patients on behavioural scales.
Gustafson et al. (1987)	Physostigmine (i.v infusion)	2 Hours for	Double-blind crossover	10 AD	Limited improvement of psychomotor performance and EEG, and increased blood flow in the most affected cortical areas mainly in the early cases.
Henderson et al. (1989)	Naloxone (i.v) (opiate antagonist) 1 or 10 mg (30 mg in 15 patients)	4 Days	Double-blind crossover	54 AD	NS improvement in neuropsychological performance.
Hyman et al. (1985)	Naltrexone (oral) (opiate antagonist) 100 mg/day	6 Weeks	Double-blind crossover	17 AD	No improvement in assessments of day-to-day living skills or on a battery of neuropsychological tests.
Kaye et al. (1982)	Combined Lecithin 60 g/day and THA 60 g/day.	3-12 Days	Double-blind	10 AD	Less impaired patients performed better on serial learning, but NS clinical improvement.
	*DDAVP (vasopressin) 20-60 g/day (internasal)	3-10 Days	Double-blind	7 AD	Enhanced arousal and increased motor and speech activity only.
Mohr et al. (1989)	Clonidine (noradrenergic) 0.1-1.2 mg/day	1-2 Weeks	Double-blind	8 AD	NS changes in cognitive function.

Mouradian et al. (1988)	RS-86 (oral) (muscarinic agonist) 10 mg/day	8-10 Days	Double-blind	7 AD (Presenile)	No consistent clinically significant cognitive improvement.
Newhouse et al. (1988)	Nicotine (i.v infusion) 0.125, 0.25 and 0.5 μg/kg/min	1 Day	Single-blind	6 AD (Non-smoking)	Reduction in intrusion errors at 0.25 μg dose. Also increase in anxiety and depression.
Peabody et al. (1986)	*DDAVP (vasopressin) 30-180 μg/day	3 Weeks	Double-blind	14 AD	3/31 measures were improved, but were too small to be of clinical significance.
Penn et al. (1988)	Bethanechol (muscarinic agonist) (i.vent) 0.3 mg/day	24 Weeks	Double-blind crossover	10 AD (Biopsy-proven)	Mild improvement on patient behaviour and mood only.
Peters and Levin (1982)	Combined Physostigmine 0.5-1.0 mg/day (titrated) Lecithin 7.2 mg/day	18 Months	Double-blind	9 AD	Augmentation of long-term verbal memory in 4/9.
Pomora et al. (1982)	Lecithin (oral) 0, 4, 12 and 12 g/day	2 Weeks per	Double-blind	4 AD	All showed improvement over placebo in memory retrieval, but the degree of change varied from 10-300%.
Serby et al. (1986)	Naltrexone (oral) (Opiate antagonist) 5, 50 and 100 mg/day	3 Weeks per dose level	Double-blind Open	9 AD 9 AD	Apparent cognitive enhancement on 100 mg/d demonstrated in 1/9. Apparent cognitive enhancement in 2/9.
Sourander et al. (1987)	Aniracetam (RO 13-5057) (nootropic)	3 Months	Double-blind	44 AD	No difference in psychometric tests between test and placebo groups.

Stern et al. (1987)	Physostigmine (oral) 13 mg/day	12 Days	Double-blind crossover	22 AD	Improvement on WAIS-revised digit symbol test and shape cancellation test only.
Stern et al. (1988)	Physostigmine (oral) 12.5-16 mg/day	5 Intervals of 4-6 weeks	Double-blind crossover	14 AD	Improvement in memory scores. 2 Patients perfomed worse on drug than placebo. 9/14 improved on 2 or more measures.
Sullivan et al. (1982)	Physostigmine (i.v) 0.25 and 0.5 mg	3 Days	Double-blind	6 Mild 5 Moderate 1 Severe AD	No reliable change in memory performance. Response to drug was variable with regard to dose and test. Generally, the milder patients showed greater improvement.
	Lecithin (oral) 30 g/day	18 Weeks	Double-blind	6 Mild 8 Moderate 4 Severe	No difference between treatment and placebo.
Summers et al. (1986)	*THA (oral) 150-200 mg/day	7-10 Days	Open	17 AD	Significant improvement in global assessment, orientation test and names learning test.
		3 Weeks	Double-blind crossover	14 AD	Significant improvement on global asssessment, orientation test, AD deficit scale and names learning test.
		3-26 months (average 12.6 months)	Open	12 AD	Some symptomatic improvement.
Tariot et al. (1987)	L-Deprenyl (*MAOi) 10 mg/day 40 mg/day	28 Days 35 Days	Double-blind	17 AD (Hospitalised, low-tyramine diet)	Improvement in total brief psychiatric rating scale for 10 mg/d and imrovement in behavioural state.

Tariot et al. (1988)	Arecoline (i.v infusion) 1, 2 and 4 mg/hour	1 Day per dose	Double-blind	12 AD	NS improvement in performance of cognitive tasks. Marginal improvement in picture recognition and in ratings of word-finding at low doses.
Thal et al. (1989)	Physostigmine (oral) Titrated doses 8-16 mg/day	12 Weeks	Double-blind	16 AD (10 Treated 6 Placebo)	Improvement on a selective reminding task and verbal memory in 7/10. No improvement on non-memory tests.

KEY:

*L-DOPA	Dopamine precursor
*THA	Tetrahydroaminoacridine (an acetylcholinesterase inhibitor)
*Thiamine	Vitamin B1
*4-Aminopyridine	Facilitates evoked ACh release at neuromuscular junctions and exerts powerful anti-curare action
*Phosphtidylserine	Plasma membrane constituent which binds to stored calcium
*DDAVP	1-desa-amino-D-arginine vasopressin
*MAOi	Monoamine oxidase inhibitor

Morphological, biochemical and behavioural abnormalities suggest that treatments which enhance central cholinergic neurotransmission should be effective therapy. Yet, there is no overwhelming evidence that this is the case. The apparent ineffectiveness of the precursor treatment could be due to inadequate subclassification of patients involved in the trials, improper dosage or insufficient duration of treatment (Drachman 1983).

In general, it is difficult to find an agent that is safe yet effective in fully restoring ACh levels or mimic its effects in AD. For example as Pomara et al. (1983) and Wurtman (1985) point out, ACh precursors have to be converted by CAT in nerve terminals, but these are likely to be the ones that are deficient. An AChEi would be an effective drug but likely to act not only in the hippocampus and cerebral cortex, but other areas in the brain leading to unwanted side-effects. According to Fibiger (1991) the idea of replacement therapy is likely to fail, because in AD there are dysfunctions in many other neurotransmitter systems, and structures such as the hippocampus and the cerebral cortex, (the target sites for drugs), are themselves damaged. Therefore, such replacement therapy would only be of use in the more mildly impaired patients with more neurologically intact populations. However, Sarter et al. (1991) have argued that the good correlation between CAT activity and cognitive impairments have not been found as consistently with the other neurotransmitter systems and so there is a need to attempt to treat symptoms with cholinergic drugs until the cause of AD has been identified.

In conclusion, there is no specific treatment for AD to date and management remains symptomatic and directed towards improving cognitive function based on replacement of neurotransmitter deficiencies or on improving overall cerebral metabolic rate. Ideally, as Drachman (1985) suggests, therapeutic strategies should be aimed at preventing the pathogenetic process but the precise aetiology is still unknown. Alternatively, strategies should be aimed at delaying the onset or slowing the progress of AD by eliminating environmental factors that contribute to its severity. But at present little is known about these factors.

4.9 Changes associated with the visual system

There are a growing number of reports describing visual impairments in AD. For example, Schlotterer et al. (1983) reported impairments in visual masking; Cogan (1985) reported poor visual acuity, alexia, agnosia and spatial disorientation which was attributed to abnormalities in the parietal cortex, in three patients; Nissen et al. (1985) reported impairments in contrast sensitivity function; Olson (1989) reported

visual complaints such as losing one's place when reading, glare, misjudging distances, clumsiness, bumping into things, knocking things over and tripping, Sadun and Bassi (1990) found that in patients with mild cognitive impairment, they often complained of an inability to read, finding difficulty in moving quickly, often bumping into furniture not seen and misreaching for objects. Colour vision can also be impaired in AD. Sadun et al. (1987) has reported abnormal colour vision in six out of twelve patients and Cogan (1985) reported that two out of the three patients studied, could identify colours but were unable to arrange them in proper order and displayed deficiencies in the turquoise zone. Therefore dyschromatopsia may be a manifestation in AD, although its significance is uncertain.

In the study by Sadun and Bassi (1990) the patients demonstrated normal Snellen acuity and usually a normal general ophthalmological examination (normal fundus appearance and no evidence of optic disc pallor). The normal visual acuity, at least during the earlier stages of the disease, has also been reported by other workers (Schlotterer et al. 1983; Wright et al. 1987). Visual field tests also appear to be normal (Cogan 1985; Sadun et al. 1987). These findings above have led to the suggestion of the involvement of specific areas in the visual pathway in AD.

4.9.1 Visual neuropathology

A loss of retinal ganglion cells has been reported (Hinton et al. 1986) especially with perimeters larger than 40 μm in patients with AD (Sadun and Bassi 1990). The degenerative features included ganglion cell swelling with microvacuolarisation. Blanks et al (1989) investigating the macular area, also found a significant loss of neurones in the ganglion cell layer of the retina, compared to the normal appearance and thickness of the the plexiform layers, inner nuclear layer, outer nuclear layer and the photoreceptor cell layer. In contrast Drucker and Curcio (1989) reported no significant loss of neurones in the central 44^{o} of retina, from the AD patients compared to their normal control group.

Hinton et al. (1986) reported widespread axonal degeneration in the optic nerves in 8 out of 10 AD sufferers. They found 15-80% depletion of axons in the optic nerves of AD patients compared to age-matched controls. In their control group, the axons were tightly packed in bundles with only infrequent glial cells, whereas in the AD group, the axons were interspersed with an increased amount of glia and not tightly packed at all. The cross-sectional axon diameter in the controls ranged from about 0.5-6 μm, but in AD it was 1-2 μm. There was no retinal neurofibrillary degeneration, plaques or amyloid angiopathy present. Sadun and Bassi (1990) confirmed these findings, reporting about 50% depletion of axons in the optic nerve as well as extensive

degeneration. The age-matched controls also demonstrated a small amount of axonal dropout but it was not as extensive as those observed in AD. These workers suggested the possibility that larger axons may initially have been involved with the subsequent involvement of all axon sizes, leading to the speculation that cells of the magnocellular pathway may be lost before cells of the parvocellular pathway.

Scholtz and Brown (1978) measured the amounts of lipofuscin in the LGN of 18 normal, 12 blind, 2 deaf and 1 AD patients as a measure of lifelong metabolic activity of neurones involved. Lipofuscin is an oxidative product which accumulates linearly with age. The workers found a linear increase in lipofuscin with age in the LGN of the normals; blind patients had less and AD patients had an intermediate amount of lipofuscin. Scholtz et al (1981) subsequently examined the striate cortex and LGN in patients with visual impairments, in patients with histologically proven AD, patients with multi-infarct dementia and age- and sex-matched controls. They found significantly reduced amounts of myelin in the outer band of Baillarger (strip of Gennari) in the visual cortex of both blind and demented patients compared with the controls. In addition, the neuronal cytoplasmic RNA, nucleolar volume and the numbers of tetraploid glial nuclei were significantly reduced in the visually impaired groups. Katz and Rimmer (1989) suggested that the demented patients may have unrecognised visual impairment, and the degree of myelination in the visual cortex may be stimulus dependent.

Lewis et al. (1987) studied the laminar and regional distributions of plaques and tangles in the visual and auditory cortices, in the brains of 8 AD patients. The primary visual cortex (area 17) displayed very few tangles, but there was a 20-fold increase in a 250 μm area in the immediately adjacent visual association area (area 18), with a further doubling in the higher-order visual association cortex of the inferior temporal gyrus (area 20). There was a similar scene for the auditory cortex with a greater density of tangles in the association area than in the primary auditory area. The highest density of tangles occurred in layers III and V. The number of plaques was equal in the three visual areas, although the type of plaque was different for these areas. In area 17, the plaques contained a dense, brightly fluorescent core, whereas those in areas 18 and 20 did not. Since the pattern of distribution of the tangles was similar to the regional and laminar locations of the long corticocortical projection neurones in homologous regions of the monkey neocortex, the tangles may be in the cell bodies of the subpopulation of pyramidal neurones along the corticocortical projection system.

Benson (1982) measuring metabolic activity with PET, found that the cerebral metabolic activity in the AD patients was below that of their age-matched normals. There was a correlation between the dementia level and the amount of isotope activity with the more severely demented patients having lower cerebral metabolic activity. The frontal and parietal association areas showed the greatest reduction in metabolic activity, whereas the calcarine cortex and the primary motor and sensory areas showed almost normal metabolic activity.

Olson (1989) reported abnormal colour vision, depth perception and ability to copy diagrams in 5 AD patients with visual symptoms. PET scans revealed reduced metabolism in the visual association cortex in these patients, whereas in 3 patients without the visual symptoms had no reduction in metabolism compared to their normal control group. These workers suggested there to be a subgroup of AD patients with the involvement of the visual system. In addition, there have been reports of a subgroup AD patients who develop Balint's syndrome, an uncommon and incompletely understood disorder of visuospatial processing (Hof et al. 1989; Mendez et al. 1990).

4.9.2 Psychophysical changes

Schlotterer et al. (1983) reported no difference in spatial frequency contrast sensitivity function (CSF) between patients with AD and age-matched controls, although both groups were impaired in comparison to the younger control group. In agreement, Wright et al. (1987) also reported no difference in the spatial contrast sensitivity function in their AD group compared to a control group of patients with non-dementing psychiatric illness. In contrast, Nissen et al. (1985) reported impaired spatial frequency contrast sensitivity between their AD and control group. These contradictory results may possibly be due to patient selection and different experimental procedures employed by the various workers.

Schlotterer et al. (1983) also investigated effect of backward masking in normal ageing and AD. Visual masking involves presentation of a visual stimulus (either a pattern or flash of light) which interferes with the perception of another stimulus presented immediately before or after the initial stimulus. Backward visual masking involves presentation of a stimulus immediately after the target stimulus. Schlotterer et al. (1983) found that AD patients were significantly more affected than the age-matched control group only when a patterned mask was used, with no difference when a homogeneous flash stimulus was used, although both groups were more affected compared to the young control group. The authors concluded that since backward patterned masking was a central phenomena while that to the flash a peripheral one,

the temporal aspects of central visual processing, which normally decreases with age, was further reduced in AD. Wright et al. (1987) also reported impaired contrast sensitivity functions at low and medium, but not to the high temporal frequencies, whereas spatial frequency CSF remained unaltered as mentioned above.

4.10 Electrophysiological changes associated with AD

4.10.1 Brainstem auditory evoked potential

The brainstem auditory evoked potential (BAEP) consists of a group of 7 peaks occurring within 10 ms of stimulus onset and conventionally labelled waves I to VII. Each component has been associated with possible generators and include, the acoustic nerve (wave I); cochlear nuclei (wave II); superior olivary complex and fibres crossing the midline (wave III); lateral lemniscus (wave IV); inferior colliculus (wave V); possibly the medial geniculate (wave VI); and possibly thalamocortical auditory radiations (wave VII) (Jewett 1970; Starr and Hamilton 1976; Buchwald and Brown 1977; Stockard and Rossiter 1977).

In AD there are reports of significant prolongation of wave V latencies and reduced amplitudes compared to normal controls and increases in the central transmission time (wave V latencies minus wave I latencies) (Fenton 1986; Patterson et al. 1983). These changes are associated with extensive loss of neurones in AD of the locus coeruleus (LC) and the projections between the LC and other brainstem regions such as the medial and lateral geniculate, inferior olive and dorsal cochlear nuclei.

4.10.2 Auditory evoked potential

Buchwald et al. (1989) reported a delay in the midlatency auditory evoked potential occurring in the 50-65 ms range (their P1). They suggested that this potential was generated by the ascending reticular activating system by presynaptic cholinergic cells in the midbrain area of the pedunculo-pontine-tegmental nucleus projecting to postsynaptic muscarinic cells in the intralaminar thalamus. Therefore, the marked degeneration in the cholinergic cells of the basal forebrain are thought to be responsible for this observed abnormality.

4.10.3 Somatosensory evoked potential

There are very few reports on the somatosensory evoked potentials (SEPs) in AD. Levy et al. (1971) compared the SEPs in controls, depressed subjects and patients with AD. They observed a delay in the five peaks measured within the first 100 ms compared to the control and depressed subjects. Only the negative peak 5 (mean

latency of 89 ms) was significantly different in the patients with AD, being absent or very flat. The ratio of the amplitude of the fifth peak to the first peak was also smaller in the AD patients. But these workers were unable to confirm their findings in later studies (Hendrickson et al. 1979). However, Huisman et al. (1985) were able to confirm the delay of the late complex latencies of the median nerve SEP.

4.10.4 Event-related potential

Long-latency event-related potentials (ERPs) (responses occurring 150 ms after stimulus) are said to be cortically mediated and affected by psychological and cognitive factors (Pedley and Miller 1983). These responses have been termed "endogenous potentials" because they arise from "intrinsic mechanisms for processing information within the central nervous system" (Pedley and Miller 1983).

4.10.4.1 Contingent negative variation

The contingent negative variation (CNV) is a small negative potential occurring at the vertex when a subject is given a warning stimulus followed by a second stimulus which requires a response (termed the imperative) (Walter et al. 1964). It is thought to be a measure of expectation and depends on the subject's awareness of having to make a response. The CNV is sensitive to voluntary changes in the subject's attitude to the task and can be consciously suppressed. In addition, if the task is complex, the potential can be attenuated (McCallum 1976) and is absent with fatigue and sleep deprivation (Naitoh et al. 1971). The CNV has been shown to appear over both hemispheres in a split brain preparation even with selective stimulation of one hemisphere (Callaway 1975).

There are very few studies of the CNV in dementia. O'Connor (1977) studied the CNV in the normal aged and dementia. Subjects were given two separate trials using an auditory stimulus. Alterations in amplitude and latency measures were reported in the normal aged as a function if intersignal interval, but only minimal adaptation occurred with the demented patients. Nakamura et al. (1979) examined the CNV in the young and elderly. The relative amplitude was found to be related to long-term memory deficits in the elderly and not related to age alone. Michaelewski et al. (1980) found the CNV amplitude was reduced in the frontal areas in the elderly, but comparable between the young and elderly in central and parietal regions. These findings were attributed to selective effects of aging on the cerebral cortex.

4.10.4.2 P300

A more commonly utilised ERP in dementia is the P300 (also known as P3), evoked using an auditory "odd-ball" paradigm. In this task two tones are repeated in a random manner with one tone (target) being presented less frequently than the other (non-target). The target tone has to be discriminated from the non-target tone by either counting or pressing a button. The resulting response takes the form of a sequence of peaks and troughs, with the component of interest being the P300, a positive wave appearing around 300 ms latency (Goodin et al. 1978; Squires et al. 1979). This is thought to reflect the central nervous system process of stimulus selection and processing. The process of discriminating a novel stimulus from a train of stimuli is thought to require "updating of working memory" (Donchin et al. 1986) and reflects transfer from short-term to long-term memory stores, stimulus assimilation and decision to respond. Such processes are thought to be impaired in dementia (O'Mahony et al. 1991). Hence the P300 may be taken as a crude measure of perception and cognition. Although the P300 has been studied most often in paradigms using auditory stimuli, it can also be elicited by other sensory modalities. Studies of the ERP, showed that 80% of presenile dementia patients had a significantly delayed latency (Goodin et al. 1978; Ford et al. 1979; Squires et al. 1979; Pfefferbaum and Rosenbloom 1984; Gordan et al. 1986). Topographical evaluation of the auditory elicited P300 revealed higher amplitudes frontally, no difference centrally and an amplitude decrement in temporoparietal areas in patients with moderate dementia (Maurer and Dierks 1991).

Studies have indicated a considerable overlap with functional disorders and has exposed limitations of the use of P300 with older and more confused patients, since the patients' active engagement in the task is required (Duffy et al. 1984). There are also many false negatives which occur and this has seriously limited the use of the P300 as a diagnostic tool in dementia of the Alzheimer-type. However, some of these problems may be overcome by the use of a passive paradigm, where the subject is blind to the relevance of the target from non-target stimuli (Polich 1987). In dementia, the P300 to this passive paradigm is also reported to be increased (Polich 1987, Surwillo and Iyer 1989; O'Mahony et al. 1991). However, the passively elicited P300 occurred in fewer patients and was of lower amplitude compared to that elicited by the active paradigm (Polich 1986; Surwillo and Iyer 1989). However, this can be overcome to an extent by accentuating the difference between the target and non-target stimuli (O'Mahony 1991).

The visual analogue of the auditory odd-ball paradigm involves a "push/wait" task (Pfefferbaum et al. 1982). Subjects press a button when the word "push" appears on a screen and withhold the response with the word "wait". The P300 to the visual stimuli occur later than those elicited from the auditory stimuli, but the scalp topography is similar and they are thought to arise from a common generating system (Snyder et al. 1980). As with the auditory stimuli, ERPs to the visual stimuli also report prolongation of the P300 component in dementia (Pfefferbaum et al. 1984; Pfefferbaum et al. 1985; De Toledo-Morrell et al. 1991; Verleger et al. 1992). Another visual analogue of the auditory odd-ball paradigm can also be used, e.g. Patterson et al. (1983) used a visual stimuli which included the number "6" as a non-target stimulus, the number "9" as the target stimulus and a novel stimuli consisting of several different randomly drawn patterns. The resultant ERP P300 component was delayed and diminished in patients with dementia compared to normal controls and depressive patients.

4.10.5 Electroencephalography

The most consistently evaluated variable of the EEG is the alpha rhythm. The mean alpha rhythm frequency in a large unselected population of young adults is 10.0-10.5 Hz. This value declines in the elderly, averaging 9.0-9.5 Hz at 70 years of age and 8.5-9.0 Hz after 80 years of age (Katz and Horowitz 1982). The faster low voltage beta activity with mean frequency approximating 18 Hz in both the young and elderly, is of larger amplitude in the elderly. The amount of theta (4-7 Hz) and delta (less than 4 Hz), both diffuse and focal slow activity, increase with age and the degree of change correlates with decline in mental function and longevity (Silverman et al. 1955; Muller and Schwartz 1978). These focal theta and delta activity have been reported to occur over temporal regions particularly on the left side in the over 60 year olds (Silverman et al. 1955).

The EEG changes occurring in AD are similar to those of normal ageing but the degree of change tends to be more pronounced in dementia. In the milder cases of AD, the EEG may be normal or show an alpha rhythm around the lower limits of normal. However, as the disease progresses there is generalised slowing of the background pattern. There is a reduction in the parieto-occipital alpha rhythm over both hemispheres, a moderate to marked amount of diffuse theta activity and delta activity and a bilaterally symmetrical decline in beta activity, (Busse 1983; Fenton 1986; Coben et al. 1990). A correlation exists between degree of dementia and amount of theta/delta activity, less beta activity the slower the alpha activity and of total activity.

In Pick's disease, even at stages of severe dementia, the EEG remains normal or near normal (Pedley and Miller 1983).

There are several improvements in EEG analysis with computer-assisted quantitative analysis of the power (a function of amplitude) and coherance (a function of regional neuronal electrical synergy) across spectrum of EEG frequencies and brain electrical activity mapping. O'Connor et al. (1979) found that AD was associated with high EEG coherence between the centroparietal and temporal areas within both hemispheres, in contrast with multi-infarct dementia, where focal lesions reduced coherence between intrahemispheric regions. Visser et al. (1985) summarised the major quantitative findings in the EEG power density spectrum that differentiated dementia from normals and non-organic behavioural disorders as: decreased mean frequency (total spectrum); decreased mean frequency (theta band); increased relative power (delta and theta bands); and decreased relative power (alpha and beta bands). Saletu (1989) also found a reduction in the relative power in the alpha and beta activity in the quantitative EEG of patients with AD. There was also increased focal alterations, a decline of differences between areas, a reduction in the overall amplitude and attenuated responses to activation procedures (hyperventilation, photic driving and eye opening). Guidi et al. (1989) compared the EEG in normal controls, depressed, mild AD and severe AD patients. The EEG power spectrum showed increased delta and total power and decrease in beta activity in frontal areas of the severe AD group, whereas the mild AD patients only showed a significant increase in the theta power. The mean frequency of the EEG at 4-12 Hz and 1-16 Hz bands was reduced in the AD patients. In the normal control group, the maximum mean frequency 4-12 Hz band occurred in the occipital area, but in AD (especially mild) patients it was more variable and generally more anterior. Guidi et al. (1989) thought this finding was influenced by the different ratio between the absolute power of theta and alpha frequency bands.

In longitudinal studies, there is progressive deterioration in the EEG but not always (Gordan 1968; Johannesson et al. 1977; Coben et al. 1985; Hooijer et al. 1990; Soinnen et al. 1991). In two studies on presenile dementia, 20-40% of patients did not shown any worsening of the EEG features over the years, with improvement occurring in some (Gordan 1968; Johannesson et al. 1977). In addition, the progressive slowing of the EEG in AD over the years tends to be unequal (Soinnen et al 1991).

There are changes in the sleep pattern in AD (Feinberg et al. 1967; Saletu 1989). During sleep, the EEG awakenings are more frequent and lengthy. The sleep becomes more fragmented, with sleep stage 4 and slightly less so stage of rapid eye movement

(REM) decline, while stage 2 increases. The total sleep time also declines as does the number of sleep spindle bursts. The reduction in REM sleep is found to be correlated with impairment in intelligence and memory tests (Feinberg et al. 1967).

The EEG in the milder stages of AD may not show any significant abnormality (Coben et al. 1990; Hooijer et al. 1990) and is of very limited use in such cases. However, patients with mild AD with a normal EEG do show increasing signs of slowing with time as the condition worsens (Hooijer et al. 1990; Soininen et al. 1991). The presence of theta activity has been judged to be the most effective indicator of dementia with a sensitivity of about 20% and a specificity of 100% (Coben et al. 1990) and suggested to be used to differentiate early AD from normal ageing and to monitor the progression of the disease (Soininen et al. 1991). But again the low sensitivity puts several constraints on the usefulness of the EEG, especially at the milder stages of AD.

The diffuse slow activity, more than other EEG variables, is related to senile intellectual deterioration (Weiner 1983). The degree of slowing is related to the severity of the impairment, in that the slower and more abundant the waves are, the greater the deterioration and severity of dementia. Experiments on cerebral blood flow and metabolism in healthy old subjects show normal tracings, cerebral metabolism and EEG (Busse 1983). But a significant relationship exists between the cerebral oxygen uptake and abundance of slow activity in elderly patients with organic brain syndrome and the diffuse slow EEG (Fenton 1986).

4.10.6 Electroretinogram

The electroretinogram (ERG) to the pattern stimulus has been reported to have lower amplitudes (Katz et al. 1988, 1989; Barris et al. 1988; Trick et al. 1989), but that to the flash stimulus was not affected (Katz et al. 1988 and 1989). The reduction in the pattern ERG amplitude was most pronounced at high temporal frequencies (Barris et al. 1988; Trick et al. 1989). The pattern ERG is thought to be generated in the retinal ganglion cell layer and the flash ERG in the photoreceptors, Müller cells and retinal pigment epithelium layer (Maffei and Fiorentini 1981; Maffei et al. 1985; Rimmer and Katz 1989; Thompson and Drasdo 1989). Barris et al. (1988) and Trick et al. (1989) attributed their findings with the selective loss in the large retinal ganglion cells which underlie the M-cell pathway that occurs in AD, which is more sensitive to low spatial and high temporal frequencies. However, since the flash stimulus is ultimately of lowest spatial frequency it is therefore, expected to be more affected than the pattern stimulus. Hence more studies need to be carried out on the flash and pattern ERGs to resolve the confusion.

4.10.7 Visual evoked potentials

One of the earliest published reports of VEP studies in dementia was by Straumanis et al. (1965), who investigated the flash VEP in patients with chronic brain syndrome associated with cerebral arteriosclerosis. They reported a delay in the components occurring after 100 ms, with the amount of delay increasing as a function of wave number. Further VEP studies in psychiatric patients with organic brain disease include Lee and Blair (1973), who described three patients with Creutzfeldt-Jakob disease. The VEP however, was only studied in detail in one patient, who showed an increase in latency during the initial stages of the disease with a reduction in amplitude of the flash VEP components.

This later provided the impetus for Visser and co-workers (1976) to investigate the flash VEP in a group of 19 patients suffering from presenile and senile dementia of the Alzheimer type. Visser et al. (1976) also found a delay in the flash VEP components occurring after 100 ms (from N2 to N3) in the patients (mean P2 latency of 155 ms) compared to the control group (mean P2 latency of 112.16 ms), findings similar to that of Lee and Blair (1973). The amplitude of the P2 and N4 components were also reported to be increased significantly. However, the control group was not age-matched with the patient group since the 19 patients were aged between 52-88 $\pm$ 71.2 years, while the 6 controls were aged between 19-25 years, hence the effect of ageing was not taken into account. Guidi et al. (1989) confirmed the findings of Visser et al (1976), of an increase in latency and amplitudes of the flash N2, P2 and N3 components in AD patients compared to normal controls. However, these workers did not publish any values or age of the patients and normal control groups, hence it is not known if the groups were age-matched, or the extent of the flash VEP delay.

Laurian et al. (1979) in an abstract, reported a normal flash VEP recorded at the Oz electrode position in 12 patients with severe dementia compared to 12 young control subjects. However, the VEP recorded at the vertex position (Cz) was of such low amplitude, that it was hardly discernible in the patient group. These workers further investigated the flash VEP and auditory evoked potentials (AEP) in 10 patients classified as suffering from "simple senile dementia" and 7 patients suffering from AD (Laurian et al. 1982). They found that in the patient group with simple dementia the vertex VER was present but of longer latency than the control, while in the AD group these responses were again not discernible. The AEP although present in both patient groups was of longer latencies than the controls.

The co-existence of the normal pattern reversal VEP with the delayed flash VEP was initially reported by Harding's group (Doggett et al. 1981; Harding et al. 1981). In a

study of 12 patients by Harding et al. (1981), 10 patients with presenile dementia displayed a delayed flash P2 component (mean latency of 162.5 ms), yet all the patients in whom a pattern reversal response was obtainable (n=9), the P100 component was of normal latency (mean latency of 102.7 ms). Eight of the ten patients also displayed increased theta activity and decreased alpha activity in the EEG. Subsequent studies by the same workers (Harding et al. 1984; Wright et al. 1984a; Harding et al. 1985; Wright et al. 1986) led Harding's group to suggest that this was a sufficiently unusual finding to be of diagnostic use for the dementias of the Alzheimer type. Wright et al. (1987) further showed that the pattern onset-offset VEP was also unaffected in AD. The flash P2 delay co-existing with a normal pattern reversal P100 has been confirmed by other independent studies (Danesi et al. 1985; Engedal et al. 1987; Philpot et al. 1989; Coburn et al. 1991; Sloan and Fenton 1992).

Cosi et al. (1982) investigated the flash VEP and its relationship with cerebral atrophy as measured by CT scans. They reported a delay in the flash VEP (mean P2 latency of 157.9 ms) in a group of patients with cerebral atrophy when compared to age-matched controls without cerebral atrophy (mean P2 latency of 139.4 ms). Patients with cerebral atrophy were further subdivided on the basis of clinical evidence of dementia. The subgroup with dementia showed a significantly greater increase in the flash VEP (mean P2 latency of 171.7 ms) than the non-demented group (mean P2 latency of 150 ms). Hence these workers concluded that the P2 delay was not directly related to presence of cortical atrophy but metabolic, biochemical and circulatory factors must also be involved.

A comparative study of the VEP, EEG, and CT scan as diagnostic tools, has shown that all three provide similar positive diagnosis in established cases of AD (Harding et al. 1984 and 1985). However, in the early cases, the VEP was reported to be superior with a 70% correct diagnosis of AD compared to 20% with the EEG and 33% with the CT scan, (Harding et al 1985). The poor relationship between the VEP and CT indicated that the VEP abnormalities may be reflecting aspects of the dementing process other than cerebral atrophy, in agreement with Cosi et al. (1982). Hence the VEP is providing a measure of function while the CT and in part the EEG mainly reflect the anatomical changes in Alzheimer patients. This is of particular interest since AD is not always accompanied by "typical" pathology. In contrast, Berg et al. (1984) reported that the EEG, VEP and CT were not helpful in predicting the clinical course of dementia in 43 mild AD patients over a twelve month period, when compared to the behavioural measures and psychometric tests as better indicators of severity of dementia.

Harding's group suggested that the difference in latency of the flash P2 and pattern reversal P100 component was sufficiently increased in AD to provide a diagnostic measure for the disease, (Wright et al. 1984a; Harding et al. 1985; Wright et al. 1986). A P2-P100 latency difference greater than 2 standard deviations above the mean for the age-matched controls was thought to be indicative of AD (Harding et al. 1981). This method resulted in a sensitivity of 80% and a specificity of 100% in differentiating patients with cerebral atrophy due to AD and those with atrophy due to other causes. Later, Wright et al. (1986) used a P2-P100 latency difference of 40 ms as the criterion, irrespective of age to be indicative of AD. Wright and Furlong (1988) used an even higher P2-P100 latency difference of 52 ms, resulting in 73% of their AD patients being correctly identified. Recently, Philpot et al. (1989) found that this P2-P100 latency difference had a sensitivity of 92% and specificity of 89% in discriminating between groups of AD patients from controls. However, the P2-P100 difference was only valid in patients younger than 75 years of age.

Sloan and Fenton (1992) investigated the P2-P100 difference in a group of AD patients, patients with multi-infarct dementia (MID), patient controls (patients with functional psychiatric illness) and normal elderly controls, over a period of 2 years at 6 monthly intervals. The P2-P100 difference increased with time in all the groups but the greatest difference was in the AD group followed by MID and then the control groups. There was no significant difference between the AD and MID group. These workers suggested that although the P2-P100 difference was useful in discriminating AD and MID in the early stages of the dementing process, in established cases, a normal P2-P100 latency difference value favours a diagnosis of MID rather than AD.

In contrast to the findings mentioned above, Coben et al. (1983) reported that the flash VEP was not significantly delayed in patients (in the milder stages of the disease) but that the later components of the pattern reversal VEP (occurring later than 170 ms), were significantly delayed. The study included elderly subjects diagnosed with mild dementia who were still living in the community. The results published did however show that the flash P2 was delayed by approximately 15 ms, in the dementia group when compared to the control group but these workers did not find this to be statistically significant. The subjects may however have been too mildly demented, as they were still able to live in the community, to be able to show a flash VEP abnormality. Philpot et al. (1989) confirmed that the flash P2 latency was not delayed in the very mild cases of AD, but the subsequent flash P3 component was delayed although it was not always identifiable and given to great variability.

The findings of Coben et al. (1983) may also be related to the inclusion of patients (14 out of the 40) who were on centrally active medications (benzodiazepines, tricyclic antidepressants, thyroid, chloral hydrate and haloperidol) which may mask the results. There is a delay in the pattern reversal components in Parkinson's disease which can be reversed upon medication in some with antipsychotic medication such as haloperidol, (Vasconetto et al 1971; Domino et al.1979). However, Wright et al. (1984a) have shown that their results were not affected by patients still on medication as their results were compared to a control group on equivalent medication. In addition, in Coben's study, (Coben et al. 1983) the patient selection was mainly based upon a 1.5 hour interview after which the patients received a clinical dementia rating (CDR) depending on the severity of the symptoms (Berg et al. 1982). Those included in the study were in CDR 1 category where there was a moderate memory loss, more marked for recent events, and sufficient to interfere with everyday activities. There was no data published regarding the changes in the EEG and CT scans. The EEG was used to monitor drowsiness in the patient and not to indicate any characteristic changes. Therefore, it is highly probable that the study may have included patients who may not have been suffering from AD (see section 4.5).

Visser and co workers (Visser et al. 1985; Huisman et al. 1987) also reported that the late components of the pattern reversal VEP (N130, P165, N220) were delayed, confirming the findings of Coben et al. (1983). The amplitude of the P100 was reduced whilst that of the P165 was increased in their dementia group compared to a control group consisting of subjects with non-organic behavioural disorders and a group of normal aged subjects. These workers had earlier reported (Visser et al. 1976) that the flash VEP was delayed in AD, however they did not record the flash VEP in their later studies.

Although AD patients tend to have normal vigilance, their ability to focus attention for a longer time is reduced, possibly due to related loss of memory function. Thus attention may directly/indirectly alter the pattern VEP by eye movement and poor fixation. However, Huisman et al. (1987) found there was no effect of attention, as measured by motor reaction times, on the pattern reversal VEP which showed a delay in the late components (N130, P165, N220).

In contrast, Philpot et al. (1989) using the same dementia classification criteria as Coben et al. (1983), did not find any significant differences in the pattern reversal VEP (N65, P100, N130 and P165 components) of the AD groups compared to the control groups. In addition, it must be considered that the later components of both the flash and pattern reversal VEPs (after 100 ms) become increasingly variable and

accurate estimation of normal values becomes extremely difficult. As a result, Harding (1974) suggested the use of the major positive component occurring at approximately 100 ms (the most consistent and easily identifiable component) for both the flash and pattern reversal VEPs.

A meta-analytic study by Pollock et al. (1989), also observed that the P100 and the later occurring pattern reversal components were delayed but not those of the flash VEP. The results published showed the flash P2 component to be approximately 12.3 ms later than the control but the delay was not statistically significant, whereas the P100 delay of 4.3 ms in the AD group was reported to be significant, when compared to the control group. The AD patients (n=13), were age and sex matched individually with a control subject (similarly to Coben et al. 1983) to "reduce the error variance" which tends to be large in VEP studies.

Ruessmann and Beneicke (1991) also reported that the flash P2 was not delayed in 13 AD patients when compared to an age-matched control group (consisting of 9 healthy volunteers, 16 patients suffering from spinal or peripheral nervous system problems and 1 migraine sufferer). The mean P2 latency was not quoted, although using their "normative values", the P2 latencies of AD patients from a study by Harding et al. (1985) were all (except 1) within normal limits. However, this may be interpreted alternatively in that the normative values of the control group of Ruessmann and Beneicke (1991) are beyond those obtained from the control group of Harding et al. (1985)! This seems to suggest that the unusually high normative P2 values of Ruessmann and Beneicke (1991), were likely to be obtained from an ill-chosen control group and further studies may be needed.

Longitudinal studies displaying the flash P2 delay with the normal pattern reversal VEP, have largely been confined to individual case studies (Harding et al. 1981; Orwin et al 1986). Orwin et al. (1986), reported on a 58 year old woman who was followed over a period of three and a half years, from the development of the earliest symptoms to complete mental incapacity. The pattern reversal response remained within normal limits for her age group, but the latency of the flash P2 component gradually slowed from 129 ms (considered to be within normal limits for the age) in 1981 to 153 ms (delayed) in 1984. EEG, CT and psychometric tests performed on this patient only indicated generalised cortical disease. It was suggested that the degree of the flash P2 delay may reflect the social ability of the patient since it was found that the P2 latency reached abnormal values when the patient could no longer cope alone.

4.11 Possible generators of the flash P2 and pattern reversal P100 components

The flash P2 delay co-existing with a normal pattern reversal P100 component in patients with AD, has provided further clues as to the pathways involved in the processing of these stimuli. The earlier VEP studies proposed that the delay in the flash VEP was attributable to the selective atrophy of the association cortical areas with the relative sparing of the primary areas (Harding et al. 1984). However, since the VEP delay may be present in the absence of cortical atrophy, (Cosi et al. 1982; Harding et al. 1985), other factors such as metabolic, biochemical and circulatory were also thought to be involved (Cosi et al. 1982). Wright et al. (1984a) agreed with these workers and additionally proposed the involvement of the cholinergic neurotransmitter system in the VEP abnormalities. Later Harding and Wright (1986) reported that in optic neuritis, the pattern reversal P100 is delayed with no effect on the flash VEP. Based on these findings, Harding and Wright (1986) proposed a model to explain this differential finding (fig. 4.3). They proposed that the flash and pattern reversal responses may be transmitted via separate subsystems to the primary visual cortex, where the flash P2 may be rerouted to the association areas (18 and 19). Alternatively, the flash P2 may be transmitted by a non-geniculate route to arrive directly at areas 18 and 19. Hence these stimuli represented different processing and/or generator sites.

Bajalan et al. (1986) provide the best evidence of a cholinergic influence on the VEP. They demonstrated that normal subjects administered with the anticholinergic agent hyoscine hydrobromide (also known as scopolamine) displayed VEPs similar to those seen in patients suffering from AD, i.e. flash P2 delay co-existing with a normal pattern reversal VEP. Post mortem studies of human brains show that the fibres of the geniculate visual pathway are not cholinergic (Shute and Lewis 1967), which may account for the normal latencies of the pattern reversal and early flash VEP components. However, post mortem studies of Alzheimer brains also indicate a reduction in CAT activity in Brodman's areas 17 as well as 19 of the visual cortex (Davies 1979; Rossor et al. 1982). But Bajalan et al. (1986) suggested that the neural input to the primary visual cortex (area 17) was not cholinergic, but ACh either functions as the synaptic transmitter for the reticular input to the cortex, or possibly as a non-synaptic neural excitant.

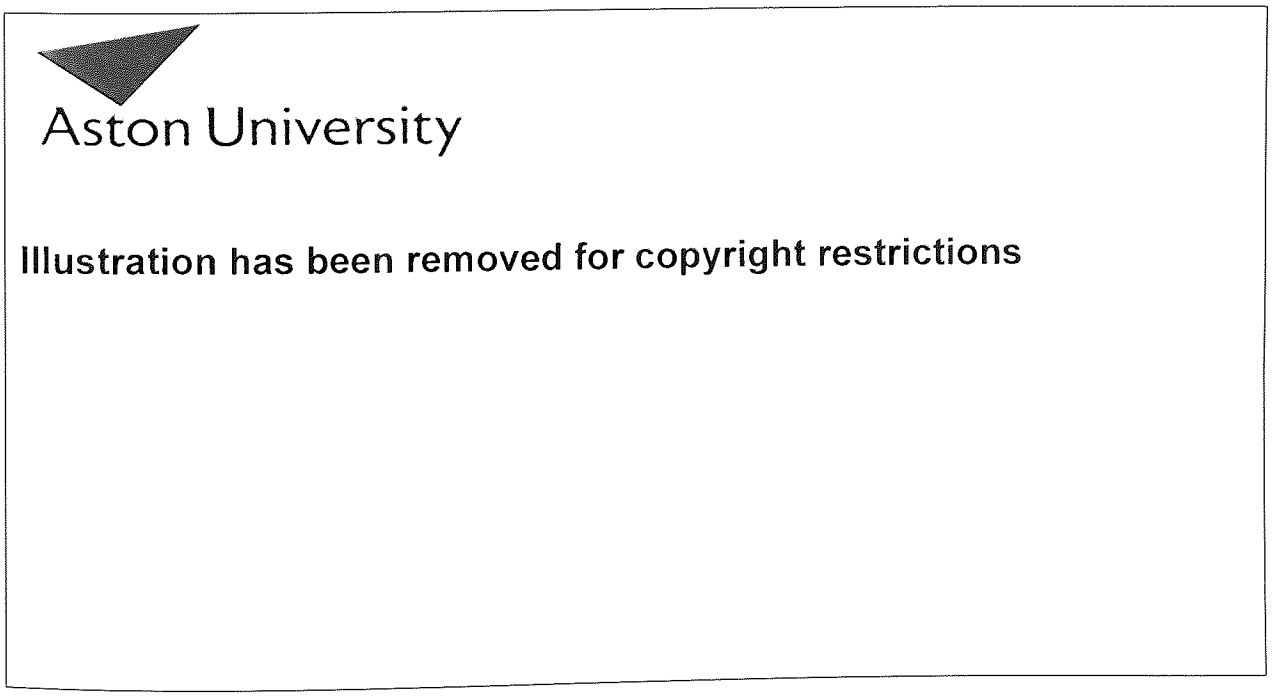
In conclusion, the flash used in conjunction with the pattern reversal VEP, would not only provide a useful diagnostic aid for AD, but also be a physiological marker

whereby therapeutic effects and the progression of the disease may be monitored quantitatively.

4.12 Summary

Alzheimer's disease is a devastating condition with little advance on improving the condition therapeutically. The pathology is generally confined to the association cortices with relative sparing of the primary cortices. The neuronal atrophy primarily affects neurones of relatively large diameters. Although many neurotransmitter systems are affected by the disease, the acetylcholine system seems to be regarded as being consistently affected and responsible for the memory and cognitive deficits associated with the disease. Hence, the major therapeutic route has been aimed at improving the cholinergic dysfunction in the disease. The use of the VEPs have shown the unusual finding of a flash P2 delay co-existing with a pattern reversal P100 component of normal latency. Similar findings have also been demonstrated with the anticholinergic agent scopolamine. Therefore, the flash and pattern reversal VEPs may be used as physiological markers whereby therapeutic effects and the progression of the disease may be monitored quantitatively.

Figure 4.3 Theoretical model for the visual processing of VEPs to the flash and pattern reversal stimuli in humans. A normal subject (**A**) shows normal latency pattern reversal VEP, presumably generated from Brodman' s area 17, and a normal flash VEP mainly generated by areas 18 and 19. In the patient with AD (**B**), the primary visual pathway is unaffected and therefore the systems subserving pattern produce a normal pattern reversal VEP but, since the pathology affects area 19 but spares area 17, the flash VEP is markedly delayed. (From Harding and Wright 1986).



CHAPTER 5

Aims

5.1 Rationale

The previous chapters have demonstrated that Alzheimer's disease is difficult to diagnose. However the flash and pattern reversal VEPs may help to armour further the battery of tests (psychometric tests, EEG, CT scans etc.) that are already used. The selective delay of the flash P2 latency co-existing with a pattern reversal P100 component of normal latency has been reported in patients with AD (section 4.10.7). Although other changes in these VEPs were also reported, the most consistent have been the P2 delay with a normal latency P100.

In addition, the disease displayed a reduction in levels of the neurotransmitter acetylcholine (ACh). Although this is not the only neurotransmitter to be reduced, it is the one reported consistently (section 4.7). This reduction in ACh levels has also been associated with the memory and cognitive symptoms occurring in the disease. Evidence has led to the formation of the cholinergic hypothesis (section 4.8.1) relating the symptoms of the disease to ACh levels in the brain and hence to therapeutic approaches aimed at reversing the cholinergic deficit. However, ACh being a neurotransmitter commonly found in most areas of the body (such as the heart, skeletal muscle and lungs), drug therapy is bound to include unwanted side-effects. It has been difficult therefore to find agents which are centrally active without peripheral effects. In addition, ACh also acts in many centres in the brain such as the vomiting centre, hence adding to the difficulty in finding an agent which would selectively act on cholinergic centres responsible for memory and cognition (e.g. hippocampus and amygdala).

The majority, if not all, therapeutic agents have had their efficacy measured using psychometric testing (table 4.1). Due to the wide variety of tests available, this has made it difficult to obtain consistent results of some agents. In addition, the disease group itself can be heterogeneous leading to inconsistent results. The disease may progress at different rates between patients thus not all patients will have the same level of memory and cognitive deficiency (section 4.5). In addition, some patients may have the classic memory and cognitive symptoms without pathology (Earnest et al. 1979; Hagnell et al. 1983), whereas others may exhibit the pathology without the symptoms (Newton 1948; Jacoby and Levy 1980). These factors all make objective testing using psychometric tests difficult.

The alternative to testing new therapeutic agents in patients is to test in healthy volunteers before the disease group. In order to achieve this, a model is needed which mimics the disease state in normal subjects. The most widely used model has been achieved with the muscarinic antagonist scopolamine (discussed in detail in section 8.2). There have been consistent reports that scopolamine produces centrally-induced deficits in memory and cognition similar to those observed in AD and which are reversible with cholinergic agents (Drachman 1977). Clinical studies using scopolamine in healthy subjects has also shown no conclusive ACh enhancing agent that would be of therapeutic value in AD (table 4.1). Therefore new agents designed to have fewer unwanted side-effects which may also significantly improve the quality of life for patients with AD are continually being developed.

In addition to the selective flash P2 delay with normal pattern reversal P100 in AD, scopolamine has also produced similar reversible results in healthy subjects (Bajalan et al. 1986; Gilles et al. 1989). Hence there may be a use of this effect in the search for therapeutic agents for AD. There have been no reports published to study whether cholinergic agents could reverse the effects of the anticholinergic agent scopolamine. The studies to be described were devised to investigate the use of the flash and pattern reversal VEPs in clinical studies of new cholinergic agents developed for treatment of AD. They were designed to investigate whether there are any changes in the flash P2 and pattern reversal P100 components when no pharmacological agents are administered. It was therefore necessary to investigate what the time of day effects were on these VEP components. In addition, a study was devised to investigate the effects of administering a cholinergic agent on the flash and pattern reversal VEPs since these studies have not been reported in humans to date. This would investigate if the VEPs to flash and pattern reversal could be affected by any cholinergic modulation or only reductions in ACh. The main investigation included demonstrating the flash P2 delay with no effects on the pattern reversal P100 with scopolamine since only some studies report this effect. In addition, once the scopolamine-induced VEP deficits have been shown, it was necessary to investigate whether they be reversed by administration of a cholinergic agent. The effects of scopolamine, although presumed to be on the central nervous system, may possibly be due to peripheral effects, such as in the retina. There are reports of retinal pathology (section 4.9.1) in AD which could account for the VEP effects observed in AD. Hence it was necessary to investigate the effects of scopolamine eye-drops as well as to systemic scopolamine.

The studies above would not only investigate the use of the flash and pattern reversal VEPs in clinical studies of new cholinergic therapeutic agents for AD, but could also explain the existence of the selective effect on the VEPs. The visual pathway can

crudely be divided into the geniculostriate pathway and the non-geniculate or tectal pathway (sections 2.1.3 and 2.1.4 respectively). Approximately 80% of the visual signal is thought to be transmitted by retinal cells via the dLGN to the striate cortex and around 20% by retinal cells to the tectal structures to the extrastriate cortex. In addition, based on the visual composition of a stimulus, the signal may be transmitted by the M- or P-cell pathways (section 2.3). It is hoped that in light of the results reported, a better understanding of the pathways responsible for transmitting the flash and pattern reversal VEP will be achieved.

5.2 Aims

Therefore based on the issues discussed above, the aims of the present study are:

1. To investigate the effectiveness of the flash and pattern reversal VEPs in clinical studies investigating new cholinergic agents developed for the treatment of AD.

2. To examine further the nature of the generators of the flash P2 and pattern reversal P100 components in relation to cholinergic function.

CHAPTER 6

Effect of Time of Day on the Flash and Pattern Reversal Visual Evoked Potentials

6.1 Introduction

The studies contained in the present thesis include investigation of the effects of cholinergic and anticholinergic agents in young healthy normal volunteers. The design of these investigations involve recording VEPs repeatedly during the day. Hence it is important to know if there is any time of day effects on the VEPs when no drug is given. In addition, VEPs are routinely used in the clinical diagnosis of many psychiatric and neurological disorders (see section 3.6). These VEPs can be recorded anytime between 9 am-6 pm during routine clinical conditions. Hence, it is useful to know the extent of time of day effects on the VEPs. This chapter, includes the investigation of the time of day effects on the flash and pattern reversal VEPs in young healthy volunteers.

Spontaneous EEG activity has been known to be affected by diurnal variation. From a review by Harding and Thompson (1977); Bjerner (1949) reported sleep-deprived subjects have the lowest frequency of the alpha activity between 4 am and 6 am; Frank (1961) and Frank et al. (1966) reported circadian variation in alpha, beta, theta, and delta activities with peak EEG output occurring between 9.30 am and 12.30 pm, the amplitude of change being enhanced in the sleep-deprived subjects; Naitoh et al. (1969) reported circadian variation to be absent during the first day of recording but enhanced after 50 hours of sleep deprivation, with maximal alpha percent times occurring at 9.00 am and 9.00 pm and minimal at 3.00 am and 3 pm; Scheich (1969) recording EEG in non-sleep deprived subjects found the consistent pattern of a high alpha index in the morning at 8.00 am, lowest before lunch at 11.00 am, an increase after lunch, and a slight decrease at 6.00 pm. Thompson (1967) and Thompson and Harding (1968) examining the relationship between biochemical and behavioural changes and circadian variation in the EEG only found changes in the harmonic mean frequency in the EEG, ranging from 0.4 to 0.6 cps, with lowered EEG frequencies occurring between 3.00 am and 4.00 am and the highest between 6.00 pm and 10.00 pm. Thus, generally the alpha frequency seems to be lowest in the morning and highest during the evening.

In contrast, relatively little work has been carried out on the diurnal effects on the VEP, eventhough the VEP has been studied in detail for over 30 years. Stolz et al.

(1987 and 1988), investigated the effects of the time of day on the VEP to the pattern reversal stimulus (to 55' checks), together with other physiological parameters (such as oral temperature, urine volume and urine potassium excretion) and psychomotor performance (such as grip strength, tapping rate, visual reaction time and performance on a letter cancellation test). Measurements taken hourly over a 30 hour period, demonstrated pronounced circadian variations for the physiological parameters and psychomotor performance. Circadian variation was also demonstrated for the latencies of the pattern reversal P100 and N140 components, which were longer during early morning (between 2 am-5 am) and shortest at 5 pm. The N75-P100 (N80-P100 in their terminology) amplitude was lowest at 5 am and reached a maximum at 11 pm. During daylight hours, the average shortening of the P100 latency was around 4 ms, being of shortest latency around midday. Unfortunately, the authors did not show any standard deviation or error values for each test session, thus the extent of the inter-individual variability remains unknown. In addition, these workers used the Oz-Cz montage, hence activity under the Cz site may have had an influence on the results (Harding 1985).

Lu and Spafford (1989) also investigated the effect of time of day on the pattern reversal VEP (to 14' checks) during daylight hours, comparing steady state and transient recordings over 9 hours during daytime. However, in contrast to the findings of Stolz et al. (1987 and 1988), these workers found no significant diurnal or fatigue effects on the VEP recordings.

The diurnal effects on the VEP to the flash stimulus, have not been investigated in such detail as those to the pattern reversal stimulus. Heninger et al. (1969), investigated the diurnal effects of the flash VEP, auditory (AEP) and somatosensory evoked potentials (SEP) together with the resting EEG and urine and plasma 17-hydroxycorticosteroid levels, over two sessions, one in the morning and one in the evening. SEPs were recorded from C3-A1, AEPs from Cz-A1 and VEPs from O1-A1, and Cz-A1. There were increases in latencies in the morning for all three modalities but reached statistical significance only in the VEP at Cz-A1 channel and the SEP. However Harding (1985), advised against the use of the vertex (Cz) and mastoids (A1 and A2) as reference sites, as they can be "too active" in relation to VEPs. In addition, since there was no significant change in the montage which included an occipital recording site (O1-A1), only in the Cz-A1 channel, this suggests that activity over the vertex is susceptible to diurnal variation, but not that over the occipital cortex. However, since the vertex is not commonly used as an active site in recording VEPs, the results of the study by Heninger et al. (1969) must be viewed with caution.

Kerkhof et al. (1980), recorded oral temperature, AEP and flash VEP at 8.00 am and 8.00 pm in "morning-" and "evening-type" subjects (as determined by a questionnaire). The morning-type subjects displayed no difference in oral temperature but larger N1-P2 amplitude of both the AEP and VEP. These results were attributed to a difference in vigilance levels in the two types of subjects. No measure of latency was undertaken, as these workers thought that the sample size (9 subjects per group) was too small. These workers also did not specify any detail recording parameters, with the results of the AEPs and VEPs being cited as taken from "Cz-recording". Therefore, as before this is an inappropriate recording site for VEPs (Harding 1985). In addition the flash was elicited by a red light emitting diode (LED) stimulator, which has to be viewed with some caution, as this stimulus being at threshold levels, is likely to be affected by ocular factors such as pupil size, which in turn will be affected by the lighting levels during the morning and evening sessions.

Since both the studies above (Heninger et al. 1967; Kerkhof et al. 1980) measured diurnal effects using only two recording sessions, one in the morning and one in the evening, the effect of the time of day still remains uncertain for the flash VEP, regardless of the stimulating procedures used.

Zimmermann et al. (1983) investigated diurnal variations of the VEP to both flash and pattern reversal (to 55' checks) stimuli, but only to evaluate possible effects on hemispheric differences. No data was presented for the actual latency or amplitude values, only the difference between the two hemispheres. These workers later investigated the effect of monotonously repeated stimulation of the VEPs on subjects grouped according to their personality types, but no significant effect was found (Görtelmeyer and Zimmermann 1984). Thus in both of these studies the diurnal effects on the recorded VEPs themselves were not investigated.

Hence there are conflicting reports of diurnal effects on the pattern reversal VEP (Stolz et al. 1987 and 1988; Lu and Spafford 1989) and no detailed investigation of the time of day effects on the flash VEP. Therefore a study of the effects of the time of day on the transient VEP to both flash and pattern reversal stimuli was carried out.

6.2 Methods

6.2.1 Subjects

5 healthy non-smoking male volunteers, mean age 26 years (range 24-26 years) were paid to participate in the study. All subjects were either staff or postgraduate members

of the Vision Sciences Department at Aston University. All subjects displayed a visual acuity of 6/6 or better with correction where needed.

6.2.2 Instrumentation

The flash stimulus was delivered by a Grass PS22 stroboscope at intensity 2 (luminance of 1363 cd/m^2) delivered at a rate of 1.7 flashes per second. The stimulator was placed 30 cm away, subtending a field of 36^o to the eye. The pattern reversal stimulus consisted of a checkerboard pattern of both 56' and 27' sized checks in a field subtending 27^o 50' x 22^o 24' to the eye and reversing at a rate of 1.7 Hz. The luminance of the white checks was 170 cd/m^2 and the contrast was 78%. The stimulus was delivered by a Hitachi TV monitor (model VM-173E/K).

The visual stimuli were triggered by a 5V TTL pulse from the Bio-Logic Traveler™ electrodiagnostic testing system with twin disk drives. The bandpass filtering was set at 0.3-30 Hz (-3dB down point), and the gain at 30,000. Two runs consisting of 50 averages were carried out for each eye, totalling in an average of 100 responses per eye.

Monocular stimulation was performed, using silver-silver chloride electrodes placed on the scalp according to the international 10-20 system (Jasper 1958). The VEPs were recorded using the standard derivations of O2 referred to C4 and O1 referred to C3 for the flash stimulation and O2 and O1 both referred to Fz for the pattern reversal stimulation (Hobley 1988; Hobley and Harding 1989). The interelectrode impedance was maintained below 5kΩ.

6.2.3 Procedure

The VEPs were recorded hourly over 13 hours, from 8 am until 8 pm (800-2000 hours), incorporating the time period when routine clinical investigations are carried out. For comparable results with normal daily clinical situations, no special experimental conditions were applied (e.g. control of absolute sleep time, dietary control, caffeine intake etc.), in order to minimise upsetting the "natural" circadian rhythm of the individuals.

6.3 Results

6.3.1 Data analysis

The latencies and amplitudes of the most consistent components of the VEPs and those most used clinically were determined (Harding 1974) as well as the later components.

Hence for the flash VEP, the N2, P2, N3 and P3 latencies and the N2-P2, P2-N3 and N3-P3 peak-to-peak amplitudes were analysed. The early N1 and P1 flash components were not consistently present in all subjects and thus not analysed. For the pattern reversal VEP, the N75, P100, N140 and P165 latencies and the N75-P100, P100-N140 and N140-P165 peak-to-peak amplitudes were analysed. For clarity, the analysis of results were confined to those from the right hemisphere and eye, as no consistent differences between the hemispheres or eyes were observed.

Kendall's W test for concordance was applied to determine the statistical significance of any time of day effects on the latencies and amplitudes of the VEPs. The level of significance was confined to $P<0.05$ level.

6.3.2 Flash VEP

The flash VEP displayed consistent responses throughout the day in all subjects. An example of the flash VEP as recorded during the day has been illustrated in fig. 6.1. The major positive P2 component was clearly defined and showed little variation throughout the day. For comparison, the flash responses were averaged from all five subjects and shown in fig. 6.2. This averaged waveform showed even less variation in response over the day. However, the averaged waveform does not account for any inter-individual variations, hence all analyses of latencies and amplitudes will be from responses from each individual.

The mean latencies for the flash VEP components have been shown in table 6.1 and illustrated in fig. 6.3. The most striking feature of the plot of the mean latencies of the flash VEP components, was that the later a components occured, the greater its variation in latency and larger standard deviation values. The increase in the inter-individual variation with increase time of occurrence of the components has been previously reported (Ciganék 1961 and 1975; Halliday 1982).

The principle N2 and P2 components showed the least change in latency over the day. The major trend in the N3 latency was an increase towards the end of the test session (1900-2000 hours), whereas the P3 latency showed an increase at 1200 and 2000 hours and a decrease at 1600 and 1800 hours. However, statistical analysis revealed no significant variation in latency in any of the flash VEP components.

The mean amplitude values for the flash VEP have been presented in table 6.2 and illustrated in fig. 6.4. The N2-P2 amplitude showed a gradual increase during the day. The P2-N3 amplitude showed an initial increase over 800-1000 hours, followed by a decrease over 1100-1300 hours before showing a gradual increase over the rest

Figure 6.1 Example of the responses to the flash VEP obtained in a single subject recorded over 13 hours. The major positive P2 component is clearly defined and shows little change over the day. The responses were recorded from the right eye and channel O2-C4.

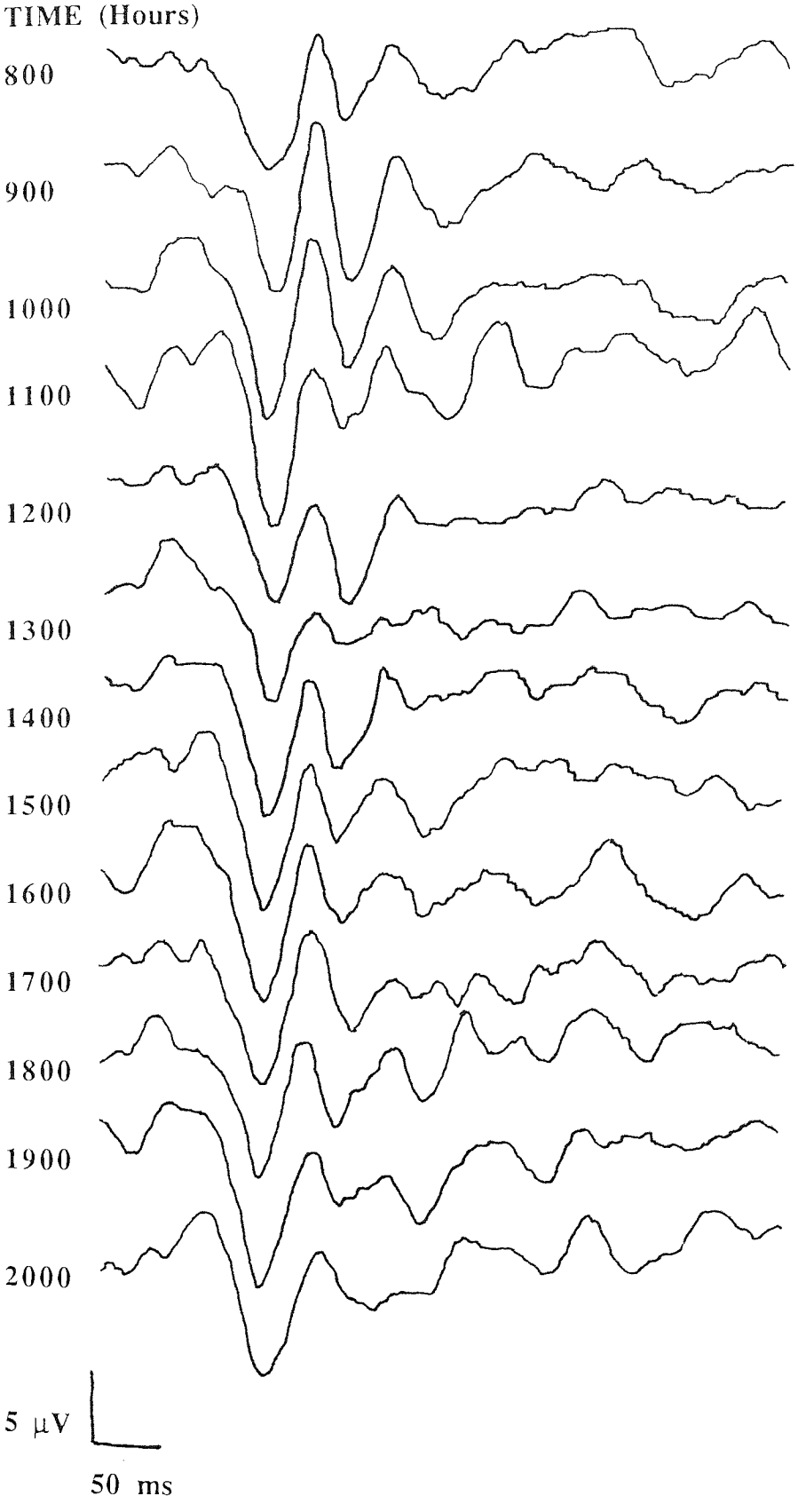


Figure 6.2 Group averaged responses for the flash VEP recorded over 13 hours. The major components are broader due to the inter-individual variation. There seems even less variation in the flash VEP throughout the day than with the individual responses. The responses were recorded from the right eye and channel O2-C4.

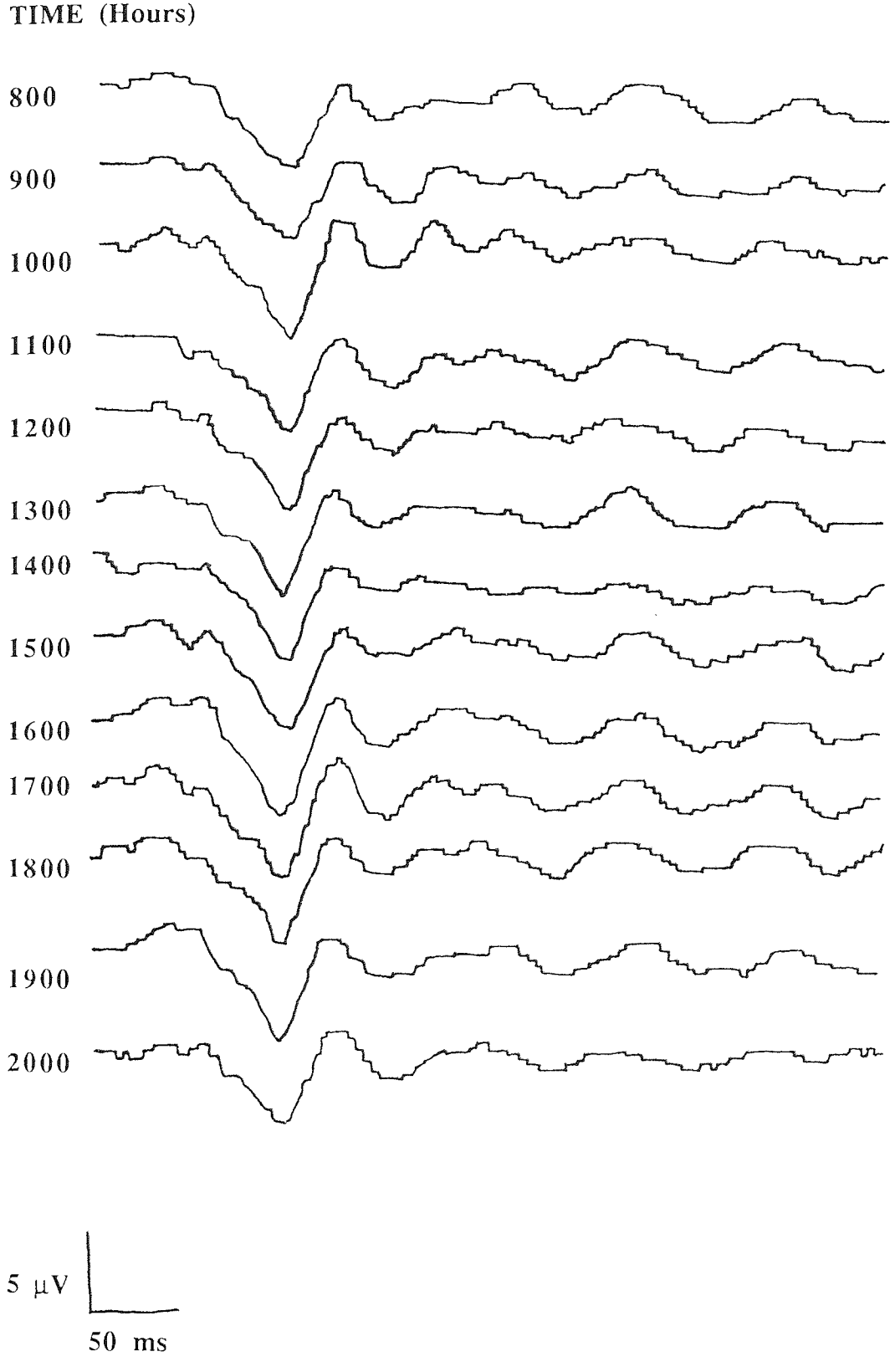


Table 6.1 Mean latency values of the flash VEP components ± standard deviation (SD) recorded from 800-2000 hours. Data from right eye and channel O2-C4.

Time	Latency (ms) ± SD			
(Hours)	N2	P2	N3	P3
800	73.24 ± 7.62	117.19 ± 12.72	154.59 ± 9.54	178.81 ± 13.58
900	73.54 ± 7.41	115.72 ± 12.03	155.28 ± 9.54	183.11 ± 15.23
1000	73.34 ± 5.14	116.90 ± 6.36	155.18 ± 4.08	185.06 ± 6.98
1100	72.85 ± 5.83	118.26 ± 11.06	154.89 ± 7.24	181.16 ± 10.94
1200	72.66 ± 6.82	118.36 ± 10.86	155.18 ± 8.93	190.68 ± 14.55
1300	73.24 ± 6.93	117.68 ± 10.49	154.10 ± 5.71	184.96 ± 19.45
1400	71.88 ± 4.90	117.29 ± 12.15	155.96 ± 11.55	182.52 ± 20.37
1500	71.68 ± 4.31	117.68 ± 11.00	154.49 ± 9.05	183.30 ± 18.55
1600	71.58 ± 4.42	117.58 ± 10.93	152.35 ± 6.14	179.10 ± 11.42
1700	70.31 ± 3.33	117.77 ± 9.71	153.71 ± 5.72	182.91 ± 7.83
1800	70.71 ± 4.65	118.33 ± 9.59	154.10 ± 4.61	180.96 ± 13.45
1900	69.93 ± 4.75	116.60 ± 10.07	157.33 ± 11.34	186.33 ± 11.47
2000	71.39 ± 4.95	116.80 ± 10.48	159.47 ± 10.86	189.94 ± 5.56

Figure 6.3 Graph of the mean latencies for the flash VEP components recorded over 13 hours. The vertical bars indicate standard deviation.

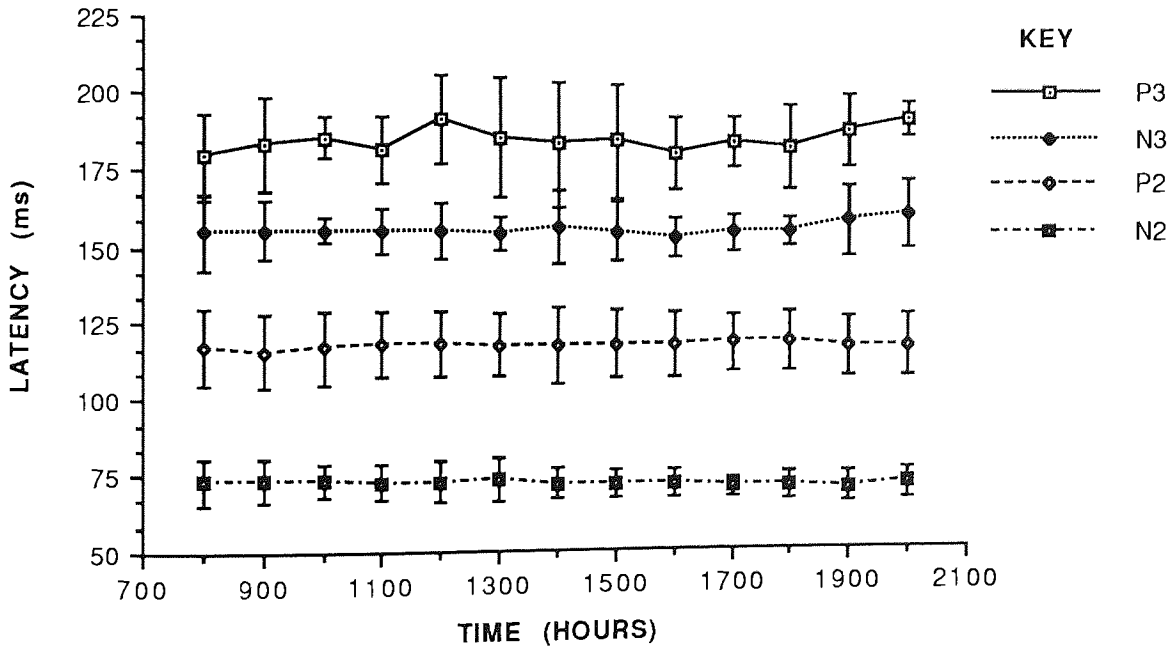


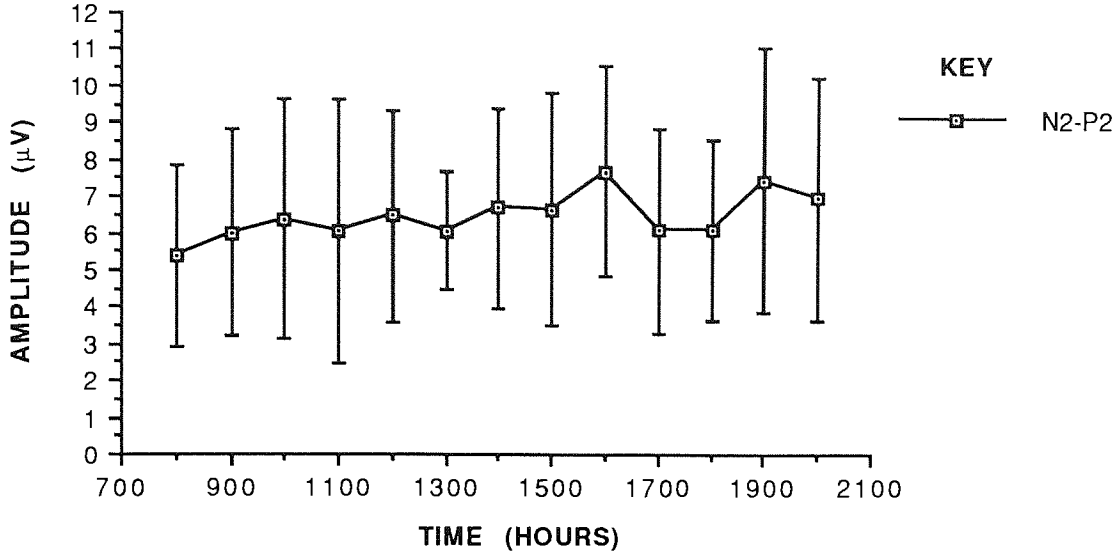
Table 6.2 Mean amplitude values of the flash VEP components ± standard deviation values (SD) recorded from 800-2000 hours. Data from right eye and channel O2-C4.

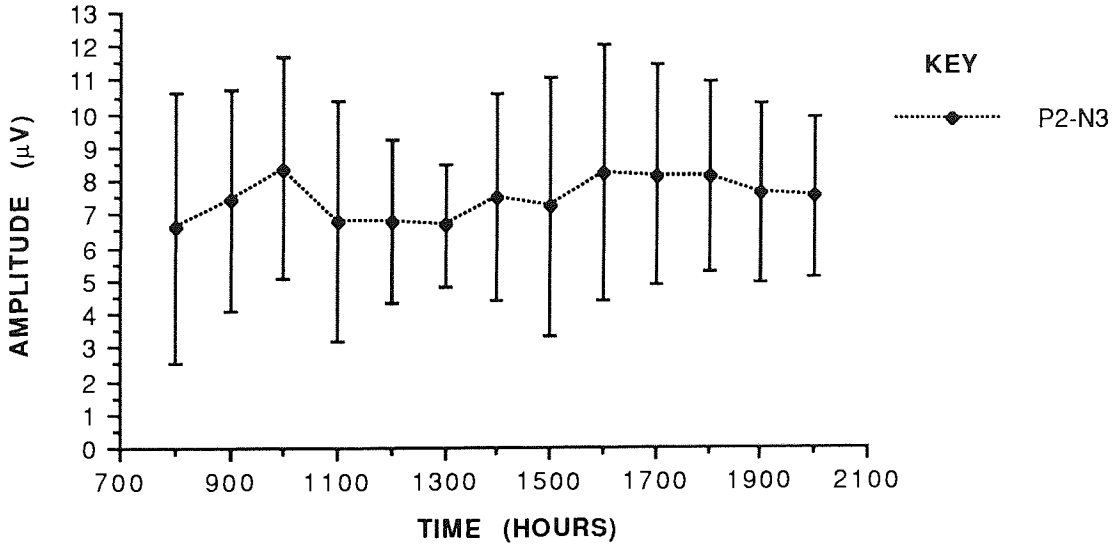
Time	Amplitude (μV) ± SD		
(Hours)	N2-P2	P2-N3	N3-P3
800	5.34 ± 2.45	6.59 ± 4.07	3.75 ± 1.55
900	5.98 ± 2.78	7.43 ± 3.32	4.91 ± 2.59
1000	6.36 ± 3.24	8.37 ± 3.30	4.50 ± 3.09
1100	6.02 ± 3.57	6.77 ± 3.58	4.44 ± 1.59
1200	6.45 ± 2.87	6.79 ± 2.43	3.67 ± 1.94
1300	6.07 ± 1.63	6.67 ± 1.87	3.64 ± 1.75
1400	6.70 ± 2.72	7.53 ± 3.08	3.61 ± 1.74
1500	6.67 ± 3.20	7.27 ± 3.89	2.95 ± 2.02
1600	7.70 ± 2.85	8.28 ± 3.83	4.24 ± 2.19
1700	6.08 ± 2.80	8.21 ± 3.29	4.04 ± 1.82
1800	6.10 ± 2.47	8.17 ± 2.84	4.03 ± 1.86
1900	7.49 ± 3.60	7.71 ± 2.71	4.02 ± 1.98
2000	6.97 ± 3.33	7.57 ± 2.39	4.54 ± 1.86

of the day. The N3-P3 amplitude also showed an increase in the morning at 900 hours, followed by a decrease in the afternoon with a trough being reached at 1500 hours before increasing towards the end of the day. The N3-P3 amplitude was about half the magnitude to that of the N2-P2 and P2-N3 amplitudes, which were of similar magnitudes. Overall, the inter-individual variation, as shown by the error bars (fig. 6.3), was much greater for the flash VEP amplitudes. This was as previously reported (Ciganék 1961 and 1974; Halliday 1982). As with the latencies, there was no statistical difference in the flash VEP amplitude values over the 13 hours.

In order to magnify and obviate the differences in both mean latency and amplitude between the subjects, the percentage deviation from the overall daily mean was calculated. The major trends in latency included the N2 component (fig. 6.5) having longer latencies during the morning session (800-1300 hours) and shorter latencies during the afternoon session. There was a small reduction in the P2 latency (1.43%) at 900 hours, but overall there was very little change from the overall mean (less than 1%). In comparison to the N2 and P2, the later occurring N3 and P3 components displayed greater change in latency throughout the day. There was little change in the N3 latency during the morning and afternoon (below 1% of mean), but there was an increase during the early evening (1800-2000 hours). The P3 displayed the shortest latency during the early morning (800 hours), followed by an increase reaching a peak

Figure 6.4 Mean amplitudes for the flash VEP components recorded over 13 hours. The data was recorded from the right eye and channel O2-C4.





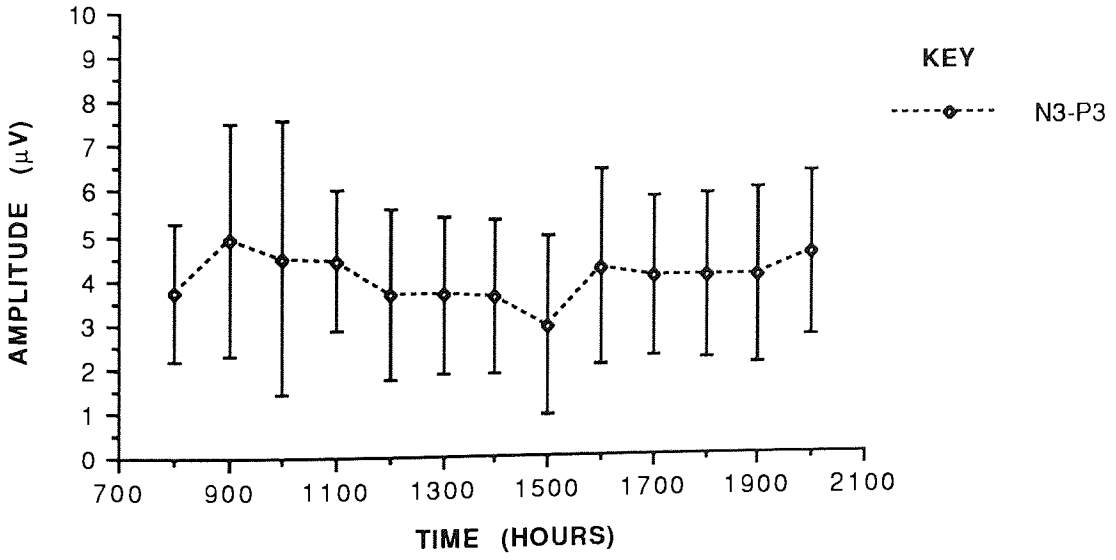
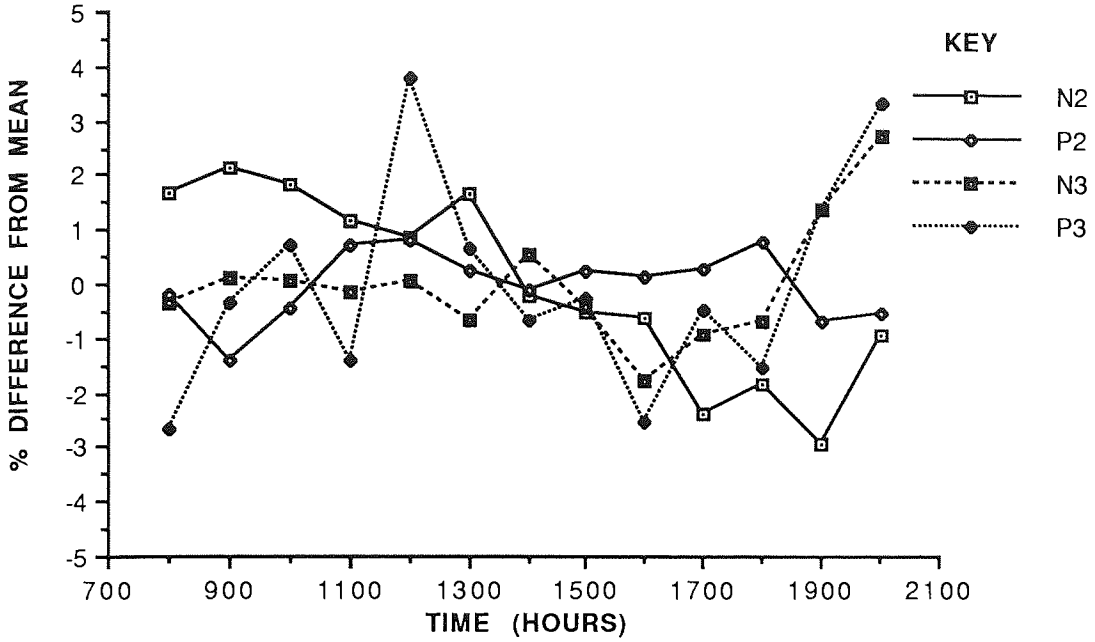


Figure 6.5 Graph showing the percentage change in latencies of the flash VEP components recorded over 13 hours.



at midday, followed by another shortening during the afternoon, but again during the evening there was an increase similar to that of the N3 component.

The percentage deviation from the mean of the flash VEP amplitudes has been shown in fig. 6.6. The percentage amplitude displayed greater deviation from the mean than did the latency values. The N3-P3 amplitude displayed the greatest percentage deviation from the mean. There was a sharp increase in the N3-P3 amplitude at 900 hours (21.84%) followed by a fall reaching a trough at 1500 hours (fall of 26.80%), after which there was another increase in amplitude during the rest of the day. The N2-P2 amplitude displayed a staggered increase in amplitude throughout the day. The P2-N3 amplitude showed a similar trend to that of the N2-P2 amplitude, except these changes were of greater magnitude with the P2-N3.

The results of the statistical analysis have been shown in table 6.3. There was no statistical circadian variation in either the latency or amplitude of the flash VEP. The range of fluctuation for the latencies of the flash VEP was 5.0%, 2.2%, 4.6%, and 6.5% for the N2, P2, N3, and P3 components respectively. Hence the later the component occurred, the greater its latency varied over the day. Similarly the range of fluctuation in the amplitude was 36.5%, 23.8%, and 48.6% for the N2-P2, P2-N3 and N3-P3 amplitudes. There was greater variation in the N2-P2 amplitude than the P2-N3, the later was also slightly larger overall.

Figure 6.6 Graph showing the percentage change in latencies of the flash VEP components recorded over 13 hours.

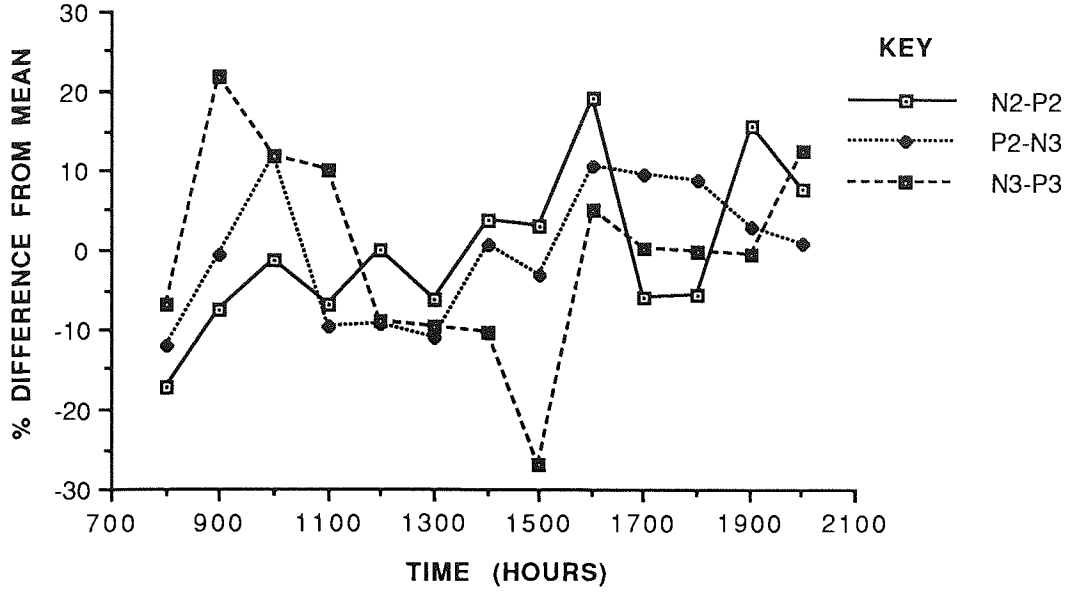


Table 6.3 Summary of the statistical analysis of the flash VEP recorded over 13 hours. The table group mean and standard deviation (**SD**) values, the difference between the maximum and minimum value (**range**), Chi square value (X^2) and significance at $P<0.05$ (**P**).

	Mean	**SD**	**Range**	X^2	**P**
Latency (ms)					
N2	72.03	1.22	3.61	19.80	NS
P2	117.40	0.76	2.61	9.07	NS
N3	155.13	1.76	7.12	5.28	NS
P3	183.76	3.65	11.87	6.35	NS
Amplitude (μV)					
N2-P2	6.46	0.65	2.36	11.73	NS
P2-N3	7.49	0.64	1.78	11.59	NS
N3-P3	4.03	0.51	1.96	9.95	NS

6.3.3 Pattern reversal VEP

The pattern reversal VEP to both the 56' and 27' check sizes were consistently recorded in all subjects throughout the 13 hours. An example of the response obtained in a single subject has been shown in fig. 6.7 to the 56' check and fig. 6.8 to the smaller 27' check. The waveforms to both check sizes displayed similar responses. The most prominent component in the pattern reversal VEPs was the major positive P100 component. From figs. 6.7 and 6.8, the shape of the P100 component seemed to vary throughout the day, although the peak latency remained unaltered. The later occurring N140 and P165 components were not as clearly identifiable throughout the day. For instance, the N140 component for the pattern reversal VEP to the 56' check size (fig. 6.7), was broader during the early morning (800-900 hours), when compared to the rest of the day. Hence the subsequent identification of the P165 component at these times became difficult.

As with the flash VEP, the waveforms of the pattern reversal VEPs were averaged and shown in fig. 6.9 for the 56' check and fig. 6.10 for the 27' check. Due to cancellation of the inter-individual variation, the waveforms were again much more consistent than the individual responses. The P100 component was more defined and showed little change throughout the day. However the later N140 and in particular the P165 components were diminished and were not clearly identified.

The mean latency values of the pattern reversal VEP components to the 56' check, have been presented in table 6.4 and illustrated in fig. 6.11. As with the flash VEP, there was greater variation in the latency and standard deviation values with the later occurring components. However, this was more pronounced for the pattern reversal than the flash VEP. The N75 component displayed little change in the latency throughout the 13 hours and was associated with the smallest standard deviation values. The P100 component also displayed little variation in the peak latency values throughout the day. The N140 component displayed a slight shortening in latency during 1100-1200 hours and a slight increase during 1500-1600 hours, but overall there was still little change. In contrast, the P165 component displayed a series of increases and decreases in latency throughout the day. There was a decrease in P165 latency during the morning until 1100 hours, followed by an increase until 1300 hours, then another decrease in latency up until 1500 hours, followed by another increase at 1600 hours, before showing another decease up until 1800 hours and finally an increase up to 2000 hours.

Figure 6.7 Example of the responses to the pattern reversal VEP to the 56' check size, obtained in a single subject recorded over 13 hours. The major positive P100 component is clearly defined and shows little change in latency over the day. The responses were recorded from the right eye and channel O2-Fz.

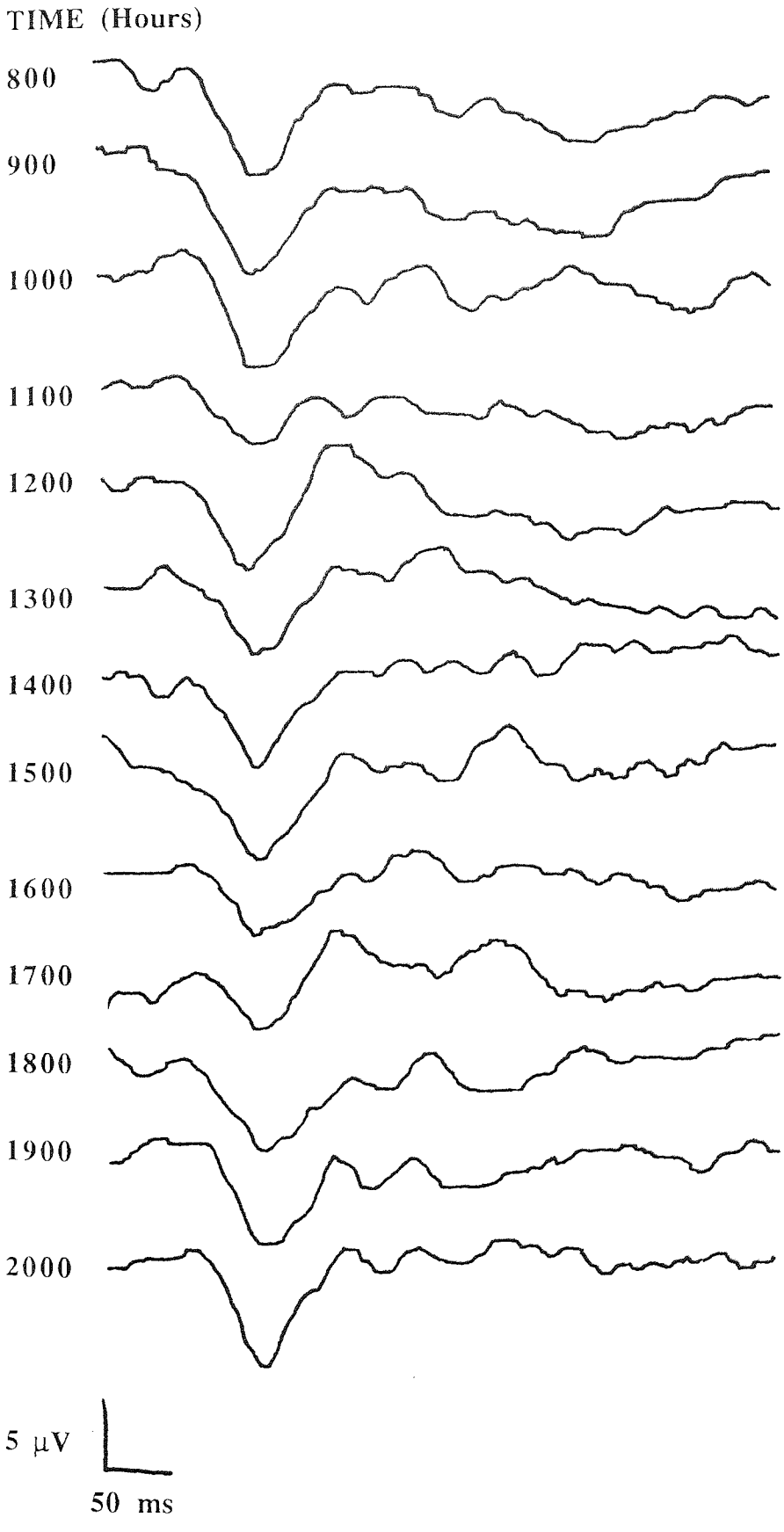


Figure 6.8 Example of the responses to the pattern reversal VEP to the 27' check size, obtained in a single subject recorded over 13 hours. The major positive P100 component is clearly defined and shows little change in latency over the day. The responses were recorded from the right eye and channel O2-Fz.

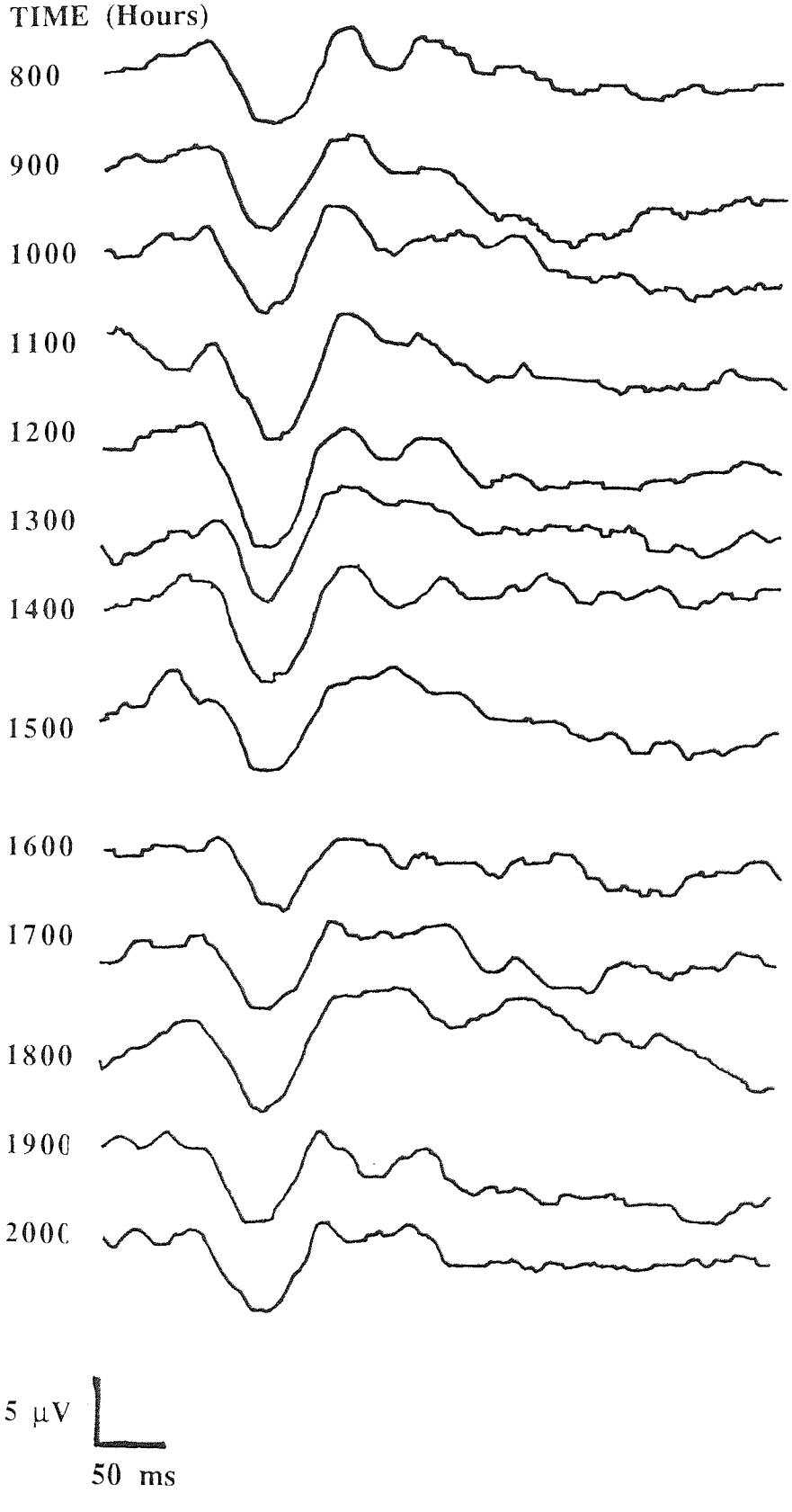


Figure 6.9 Group averaged responses for the pattern reversal VEP to the 56' check size recorded over 13 hours. The major P100 component is broader due to the inter-individual variation. There seems even less variation in the waveform throughout the day than with the individual responses. The responses were recorded from the right eye and channel O2-Fz.

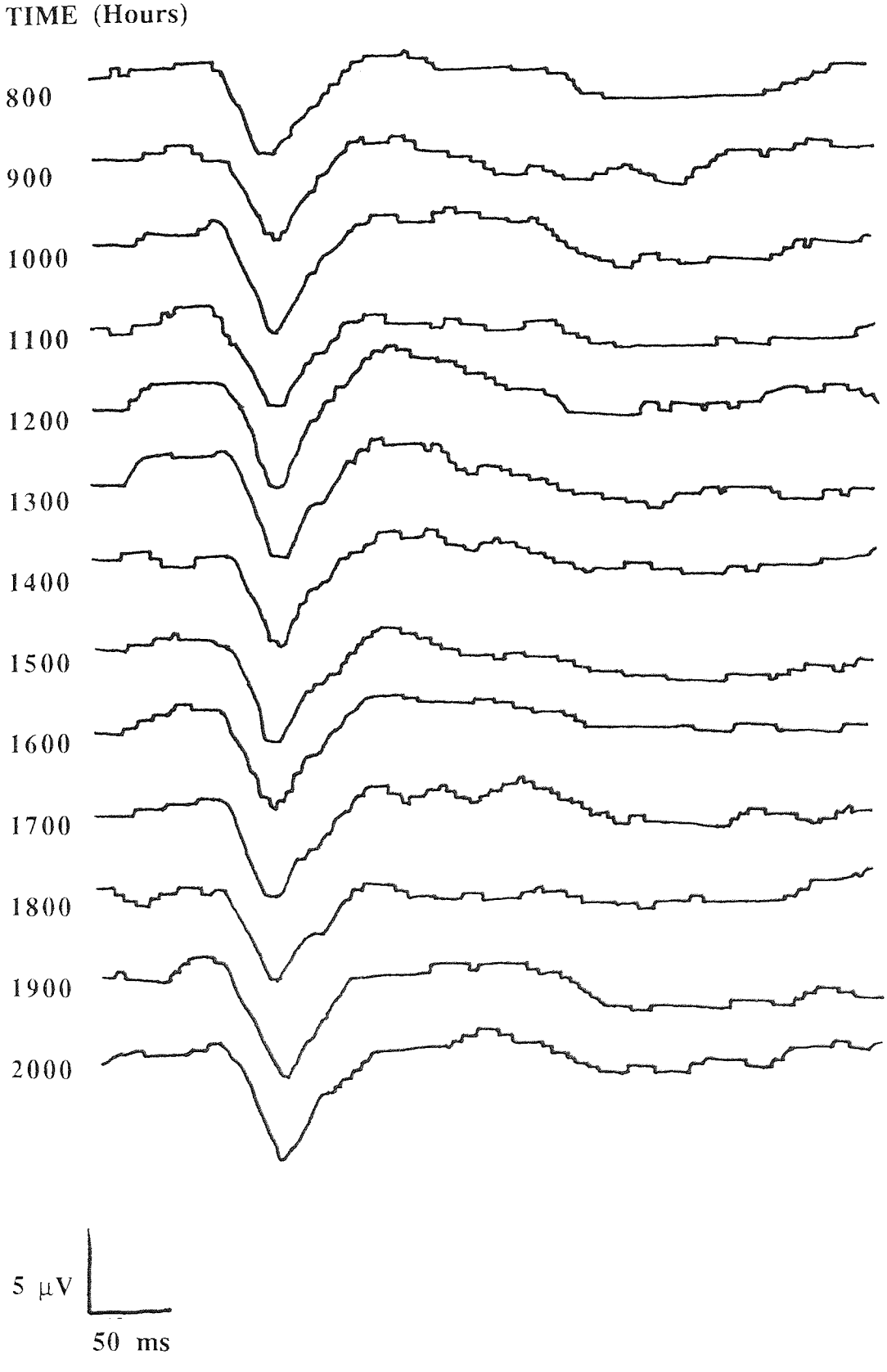
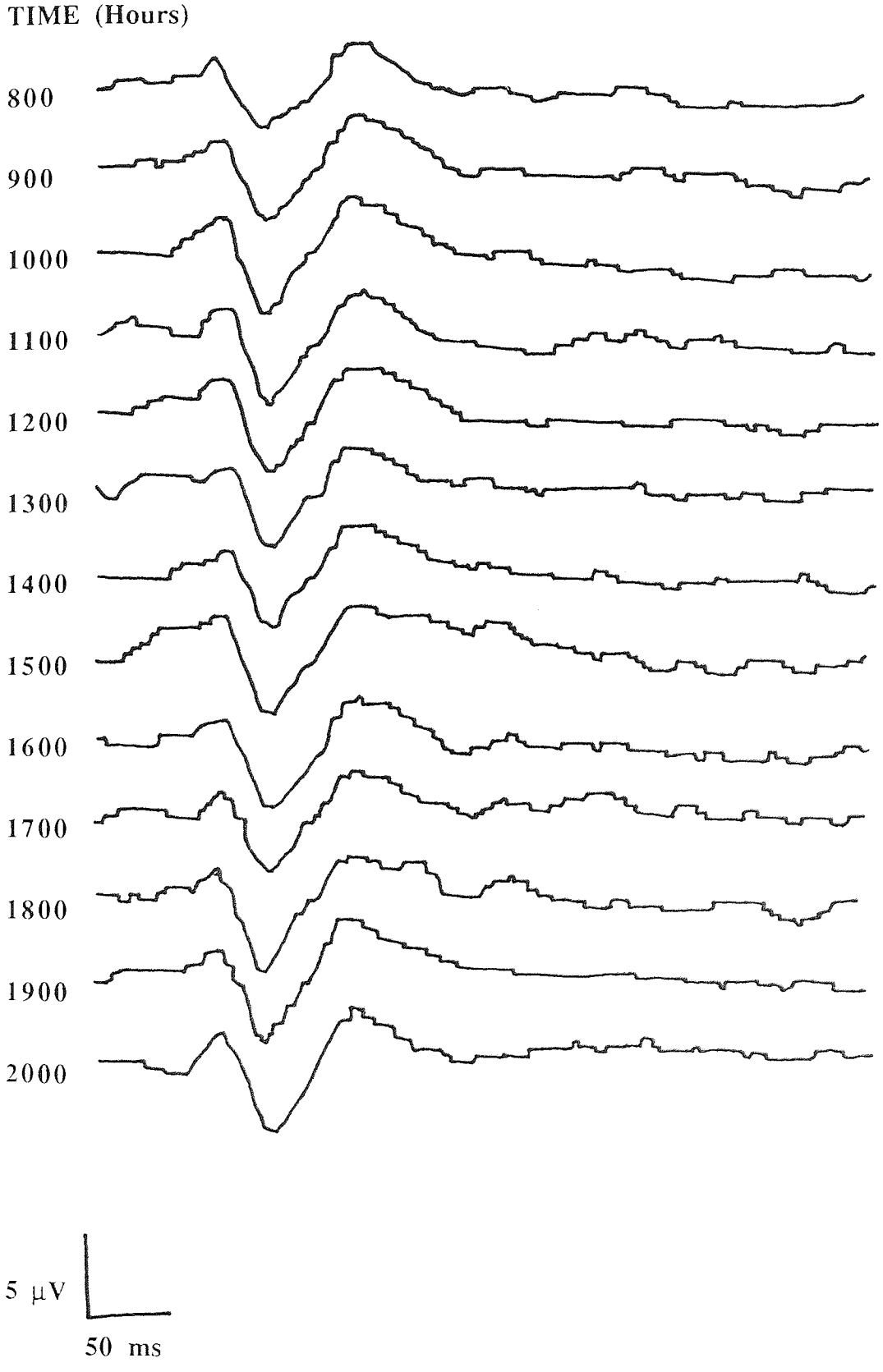


Figure 6.10 Group averaged responses for the pattern reversal VEP to the 27' check size recorded over 13 hours. The major P100 component is again broader due to the inter-individual variation. The P165 component is diminished. There seems even less variation in the waveform throughout the day than with the individual responses. The responses were recorded from the right eye and channel O2-Fz.



The mean latencies for the pattern reversal VEP to the smaller 27' have been presented in table 6.5 and illustrated in fig. 6.12. The mean latency of the pattern reversal VEP components to the 27' check size displayed similar trends to those obtained with the larger 56' check size. The N75 component to the smaller check, displayed the smallest standard deviation values of the three stimuli examined. As before (with 56' check), there was very little variation in the N75 latency over the 13 hours. The P100 component also showed fairly consistent latency and standard deviation values throughout the day. The N140 component of the pattern reversal VEP to 27' check, displayed smaller standard deviation values when compared to 56' check and there were only small variations in latency over the day. The P165 component displayed the greatest standard deviation values, as expected for a late occurring component. There was a shortening in the P165 latency around 1400 hours and again at 1700-1800 hours, similar to that obtained with the 56' check. Overall, the difference in the check size of the pattern reversal stimuli made little difference to the latency of the VEP components over the 13 hours.

Table 6.4 Mean latency values of the pattern reversal VEP (56' check size), components ± standard deviation (SD) recorded from 800-2000 hours. Data from right eye and channel O2-Fz.

Time	Latency (ms) ± SD			
(Hours)	N75	P100	N140	P165
800	74.71 ± 3.31	117.29 ± 11.98	167.19 ± 21.53	196.78 ± 26.90
900	76.86 ± 5.63	117.87 ± 11.71	167.87 ± 18.40	192.63 ± 21.08
1000	75.59 ± 3.91	116.60 ± 9.17	169.34 ± 22.96	188.80 ± 25.96
1100	73.83 ± 4.11	117.09 ± 10.64	163.18 ± 21.32	186.89 ± 23.41
1200	74.91 ± 5.60	115.34 ± 9.59	163.68 ± 22.24	193.36 ± 24.77
1300	76.27 ± 4.40	116.41 ± 8.28	169.34 ± 28.91	205.41 ± 9.87
1400	76.27 ± 4.84	115.24 ± 9.86	164.68 ± 21.14	196.78 ± 25.78
1500	76.57 ± 5.28	115.63 ± 9.32	170.90 ± 24.31	184.41 ± 22.99
1600	75.79 ± 3.77	115.33 ± 9.24	169.83 ± 23.62	200.19 ± 8.46
1700	75.98 ± 3.55	116.02 ± 8.68	165.14 ± 19.83	188.11 ± 21.31
1800	74.32 ± 3.07	114.75 ± 8.63	167.09 ± 24.07	180.01 ± 32.51
1900	73.44 ± 3.43	116.80 ± 8.50	166.02 ± 16.20	186.28 ± 19.70
2000	74.51 ± 4.15	116.02 ± 9.14	166.89 ± 21.51	187.63 ± 23.26

Figure 6.11 Graph of the mean latencies for the pattern reversal VEP (to 56' check size) components recorded over 13 hours. The vertical bars indicate standard deviation.

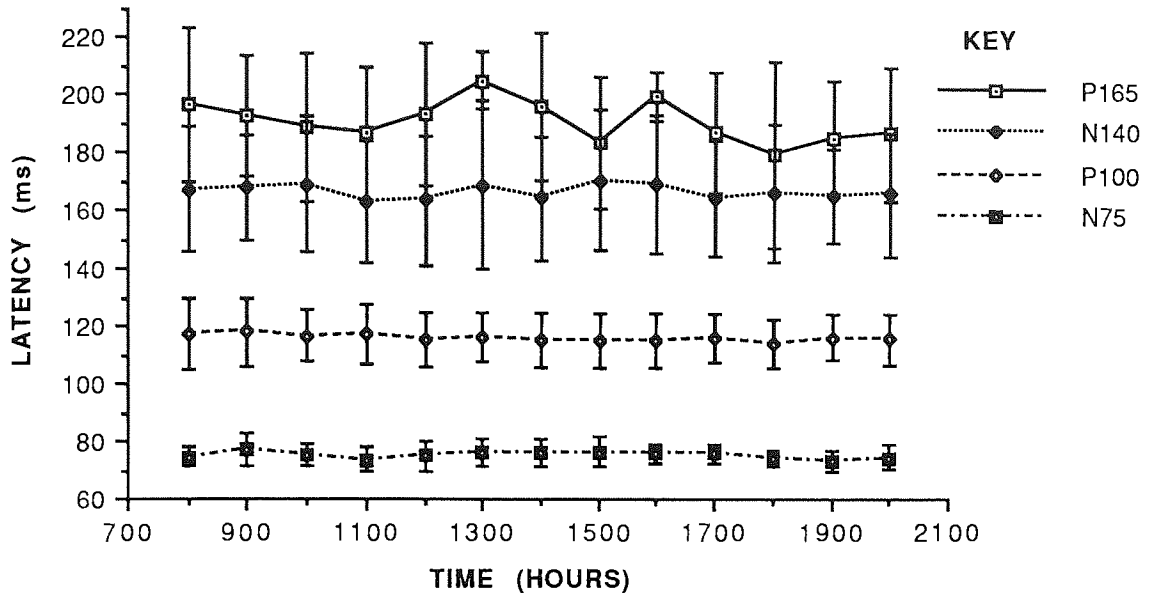
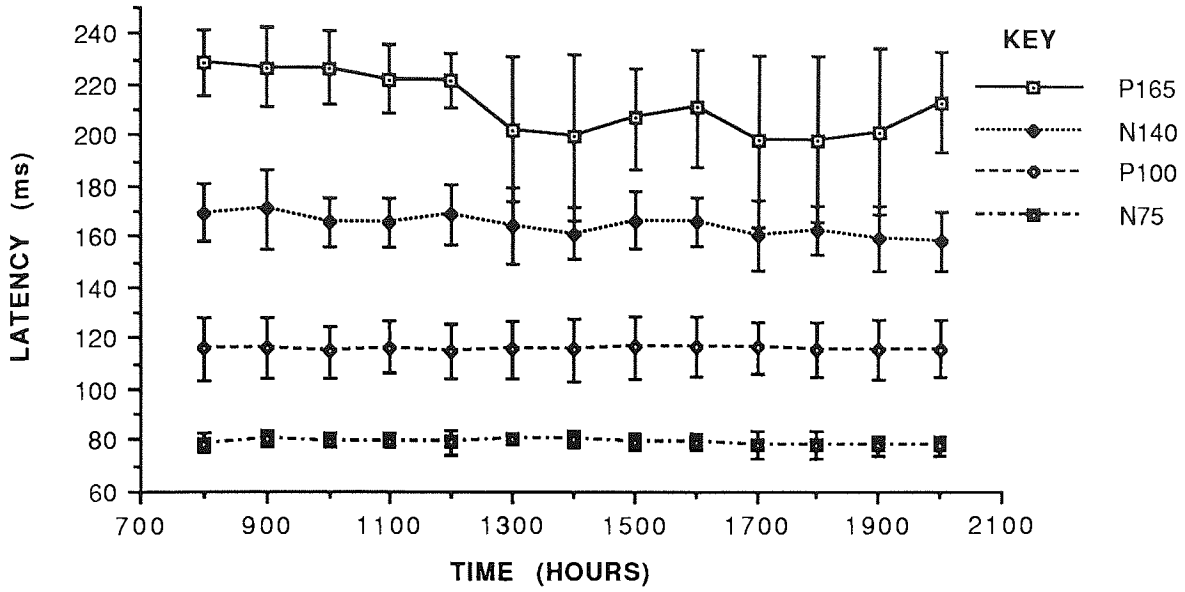


Table 6.5 Mean latency values of the pattern reversal VEP (27' check size), components ± standard deviation (SD) recorded from 800-2000 hours. Data from right eye and channel O2-Fz.

Time	Latency (ms) ± SD			
(Hours)	N75	P100	N140	P165
800	78.52 ± 3.64	115.73 ± 12.52	169.34 ± 11.12	228.72 ± 12.85
900	80.37 ± 3.17	116.41 ± 11.92	170.90 ± 15.50	226.37 ± 15.57
1000	79.88 ± 2.62	114.95 ± 10.36	165.53 ± 9.87	226.27 ± 14.57
1100	79.83 ± 2.85	116.51 ± 10.58	166.11 ± 9.85	222.17 ± 13.34
1200	79.01 ± 4.73	114.94 ± 10.50	169.14 ± 11.89	221.56 ± 10.64
1300	80.57 ± 1.86	116.02 ± 11.38	164.75 ± 15.43	202.64 ± 28.62
1400	80.67 ± 3.35	115.82 ± 12.62	161.92 ± 10.41	200.10 ± 32.91
1500	79.59 ± 3.18	116.90 ± 12.54	167.00 ± 11.35	207.65 ± 19.94
1600	79.59 ± 3.26	117.39 ± 11.96	166.60 ± 9.59	211.92 ± 23.25
1700	78.62 ± 5.20	116.70 ± 10.25	161.33 ± 14.33	199.12 ± 34.02
1800	78.32 ± 4.96	116.41 ± 10.96	163.94 ± 9.66	199.80 ± 32.55
1900	77.93 ± 3.36	115.92 ± 11.96	160.06 ± 13.07	203.03 ± 33.41
2000	78.22 ± 3.90	116.12 ± 11.33	159.28 ± 12.34	214.60 ± 19.88

Figure 6.12 Graph of the mean latencies for the pattern reversal VEP (to 27' check size) components recorded over 13 hours. The vertical bars indicate standard deviation.



The mean amplitude values for the pattern reversal VEP (to 56' checks) have been presented in table 6.6 and illustrated in fig. 6.13. The N7-P100 amplitude displayed maximum values at 1000, 1300 and 1900 hours and lowest values at 900 and 1800 hours. The P100-N140 amplitude showed a maximum during 1200 hours and minimum values at 1100 and 1800 hours. The N140-P165 amplitude gradually increased during the morning up until 1200 hours, followed by a small fall at 1300 hours, after which there was no consistent change. Overall, the changes in the amplitude values were quite small.

The mean amplitude values for the pattern reversal VEP (to 27' checks) have been presented in table 6.7 and illustrated in fig. 6.14. The N75-P100 amplitude displayed larger values during the afternoon-evening session (1500-2000 hours) and lower values during the morning. The P100-N140 amplitude showed an increase from 800-1100 hours after which it became steady until a fall at 1700 hours. The N140-P165 amplitude displayed a staggered increase and decrease in values, but there was an overall decrease during the day.

The amplitude values of the N75-P100 and P100-N140 were of similar magnitude for both check sizes. However, the N140-P165 amplitude was slightly larger for the smaller 27' check size. There was no overall trend displayed by the amplitude of the pattern reversal VEP, mainly due to large inter-individual variations.

Table 6.6 Mean amplitude values of the pattern reversal VEP (to 56' check size) components ± standard deviation values (SD) recorded from 800-2000 hours. Data from right eye and channel O2-Fz.

Time	Amplitude (μV) ± SD		
(Hours)	N75-P100	P100-N140	N140-P165
800	7.12 ± 1.69	7.26 ± 1.63	2.74 ± 2.15
900	6.22 ± 1.58	7.28 ± 1.59	3.05 ± 2.00
1000	7.65 ± 1.75	7.91 ± 2.14	3.27 ± 1.76
1100	6.77 ± 1.98	7.19 ± 2.08	3.49 ± 2.22
1200	7.49 ± 1.82	9.51 ± 1.96	3.71 ± 2.37
1300	7.63 ± 3.06	8.78 ± 2.82	2.69 ± 1.74
1400	7.23 ± 1.80	8.68 ± 1.97	2.92 ± 2.05
1500	7.02 ± 2.05	8.48 ± 2.36	3.28 ± 1.36
1600	6.85 ± 2.33	8.19 ± 2.84	2.91 ± 2.18
1700	7.22 ± 2.50	8.13 ± 1.06	3.33 ± 1.04
1800	6.71 ± 2.27	7.65 ± 2.13	2.70± 1.98
1900	7.86 ± 2.29	8.49 ± 2.10	2.81 ± 1.39
2000	8.17 ± 2.44	8.65 ± 2.22	3.16 ± 2.48

Table 6.7 Mean amplitude values of the pattern reversal VEP (to 27' check size) components ± standard deviation values (SD) recorded from 800-2000 hours. Data from right eye and channel O2-Fz.

Time	Amplitude (μV) ± SD		
(Hours)	N75-P100	P100-N140	N140-P165
800	5.38 ± 0.52	6.21 ± 1.17	4.56 ± 1.84
900	6.05 ± 1.63	7.55 ± 2.38	5.66 ± 2.11
1000	6.85 ± 2.00	8.12 ± 1.85	5.18 ± 2.23
1100	6.67 ± 0.58	7.93 ± 0.87	4.42 ± 1.76
1200	6.54 ± 2.03	7.89 ± 1.69	5.10 ± 3.29
1300	6.05 ± 1.33	7.41 ± 1.58	3.75 ± 2.25
1400	5.82 ± 1.10	7.72 ± 2.53	4.69 ± 2.66
1500	7.20 ± 1.55	7.72 ± 2.51	3.67 ± 1.97
1600	7.00 ± 2.64	8.15 ± 2.28	4.26 ± 1.99
1700	6.55 ± 1.63	7.00 ± 1.31	4.24 ± 2.26
1800	7.08 ± 3.11	7.79 ± 2.02	4.15± 1.96
1900	6.87 ± 2.11	8.60 ± 2.89	4.90 ± 1.99
2000	7.48 ± 1.80	7.92 ± 2.36	4.31 ± 1.61

As with the flash VEP, in order to magnify any trends in the latency and amplitude, the percentage deviation from the mean was calculated. The percentage change in the latency of the pattern reversal VEP to 56' check has been shown in fig. 6.15. The N75, P100 and N140 components all displayed little change in latency over the 13 hours. The later P165 component however, displayed greater variation in the latency values over the day, particularly after midday. Longest peak P165 latency values occurred at 1300 and 1600 hours and shortest at 1100, 1500 and 1800 hours.

The percentage change in latency of the pattern reversal VEP to 27' checks has been shown in fig. 6.16. Again the earlier N75, P100 and N140 components displayed little change in latency over the day. However, the P165 latency displayed a gradual shortening of latency from 800-1200 hours, after which there was a sharp decrease in latency until 1400 hours, followed by an increase until 1600 hours, a decrease again at 1700 hours and finally an increase until 2000 hours.

Therefore there was little change in the latency values of the earlier N75, P100 and N140 components to the pattern reversal VEP, regardless of the check size. The later P165 however seemed prone to variation in latency throughout the day. The pattern reversal VEP also displayed less variation in latency in comparison to the percentage change in latency of the flash VEP (fig. 6.5).

The percentage change in the amplitude values of the pattern reversal VEP to 56' checks have been shown in fig. 6.17. All three amplitude measures displayed an alternating increase and decrease in values over the day. The N140-P165 displayed change of the greatest magnitude and the N75-P100 the least, through the day.

The percentage change in the amplitude of the pattern reversal VEP to 27' check has been shown in fig. 6.18. As before, the amplitude measures displayed an alternating increase and decrease in values. The N75-P100 and P100-N140 amplitudes displayed similar trends with lower amplitudes during the early morning and higher amplitudes during the evening. This was also reported by Stolz et al. (1987 and 1988). The N140-P165 amplitude again displayed greatest magnitude of change with higher amplitudes during the morning and lower in the afternoon.

The results of the statistical analysis has been shown in table 6.8 and 6.9 for the pattern reversal VEP to 56' and 27' checks respectively. There was no statistical circadian variation in either the latency or amplitude of the pattern reversal VEP to either check size.

Figure 6.13 Mean amplitudes for the pattern reversal VEP (to 56' check size) components recorded over 13 hours. The data was recorded from the right eye and channel O2-Fz

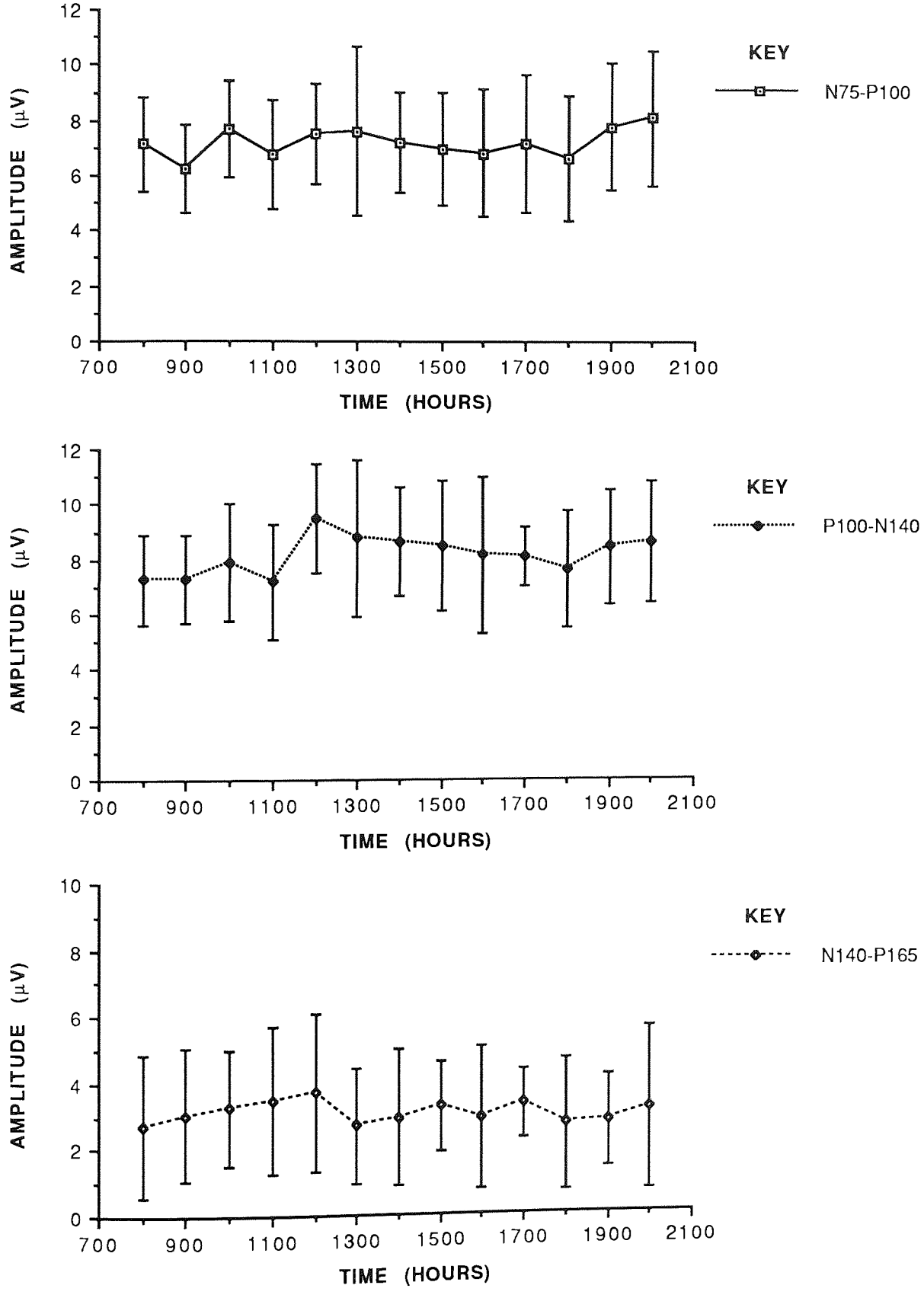


Figure 6.14 Mean amplitudes for the pattern reversal VEP (to 27' check size) components recorded over 13 hours. The data was recorded from the right eye and channel O2-Fz

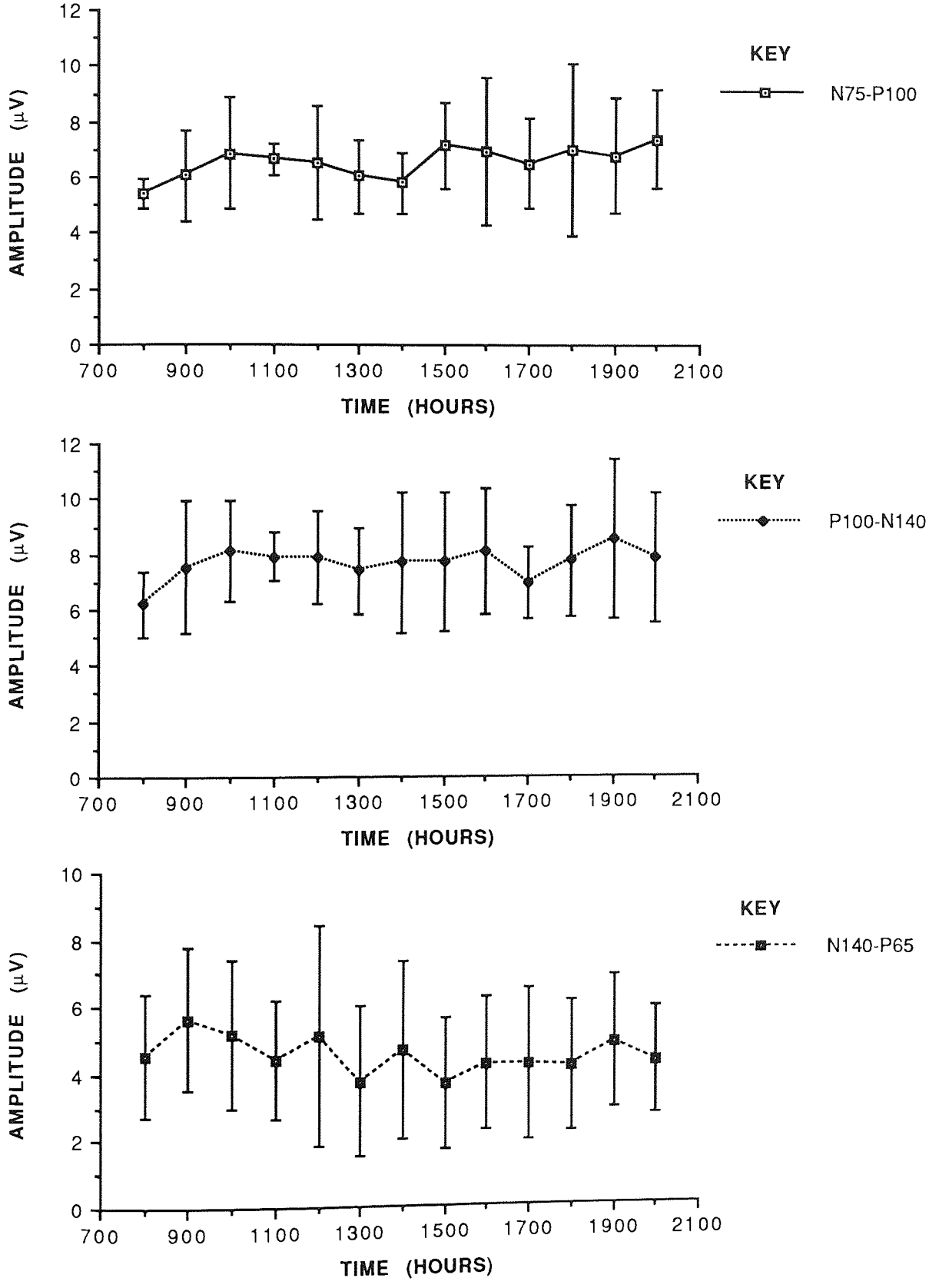


Figure 6.15 Graph showing the percentage change in latencies of the pattern reversal VEP (to 56' check size) components recorded over 13 hours.

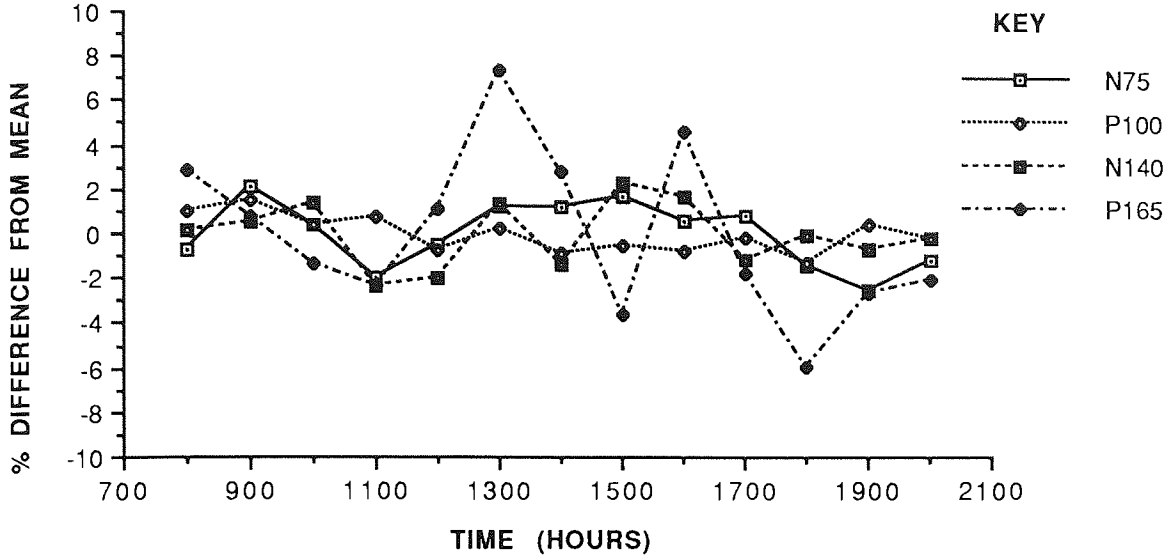


Figure 6.16 Graph showing the percentage change in latencies of the pattern reversal VEP (to 27' check size) components recorded over 13 hours.

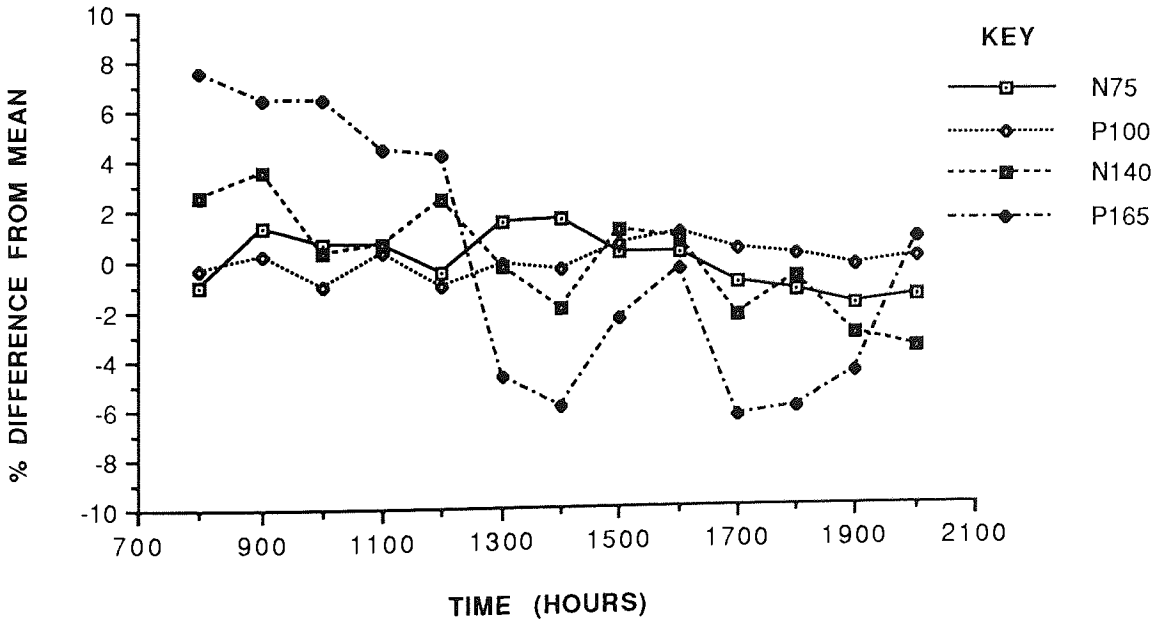
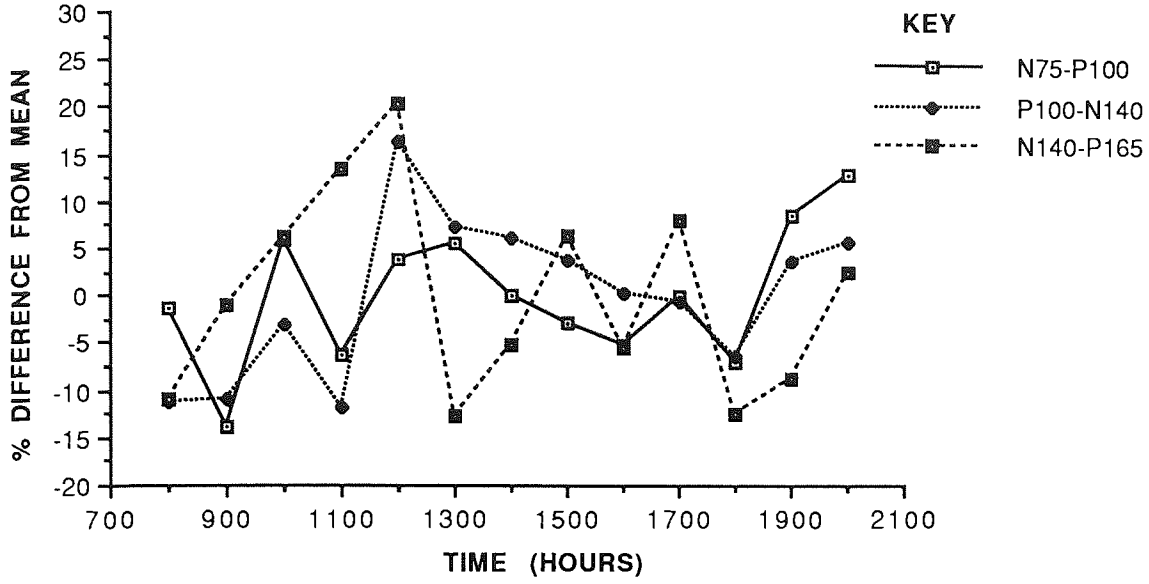


Figure 6.17 Graph showing the percentage change in amplitude values of the pattern reversal VEP (to 56' check size) components recorded over 13 hours.



The range in variation (difference between the maximum and minimum values) was very similar for the pattern reversal VEP to both check sizes. The N75 latency changed by 4.54% and 3.45%, the P100 latency by 2.69 % and 2.11%, the N140 latency by 4.62% and 7.04% and the P165 by 13.28% and 13.46% for the 56' and 27' check sizes respectively. Hence the P100 latency displayed the least and the P165 the greatest amount of variation during the day.

The amplitudes also displayed similar changes for the different check sizes. The N75-P100 changed by 27.15% and 31.91%, the P100-N140 by 28.40% and 31.08%, and N140-P165 by 33.12% and 43.93% for the 56' and 27' check sizes respectively. The amplitude of the later components again showed the greatest amount of variation during the day.

Figure 6.18 Graph showing the percentage change in amplitude values of the pattern reversal VEP (to 27' check size) components recorded over 13 hours.

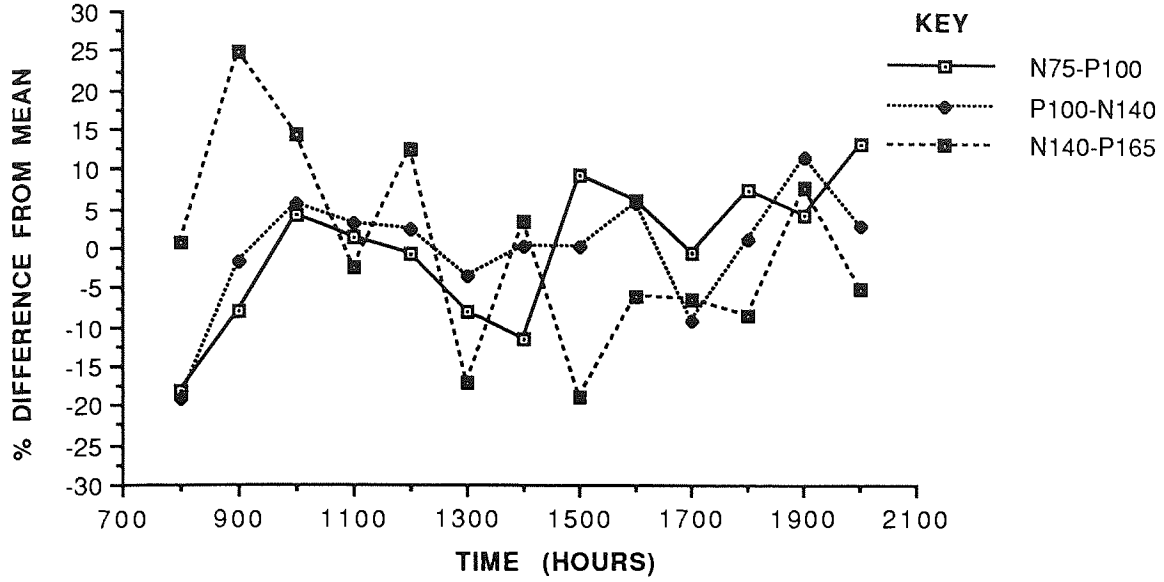


Table 6.8 Summary of the statistical analysis of the pattern reversal VEP to 56' check size recorded over 13 hours. The table group mean and standard deviation (**SD**) values, the difference between the maximum and minimum value (**range**), Chi square value (X^2) and significance at $P<0.05$ (**P**).

	Mean	**SD**	**Range**	X^2	**P**
Latency (ms)					
N75	75.31	1.10	3.42	11.07	NS
P100	116.18	0.92	3.12	17.20	NS
N140	167.01	2.42	7.72	14.42	NS
P165	191.33	7.03	25.40	7.89	NS
Amplitude (μV)					
N75-P100	7.22	0.53	1.96	11.58	NS
P100-N140	8.17	0.69	2.32	20.01	NS
N140-P165	3.08	0.32	1.02	6.10	NS

Table 6.9 Summary of the statistical analysis of the pattern reversal VEP to 27' check size recorded over 13 hours. The table group mean and standard deviation (**SD**) values, the difference between the maximum and minimum value (**range**), Chi square value (X^2) and significance at P<0.05 (**P**).

	Mean	SD	Range	X^2	P
Latency (ms)					
N75	79.32	0.94	2.74	16.21	NS
P100	116.14	0.70	2.45	8.73	NS
N140	165.07	3.65	11.62	18.78	NS
P165	212.61	11.29	28.62	11.61	NS
Amplitude (μV)					
N75-P100	6.58	0.60	2.10	13.74	NS
P100-N140	7.69	0.59	2.39	11.85	NS
N140-P165	4.53	1.57	1.99	17.76	NS

6.4 Discussion

The present study was carried out to determine the effect of time of day on the flash and pattern reversal VEPs (to 56' and 27' check sizes). This was carried out as the following studies in the thesis will incorporate multiple recording of the VEP during the day and therefore it was important to know the extent of time of day variation in the VEP.

The present study found no statistically significant variation in either the latency or amplitude values of the flash and pattern reversal VEP. Generally, the later the component occurred, the greater its variation in latency and amplitude. The amplitude also displayed greater variation than the latencies. However, both observations have been previously reported (Ciganék 1961 and 1975; Halliday 1982). In addition, Brocklin et al (1979) reported transient VEP amplitudes to be unreliable, such that changes of approximately 30% would be required under "good" conditions and up to 60% under the "worst" conditions to identify any significant changes in recordings. The variations in the present study were within these limits for the pattern reversal VEP (to 56' checks) N75-P100 and P100-N140 amplitudes. The amplitudes for the other VEPs exceeded the 30% but were below the 60% variation. However, no

statistical significance was obtained in any of the amplitude measurements, probably due to the high inter-individual variation associated with amplitude measures.

The lack of any significant changes observed in this study were contradictory to the findings of Stolz et al. (1987 and 1988). These workers found a clear circadian variation in the pattern reversal VEPs over 24 hours (fig. 6.19A). However, if the published waveforms recorded over the daylight hours are extracted to include only those contained in the present study (8 am-8 pm) (fig. 6.19B), it can be seen that very little if any variation seems to occur. Stolz et al. (1988) however also found more distinct circadian variation in latencies the later the component occurred. These workers published the mean, standard errors and range of fluctuations similar to those of the present study (table 6.10).

Table 6.10. Means, standard error (SE) and range of fluctuation within subjects across 24 hours for pattern reversal VEP (to 55' check size). From Stolz et al. (1988).

Parameter	Mean	SE	Range
N80 latency (ms)	70.1	1.7	2.2
	70.3	1.9	3.7
P100 latency (ms)	100.4	2.5	6.5
	98.8	1.8	5.1
N140 latency (ms)	134.2	7.4	8.9
	133.5	6.9	8.5
N80-P100 amplitude (μV)	3.7	0.53	1.3
	3.5	0.53	0.6
P100-N140 amplitude (μV)	3.5	0.42	0.6
	3.7	0.45	0.7

These results above, when compared to those from the present study (table 6.8), reveal that the latencies of the N75, P100 and N140 components occurred later in the present study. However, the standard deviation values (which tend to be larger than standard error values) were lower for the latencies in the present study. The range in fluctuation for the present study was similar for the N75 and N140 components but almost half for the P100 component. The amplitude values in the study by Stolz et al.

Figure 6.19 **A** Pattern reversal VEP (55' check) waveforms from a single subject recorded over two 24-hour cycles . The responses were recorded binocularly from Oz-Cz derivation. **B** Responses extracted from the 24 hour recording to only include those recorded over the daylight hours (8 am-8 pm). From Stolz et al. (1988).

(1988) were much smaller than those obtained in the present study, yet the standard deviation and standard errors were of similar magnitude. But the range of fluctuation in amplitude was much greater in the present study, as expected due to larger values. Therefore there are several similarities and differences in the results of the two studies. Stolz et al. (1988) examined the VEP over 24 hours in 8 subjects, whereas the present study examined the VEP over 13 (daylight) hours in 5 subjects. The significant variation reported by Stolz et al. (1988) was likely to be due to greater circadian effects expected on a 24 hour VEP recording than on a recording over waking daylight hours. In addition, there may have been contamination of data in the study by Stolz and workers, due to recordings over the vertex. This may be resolved by using other reference electrode sites as well as Cz to examine its influence. However, the studies in the present thesis will only be using electrode montages used by Harding, i.e. O1-C3 and O2-C4 for the flash VEP and O1 and O2 referred to Fz for the pattern reversal VEP (Hobley and Harding 1989).

The results obtained in the present study are however in agreement with those of Lu and Spafford (1989) (fig. 6.20). These workers examined the pattern reversal VEP in 10 subjects over 9 hours. The active electrode was placed 10% above the inion and the reference site was 20% above the inion. These would approximate to Oz-Pz, where the reference site is quite close to the active site. Hence any circadian influences over the active site may also affect the reference site. However, comparison of the results in fig. 6.20 with those in the present study (figs. 6.12 and 6.14) show similar effects of time of day on the pattern reversal VEP.

Overall, studies which have reported a significant circadian effect on the flash VEP (Heninger et al. 1969; Kerkhof et al. 1980) and the pattern reversal VEP (Stolz et al. 1987 and 1988) have all used Cz as a recording (either as an active or reference) site. Whereas those studies which have not found any circadian effect (Lu and Spafford 1989; present study) did not use the vertex site. This suggests that the Cz may be under circadian influences, although further studies should be carried out in order to substantiate this inference. However such studies are beyond the scope of the present thesis since no time of day effects on the VEPs (recorded with the electrode montages used in the present study) were found that will be used in subsequent studies.

Circadian rhythms have been reported to affect many of the physiological and psychological functions such as psychomotor performances (Aschoff and Wever 1981); reaction times (Hildebrandt and Engel 1972); metabolic rate, body temperature, and heart rate (Colquhoun 1972); and neurotransmitter levels and receptor affinity (Van Gool and Mirmiran 1986). But as the present study suggests, there seems little

effect on the VEP eventhough the EEG may be influenced by circadian rhythms. The reason for the apparent lack of influence on the VEP may be that attention of the subject is required for the test to a certain extent (especially for the pattern reversal VEP) and so the neuronal firing responsible for the generation of the response will to an extent be firing in synchrony. Whereas in recording the EEG, the subject is not required to be as attentive and so the neurones could settle into their own rhythm and thus likely to be under greater influence of circadian variation. This is supported by the data from Stolz et al. (1987 and 1988) where the VEP was under circadian influence mainly during the early morning and late evening periods when the attention and vigilance span of the subjects were low and hence be expected to be under greater influence of the circadian rhythm.

In conclusion, there are no significant time of day effects on the flash and pattern reversal VEPs during normal waking hours. However, different recording electrode montages may influence the results. Therefore, the effect of time of day effects should be examined on the particular montages to be used by various investigators.

Figure 6.20 Group averaged data for pattern reversal VEP (14' checks) recorded over 9 hours using Oz-Pz derivation. **A** The plot of the P100 latency. **B** The plot of the N75-P100 amplitude. The vertical bars indicate ± 1 standard error of mean. From Lu and Spafford (1989).

CHAPTER 7

The Effects of a Muscarinic Agonist on the Flash and Pattern Reversal Evoked Potential.

7.1 Introduction

There is a great deal of evidence supporting the use of cholinomimetics in the treatment of AD (see section 4.8), e.g. the use of ACh receptor agonists such as arecoline (table 4.1). But these drugs have either shown no cognitive improvement, have a narrow dose range, or have potentially serious side-effects. Therefore, new drugs are continually being developed which will be of therapeutic benefit. Sandoz Ltd., developed SDZ 210-086 ((-) [2-methyl-spiro-(1,3,-dioxolan-4,4']-1'-methyl-piperidin-hydrogenmalate) specifically as a potential therapeutic agent for AD. SDZ 210-086 is an analogue of dioxolan with specific agonist effects on both central and peripheral cholinergic muscarinic receptors (Palacios 1987). The pharmacological, toxicological and clinical data obtained for SDZ 210-086 have been presented in appendix 1. Based upon the laboratory and clinical findings (appendix 1), SDZ 210-086 was proposed as potential therapeutic agent for AD.

The diagnosis of AD (without the use of VEPs) remains largely one of exclusion of other disorders, with success rates of between 50-85% (Holmes et al. 1988). But the success rate is much improved with the use of the flash and pattern reversal VEPs (section 4.10.7). Since the delayed flash P2 co-existing with a normal pattern reversal P100 component occurring in AD can also be mimicked in normal volunteers with the use of the anticholinergic agent scopolamine (Bajalan et al. 1986), it may be possible that the VEP represents a marker both for the presence of the disease process itself and for the central cholinergic effects of the drug.

In addition, the effect of a cholinergic agonist on the human flash and pattern reversal VEP has not yet been reported in the literature. Therefore, the current study was designed to examine the effects of repeated administration of SDZ 210-086 on the flash and pattern reversal evoked potentials in healthy male volunteers. The effect on the VEP was an integral part of a multiple-dose tolerability trial of SDZ 210-086 in healthy male volunteers (Sandoz study code AC A 105). The study was carried out at the Inveresk Research Centre, Musselburgh, Scotland and funded by Sandoz Ltd.

7.2 Methods

7.2.1 Subjects

Two groups of 9 healthy male volunteers, each of whom had fulfilled the inclusion criteria (detailed in appendix 2) participated in a randomised double-blind placebo-controlled study for which they were paid. In each group, 6 volunteers received SDZ 210-086 and 3 received a placebo for 12 days. Results of the placebo subjects (age range 20-28 years, mean 24.2 ±2.8 years) were pooled in order to form a group containing equal number of subjects as the treated groups. The 6 treated group 1 subjects (age range 21-41 years, mean 30.5 ±7.2 years) received 0.5 mg of SDZ 210-086 and the 6 treated group 2 subjects (age range 20-35 years, mean 28.0 ±6.2 years) received 1.0 mg of SDZ 210-086 (administered as 0.5 mg twice a day). SDZ 210-086 and the placebo were administered orally with 100 ml of tap water. The subjects were institutionalised for the duration of the study in a hospital-type environment. Meals were standardised and alcohol was restricted for the duration of the study.

7.2.2 Instrumentation

The flash stimulus was generated from a Medelec OS5 unit and delivered by a Grass PS22 stroboscope at intensity 2 (luminance of 1363 cd/m^2), 30 cm away and delivered at a rate of 1.7 Hz. The pattern reversal stimulus consisted of a checkerboard pattern of 56' checks in a 23^o 50' x 18^o 24' field and reversing at a rate of 1.7 Hz. The luminance of the white checks was 170 cd/m^2 and a contrast of 78%. The stimulus was generated onto a Cadwell TV monitor by a Cadwell pattern generator unit. Both visual stimuli were triggered by a 5V TTL pulse from the Bio-Logic Traveler™ electrodiagnostic testing system with twin disk drives. The bandpass filtering was set at 1-30 Hz (-3dB down point), and the gain at 30,000. Two runs each consisting of 50 averages were carried out for each eye, totalling in an average of 100 responses per eye.

7.2.3 Procedure

The drug/placebo treatment was administered at 800 hours to both group 1 and group 2 subjects, after a 12 hour overnight fast during which fluids were permitted. Group 2 subjects were administered the second dose of treatment at 2000 hours. The VEPs were scheduled to be recorded between 2.0-6.5 hours after drug administration, since during this period, blood levels of SDZ 210-086 were thought to reach a peak (Palacios 1987). The time of day and the interval from drug administration was kept constant for each subject where possible. However, the recording of the VEPs during

the control run was dependent upon the time of arrival of the subjects and for group 1 subjects, the control VEPs were recorded at 1400 hours (see appendix 3).

VEPs were recorded on the day of arrival of the subjects to the centre (day 0) when no treatment was administered. The placebo/drug treatment was administered the following day (day 1). Since the VEPs could not be recorded between the 2.0-6.5 hours post dosing in all subjects, VEPs were recorded on days 2 and 10 for subjects GRey, DC, AD, MT, GRei, SCo, JA, BB, BM, and BW and on day 3 and 11 for subjects KA, SCr, DR, GM, KM, KB, IM, and HA.

Monocular VEPs were recorded from both eyes, using silver-silver chloride electrodes placed on the scalp according to the international 10-20 system (Jasper 1958). The VEPs were recorded using the standard derivations of O2 referred to C4 and O1 referred to C3 for the flash stimulation and O2 and O1 both referred to Fz for the pattern reversal stimulation. The interelectrode impedance was maintained below 5kΩ.

7.3 Results

7.3.1 Data analysis

Analysis of the flash VEP components were confined to measurement of the N2 and P2 latency and the N2-P2 peak-to-peak amplitude. The early P1 and N1 components were not present in all subjects due to the relatively young age of the subjects as expected (Wright et al. 1985). For the pattern reversal VEP, the N75 and P100 latency and the N75-P100 amplitude were also analysed. An analysis of variance with a nested classification revealed no significant difference in responses between the eyes, hemispheres or runs. Therefore, for simplicity, only the responses from the right eye and the right hemisphere have been analysed.

Statistical analysis of the results was carried out using a two-way analysis of variance. The results of the 3 placebo subjects from both group 1 and 2 were combined since equal subject numbers are required for the analysis. A further split plot, two-factorial analysis of variance in a randomised design with the groups as the major and days as the minor factor was also carried out. This test was used to examine the variance between the groups, between recording days and the interaction of the two, i.e. variation between the groups over the days.

7.3.2 Flash N2 latency

The results of the flash N2 latency measurements have been summarised in table 7.1 and the group mean values shown in fig. 7.1. The placebo and group 1 subjects demonstrated a difference in the mean N2 latency of less than 1 ms over the days (fig. 7.1). In contrast, group 2 subjects showed an increase in the mean N2 latency of 6.83 ms and 5.21 ms on day 2/3 and day 10/11 respectively, however this did not reach statistical significance.

In general, the mean N2 latency for group 1 subjects was longer when compared to the other groups (fig. 7.1). Interestingly, there were 4 subjects in group 1 who displayed a N2 latency above 80 ms (table 7.1). In particular, subject MT displayed the latest occurring N2 component with latencies occurring above 90 ms, however this was still within the normal limits (Wright et al. 1985) for his age (41 years).

In order to magnify any changes in the flash N2 latency after administration of SDZ 210-086, the percentage difference between the baseline (day 0) and day 2/3 and day 10/11 were plotted (fig. 7.2). No group showed any shortening of the N2 latency. The placebo group showed no change in the N2 latency on day 2/3 and only a very slight increase of 0.5% on day 10/11. Both of the treated groups showed an increase in the N2 latency which was greater on day 2/3 than day 10/11. Group 2 subjects displayed the greatest magnitude of change with an increase in N2 of 11.2% and 8.5%, compared to group 1 with an increase of only 1.3% and 0.42% on days 2/3 and 10/11 respectively.

Statistical analysis (table 7.1) revealed no significant change in the N2 latency over the days in either the placebo or the treated groups. There was significant inter-individual variation ($P<0.005$) in the N2 latency in each group, as illustrated by the large standard deviation values. The additional two-factorial ANOVA revealed no significant difference in N2 latency between the groups, no difference in values over the days and no interaction between the two factors. Therefore SDZ 210-086 did not have any significant effect on the flash N2 latency, eventhough there was an increase in the treated groups compared to placebo subjects (fig. 7.2). The primary reason for no significant difference between the placebo and treated groups may be the high and overlapping standard deviation values present in each group (fig. 7.1).

Table 7.1 Summary of the data for the flash N2 latency for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086) subjects.

SUBJECTS	N2 LATENCY			STATISTICS	
PLACEBO	DAY 0	DAY 2/3	DAY 10/11		
GRey	61.52	62.50	65.43	F Ratio 1.54×10^{-2}	
KA	89.84	92.77	93.75	NS	
SCr	64.45	65.43	65.43		
DC	63.48	61.52	55.66	F' Ratio	
	242.38				
DR	60.55	58.59	57.62	P<0.005	
GM	55.66	54.69	55.66		
MEAN	65.92	65.92	65.59		
SD	12.11	13.65	14.52		
SE	4.95	5.57	5.93		
GROUP 1					
AD	43.95	41.02	42.97	F Ratio 4.55×10^{-2}	
MT	100.59	96.68	91.80	NS	
GRei	83.01	83.98	86.91		
KM	86.91	86.91	82.03	F' Ratio	
	215.17				
KB	83.98	93.75	91.80	P<0.005	
IM	69.34	71.29	74.22		
MEAN	77.96	78.94	78.29		
SD	19.42	20.59	18.53		
SE	7.93	8.41	7.57		
GROUP 2					
SCo	66.41	61.52	61.52	F Ratio	1.69
JA	62.50	74.22	70.31	NS	
BB	69.34	70.31	67.38		
BM	48.83	59.57	57.62	F' Ratio	13.73
BW	61.52	75.20	76.17	P<0.005	
HA	58.59	67.38	65.43		
MEAN	61.50	68.03	66.41	Fg ratio 1.71	NS
SD	7.14	6.47	6.54	Fd ratio 2.59	NS
SE	2.92	2.64	2.67	Fi ratio 1.91	NS

KEY: F ratio- variation across the days and F' ratio- inter-individual variation from a two-way analysis of variance, Fg ratio- variance between groups; Fd ratio- variance between days and Fi ratio- interaction of the two, from a split-plot two-factorial analysis of variance.

Figure 7.1 Graph showing the mean N2 latency values of the flash VEP for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086) subjects. Data represents responses recorded from the right eye and channel O2-C4.

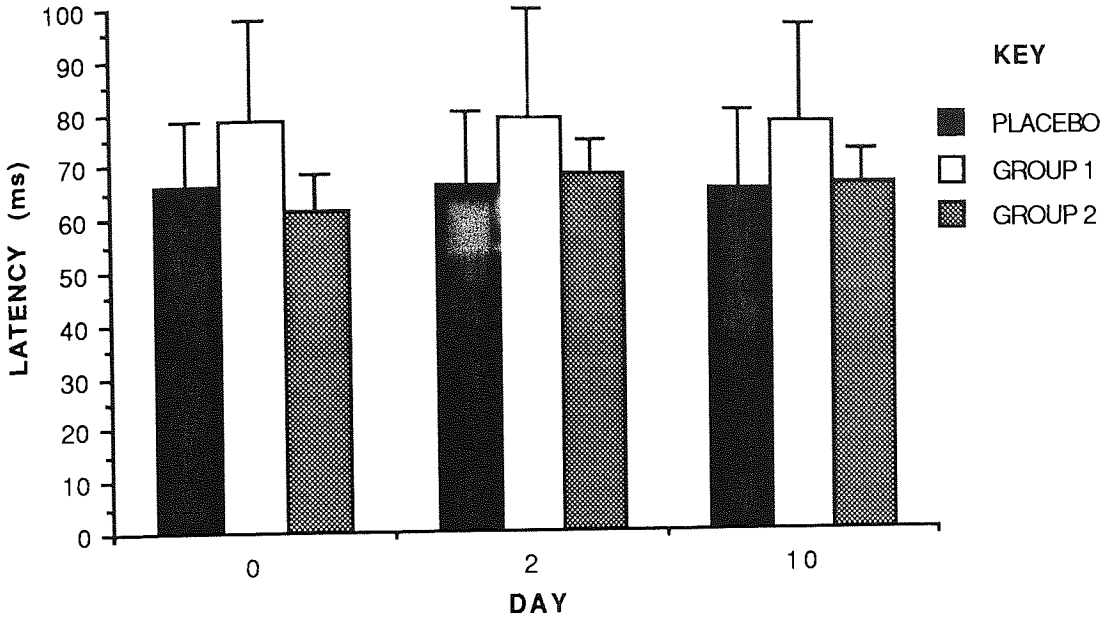
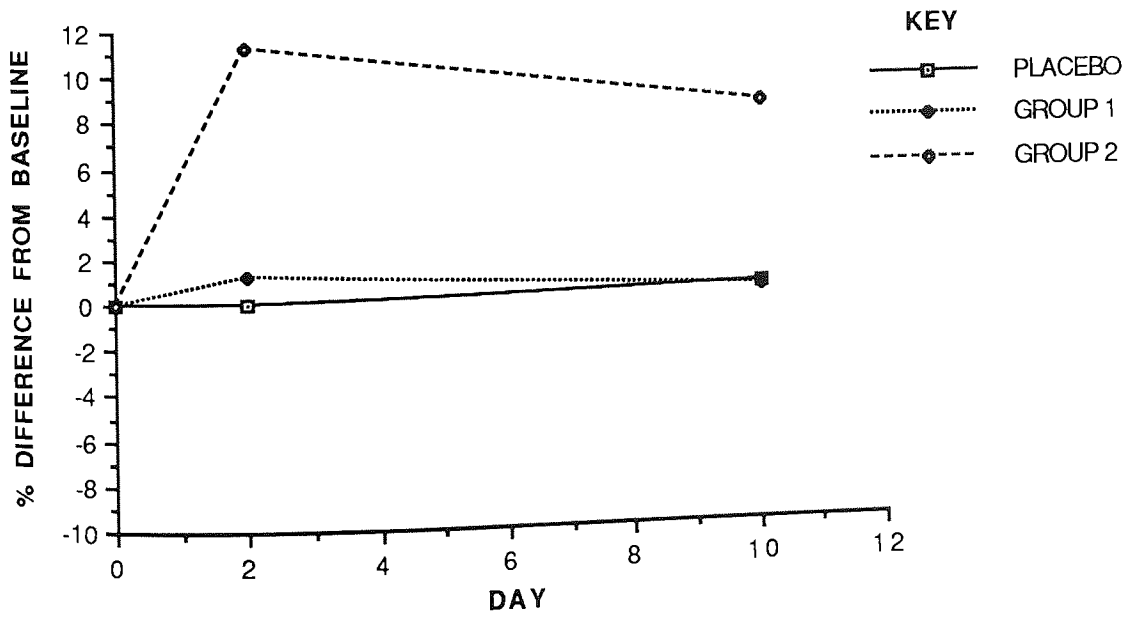


Figure 7.2 Graph showing the percentage difference in flash N2 latency after administration of SDZ 210-086 for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086) subjects.



7.3.3 Flash P2 latency

The results of the flash P2 latency measurements have been summarised in table 7.2 and the group mean values plotted in the graph shown by fig. 7.3. There was a P2 latency fluctuation of less than 2 ms over the days for the treated groups. The placebo group, however showed a shortening of the P2 latency on day 10/11 by 5.7 ms, but this did not reach statistical significance (table 7.2).

The percentage change in the flash P2 latency (fig. 7.4) revealed variable effects. There was a gradual shortening of the P2 latency over the days in both the placebo and group 2, with the magnitude of change being greater for the placebo group. Group 1 displayed a slight increase in the P2 latency over day 2/3 (1.2%) and slight shortening over day 10/11 (0.8%) compared to the baseline values. Overall, these changes in the P2 latency were much less (below 5%) than for the N2 latency, (reaching 11.2%).

As with the flash N2 latency, there was significant ($P<0.005$) inter-individual variation in the P2 latency values. But as indicated by the error bars (fig. 7.3) these were not as large as those for the N2 latency (fig. 7.1) for the placebo and group 1, although group 2 displayed similar standard deviation levels for both the N2 and P2 components. The two-factorial ANOVA (table 7.2) also revealed no significant difference in the P2 values between the groups or over the days. Hence SDZ 210-086 had no significant effect on the flash P2 latency.

Figure 7.3 Graph showing the mean P2 latency values for the flash VEP for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086) subjects. Data represents responses recorded from the right eye and channel O2-C4.

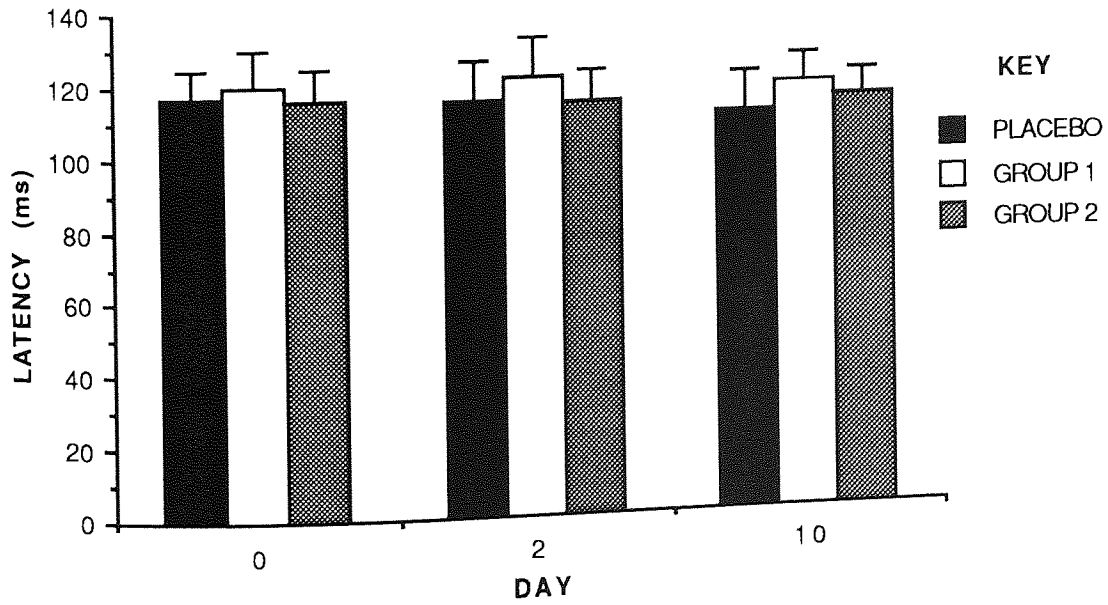
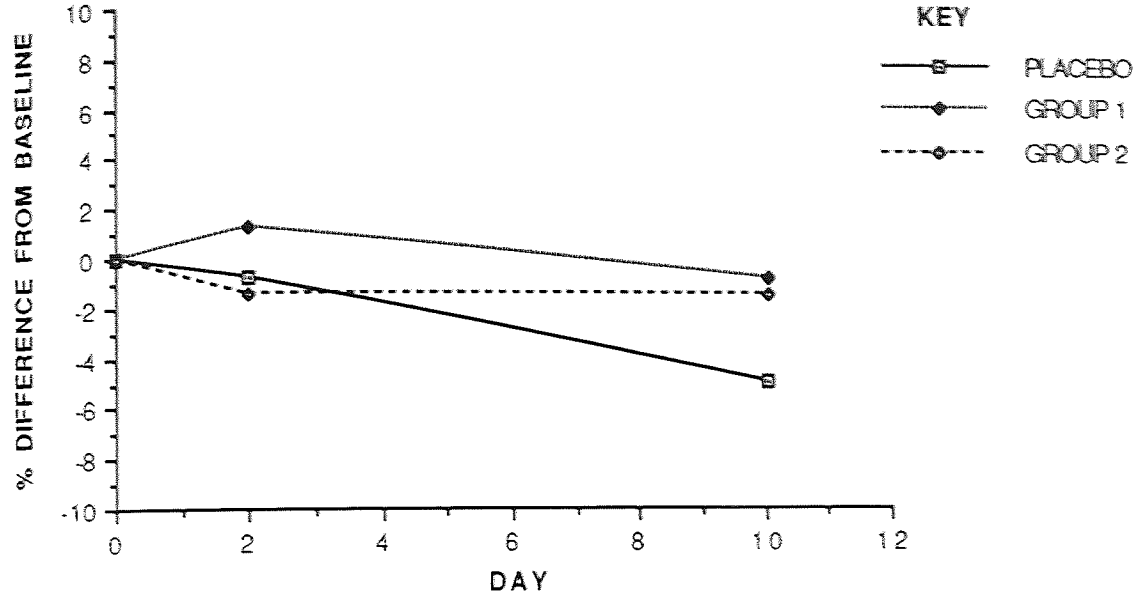


Table 7.2 Summary of data for the flash P2 latency for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086) subjects.

SUBJECTS	P2 LATENCY			STATISTICS	
PLACEBO	DAY 0	DAY 2/3	DAY 10/11		
GRey	114.26	116.21	115.23	F Ratio	2.46
KA	123.05	123.05	124.02	NS	
SCr	125.98	125.00	115.23		
DC	102.54	97.66	92.77	F' Ratio	74.22
DR	114.26	110.35	105.47	P<0.005	
GM	119.14	121.09	112.30		
MEAN	116.54	115.56	110.84		
SD	8.30	10.23	10.67		
SE	3.39	4.18	4.36		
GROUP 1					
AD	106.45	107.42	113.28	F Ratio	0.29
MT	138.67	137.70	131.84	NS	
GRei	118.16	112.30	110.35		
KM	115.23	118.16	117.19	F' Ratio	52.40
KB	122.07	125.98	120.12	P<0.005	
IM	117.19	125.00	119.14		
MEAN	119.63	121.09	118.65		
SD	10.67	10.84	7.43		
SE	4.36	4.43	3.03		
GROUP 2					
SCo	112.30	114.26	121.09	F Ratio	0.12
JA	111.33	113.28	115.23	NS	
BB	106.45	105.47	101.56		
BM	123.05	120.12	120.12	F' Ratio	19.12
BW	114.26	104.49	111.33	P<0.005	
HA	128.91	127.93	116.21		
MEAN	116.05	114.26	114.26	Fg ratio 0.71	NS
SD	8.32	8.89	7.15	Fd ratio 2.96	NS
SE	3.40	3.63	2.92	Fi ratio 1.05	NS

KEY: F ratio- variation across the days and F' ratio- inter-individual variation from a two-way analysis of variance, Fg ratio- variance between groups; Fd ratio- variance between days and Fi ratio- interaction of the two, from a split-plot two-factorial analysis of variance.

Figure 7.4 Graph showing the percentage difference in flash P2 latency after administration of SDZ 210-086 for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086).



7.3.4 Flash N2-P2 amplitude

[illegible] in table 7.3 and the group mean values plotted in fig. 7.5. The results of the N2-P2 amplitude displayed greater variability than the latency results, a finding typical for VEPs (Ciganék 1961 and 1975; Halliday 1982). The placebo group showed a gradual increase over the days whereas both group 1 and 2 showed a slight decrease in amplitude post dosing.

The percentage change in the flash N2-P2 amplitude (fig. 7.6) showed a large increase in amplitude for the placebo group which almost reached 40% on day 10/11. In contrast both the treated groups displayed a decrease in amplitude of similar magnitude post dosing.

Statistical analysis (table 7.3) revealed a significant difference in the N2-P2 amplitude between the groups (Fg ratio $P<0.01$). This was illustrated by both groups 1 and 2 displaying a fall in amplitude values after drug administration, whereas the placebo group displayed an opposite increase in amplitude. However, the increase in N2-P2 amplitude within the placebo group and the decrease within the treated groups, did not reach statistical significance, probably due to the large inter-individual variation present within each group. The error bars in fig. 7.5 were large and overlap in each group over the days (similarly to N2 latency).

Table 7.3 Data for the flash N2-P2 amplitude for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086) subjects.

SUBJECTS	N2-P2 AMPLITUDE			STATISTICS	
PLACEBO	DAY 0	DAY 2/3	DAY 10/11		
GRey	1.38	8.06	14.24	F Ratio	0.46
KA	4.01	3.58	3.42	NS	
SCr	8.39	6.17	6.94		
DC	4.15	6.86	7.78	F' Ratio	9.41
DR	8.65	6.62	7.85	P<0.005	
GM	13.52	12.24	13.92		
MEAN	6.68	7.26	9.03		
SD	4.36	2.85	4.24		
SE	1.78	1.17	1.73		
GROUP 1					
AD	5.22	4.02	6.03	F Ratio	0.39
MT	7.91	4.43	3.33	NS	
GRei	5.07	5.89	3.65		
KM	4.51	5.68	4.65	F' Ratio	4.75
KB	4.69	2.47	3.39	P<0.025	
IM	6.80	5.90	7.75		
MEAN	5.70	4.73	4.80		
SD	1.35	1.37	1.77		
SE	0.55	0.56	0.72		
GROUP 2					
SCo	10.64	10.69	11.21	F Ratio	0.56
JA	15.01	10.46	9.01	NS	
BB	11.50	11.46	5.14		
BM	10.58	9.42	10.47	F' Ratio	6.78
BW	12.86	10.52	11.00	P<0.025	
HA	17.39	11.78	19.58		
MEAN	13.00	10.72	11.07	Fg ratio 12.7	P<0.01
SD	2.72	0.83	4.74	Fd ratio 0.64	NS
SE	1.11	0.34	1.93	Fi ratio 1.48	NS

KEY: F ratio- variation across the days and F' ratio- inter-individual variation from a two-way analysis of variance, Fg ratio- variance between groups; Fd ratio- variance between days and Fi ratio- interaction of the two, from a split-plot two-factorial analysis of variance.

Overall, SDZ 210-086 may be responsible for reducing the flash N2-P2 amplitude, since both of the treated groups showed this effect but not the placebo group. However, other observations do not support this. For instance, the placebo group displayed change of the greatest magnitude in amplitude over the days. In addition, if SDZ 210-086 administration was to result in a decrease in the N2-P2 amplitude, the magnitude of this decrease was expected to be greater in group 2 subjects as they received a higher dose of the drug, yet no difference between the treated groups was obtained. This lack of specificity between doses may be that the effect of SDZ 210-086 is not directly correlated with its dosage. However, factors affecting the placebo group are also likely to affect the treated groups such that they too should have shown an increase in amplitude if there was no effect of SDZ 210-086, but there was an opposing effect on the flash amplitude between the placebo and treated groups. Therefore, the reduction in amplitude in the treated groups was likely to be due to the administration of SDZ 210-086.

Figure 7.5 Graph showing the mean N2-P2 amplitude values for the flash VEP for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086). Data represents responses recorded from the right eye and channel O2-C4.

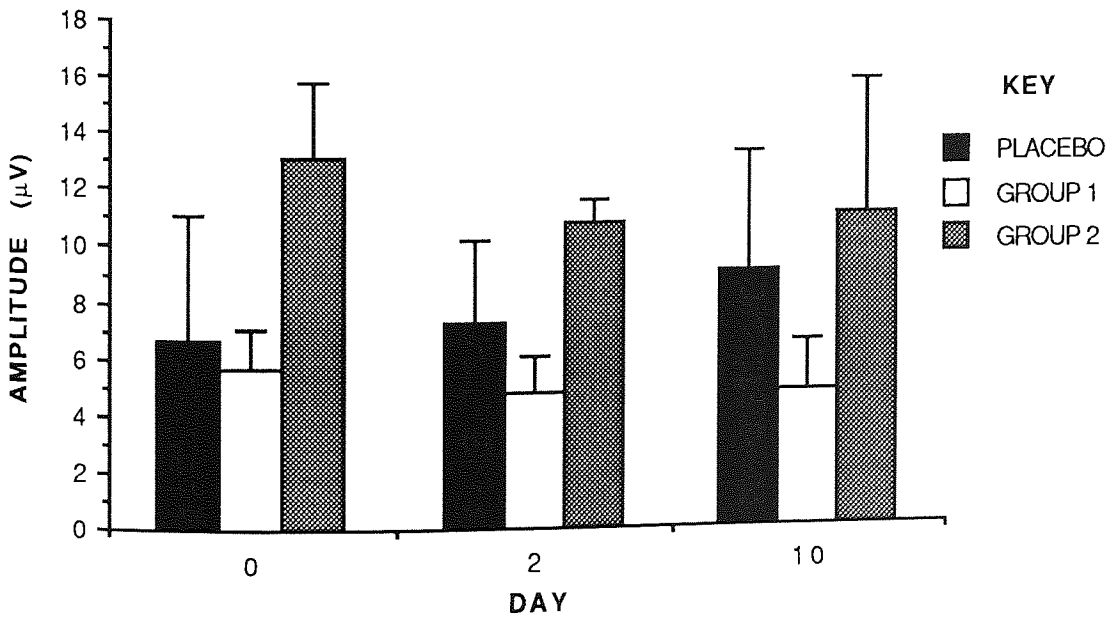
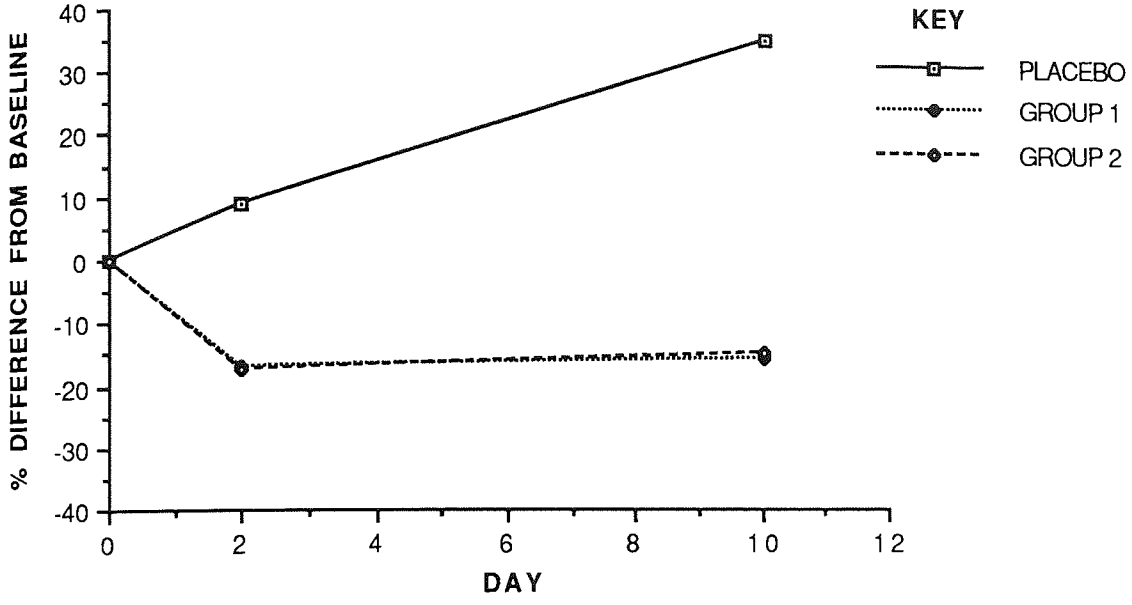


Figure 7.6 Graph showing the percentage difference in flash N2-P2 amplitude after administration of SDZ 210-086 for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086).



7.3.5 Pattern reversal N75 latency

The results of the measurement of the latency of the N75 component of the pattern reversal VEP have been summarised in table 7.4 and the group mean values shown in fig. 7.7. Each group displayed little change in the N75 latency over the days.

The percentage difference in the N75 latency (fig. 7.8) was similar in all three groups. The placebo group showed a small increase in latency on day 10/11 (of 6.5%), while the treated subjects showed a small decrease in latency on day 2/3 (2.2% for group 1 and 2.5% for group 2), but on day 10/11, group 2 showed a small increase (of 5.1%) and group 1 showed N75 latency similar to baseline values. Thus there was no consistent effect on the N75 latency.

As with the flash VEP, there was significant ($P<0.005$) inter-individual variation within each group. Group 2 subjects displayed the greatest inter-individual variation with the N75 latency range of 65 ms on day 0. Subjects SCo displayed an early N75 latency occurring before 50 ms, while subject BW displayed a considerably delayed N75 latency occurring over 100 ms. However no statistically significant difference was found in the N75 latency values between the groups, days or any interaction between the two.

Table 7.4 Data for the pattern reversal N75 latency for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086).

SUBJECTS	N75 LATENCY			STATISTICS	
PLACEBO	DAY 0	DAY 2/3	DAY 10/11		
GRey	80.08	77.15	78.13	F Ratio	0.54
KA	61.52	60.55	58.59	NS	
SCr	58.59	61.52	64.45		
DC	80.08	69.34	83.98	F' Ratio	17.28
DR	69.34	66.41	73.24	P<0.005	
GM	53.71	69.34	71.29		
MEAN	67.22	67.39	71.61		
SD	11.18	6.08	9.16		
SE	4.56	2.48	3.74		
GROUP 1					
AD	71.29	65.43	71.29	F Ratio	0.11
MT	87.89	83.98	77.15	NS	
GRei	62.50	58.59	69.34		
KM	78.13	76.17	79.10	F' Ratio	20.34
KB	72.27	76.17	78.13	P<0.005	
IM	76.17	78.13	72.27		
MEAN	74.71	73.08	74.55		
SD	8.42	9.29	4.08		
SE	3.44	3.79	1.67		
GROUP 2					
SCo	41.02	46.88	46.88	F Ratio	1.48
JA	74.22	73.24	75.20	NS	
BB	51.76	53.71	60.55		
BM	68.36	70.31	74.22	F' Ratio	
	636.36				
BW	106.45	105.47	105.47	P<0.005	
HA	77.15	80.08	78.13		
MEAN	69.83	71.62	73.41	Fg ratio 0.24	NS
SD	22.68	20.76	19.61	Fd ratio 2.35	NS
SE	9.26	8.48	8.01	Fi ratio 0.67	NS

KEY: F ratio- variation across the days and F' ratio- inter-individual variation from a two-way analysis of variance, Fg ratio- variance between groups; Fd ratio- variance between days and Fi ratio- interaction of the two, from a split-plot two-factorial analysis of variance.

Figure 7.7 Graph showing the mean N75 latency of the pattern reversal VEP for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086). Data represents responses recorded from the right eye and channel Oz-Fz.

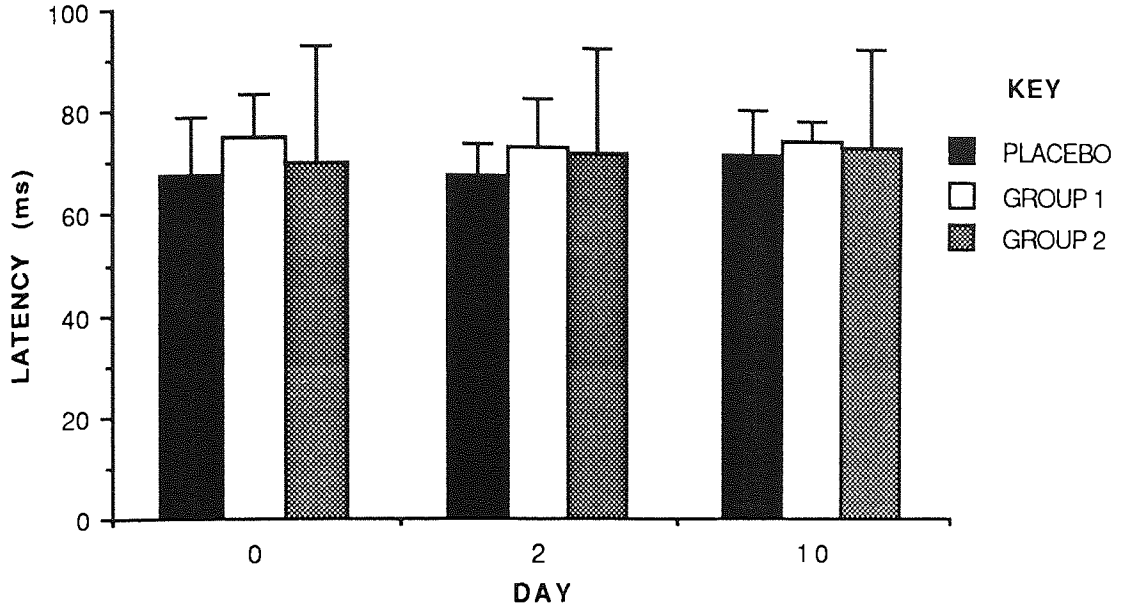
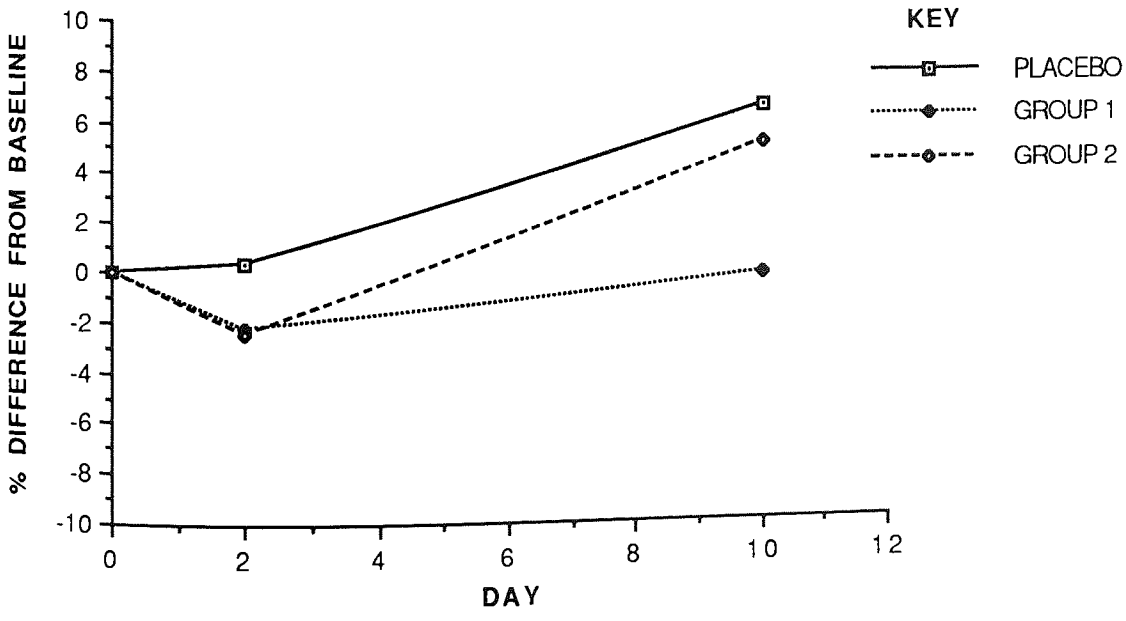


Figure 7.8 Graph showing the percentage difference in the pattern reversal N75 latency after administration of SDZ 210-086 for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086).



7.3.6 Pattern reversal P100 latency

The results of the measurement of the latency of the pattern reversal P100 component have been summarised in table 7.5, and the group mean values plotted in fig. 7.10. The P100 component showed very little difference in latency between the groups.

Both the placebo and group 2 subjects showed a small increase in the percentage change in P100 latency on day 2/3 which increased slightly further on day 10/11 (fig. 7.11). The group 1 subjects also displayed an increase in the P100 latency which was higher on day 2/3 than 10/11. However these changes in the P100 latency have been magnified in fig. 7.11, and the actual increases were below 5%.

There was also less inter-individual variation of the P100 latency when compared to the N75 component, but still reached significance ($P<0.005$). Subject BW (aged 26 years) displayed P100 latencies occurring after 135 ms, well above the normal limits for an individual in that age range (Wright et al. 1985). Statistical analysis also revealed significant variation in the P100 latency over the days (Fd ratio, $P<0.01$) in all 3 groups. Hence the change in the P100 latency cannot be attributed to SDZ 210-086 as a similar trend was present in the placebo as well as the treated groups.

Figure 7.9 Graph showing the mean P100 latency for the pattern reversal VEP for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086). Data represents responses recorded from the right eye and channel Oz-Fz.

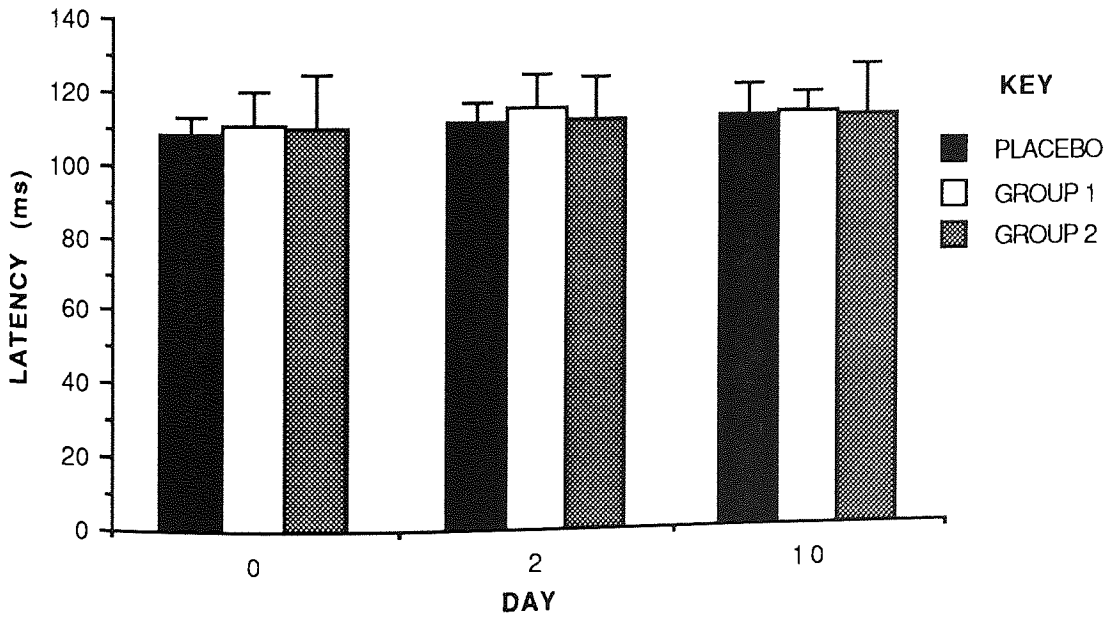
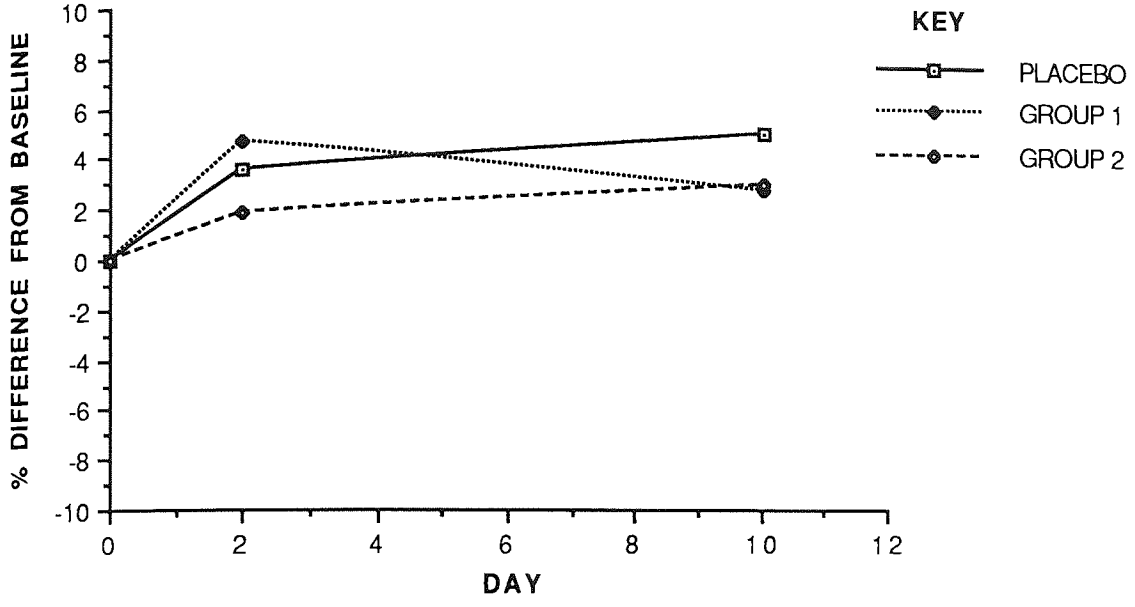


Table 7.5 Data for the pattern reversal P100 latency for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086).

SUBJECTS	P100 LATENCY			STATISTICS	
PLACEBO	DAY 0	DAY 2/3	DAY 10/11		
GRey	111.33	114.26	123.06	F Ratio	1.75
KA	107.42	111.33	108.40	NS	
SCr	98.63	103.52	103.52		
DC	114.26	112.30	120.12	F' Ratio	24.23
DR	106.45	109.38	106.45	P<0.005	
GM	109.38	119.14	118.16		
MEAN	107.91	111.66	113.28		
SD	5.34	5.19	8.15		
SE	2.18	2.12	3.33		
GROUP 1					
AD	102.54	104.49	108.40	F Ratio	0.55
MT	123.05	116.21	113.28	NS	
GRei	116.21	117.19	112.30		
KM	96.68	109.38	111.33	F' Ratio	12.87
KB	109.38	114.26	111.33	P<0.005	
IM	114.26	130.86	124.02		
MEAN	110.35	115.40	113.44		
SD	9.59	8.94	5.43		
SE	3.92	3.65	2.22		
GROUP 2					
SCo	108.40	108.40	10.35	F Ratio	0.67
JA	107.42	107.42	108.40	NS	
BB	107.42	106.45	111.33		
BM	95.70	105.47	108.40	F' Ratio	
	135.91				
BW	138.67	136.72	139.65	P<0.005	
HA	102.54	107.42	101.56		
MEAN	110.03	111.98	113.28	Fg ratio 0.01	NS
SD	14.82	12.16	13.36	Fd ratio 5.22	
	P<0.01				
SE	6.05	4.97	5.461	Fi ratio 0.72	NS

KEY: F ratio- variation across the days and F' ratio- inter-individual variation from a two-way analysis of variance, Fg ratio- variance between groups; Fd ratio- variance between days and Fi ratio- interaction of the two, from a split-plot two-factorial analysis of variance.

Figure 7.10 Graph showing the percentage difference in the pattern reversal P100 latency after administration of SDZ 210-086 for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086).



7.3.7 Pattern reversal N75-P100 amplitude

The results of the measurements of the N75-P100 amplitude have been summarised in table 7.6 and the mean values shown in fig. 7.11. There was a variable trend in the amplitude measurements over the days. Both the placebo and group 1 subjects displayed an increase, whereas group 2 subjects displayed a decrease in amplitude over the days. The placebo and group 1 subjects showed a large increase in the N75-P100 amplitude (fig. 7.11) on day 2/3 (34.1% for placebo and 21.5% for group 1), which was lower on day 10/11 (19.7% for placebo and 4.8% for group 1). In contrast, group 2 displayed a decrease in amplitude over both day 2/3 (15.4%) and day 10/11 (29.3%).

Statistical analysis (table 7.3) revealed no significant variation in amplitude over the days for each group. The inter-individual variation again reached significance ($P<0.005$), as illustrated by the large error bars in fig. 7.11. However, the two-factorial ANOVA revealed a significance ($P<0.001$ for Fd and Fi ratios) variance over the days and for the interaction between the values in the groups recorded over the days. Hence the decrease in the N75-P100 amplitude was significantly different from the increase in N75-P100 amplitude observed in group 1 and placebo subjects. Therefore, SDZ 210-086 may be responsible for reducing the N75-P100 amplitude but only at the higher (1.0 mg/day) dose levels.

Figure 7.11 Graph showing the mean N75-P100 amplitude for the pattern reversal VEP for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086). Data represents responses recorded from the right eye and channel Oz-Fz.

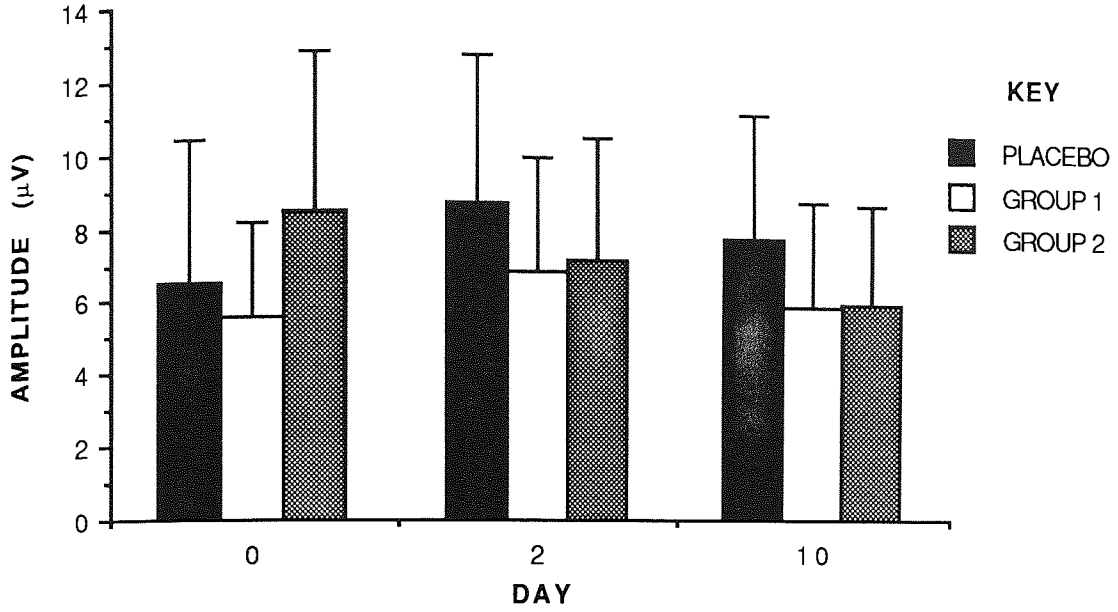


Figure 7.12 Graph showing the percentage difference in the pattern reversal N75-P100 amplitude after administration of SDZ 210-086 for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086).

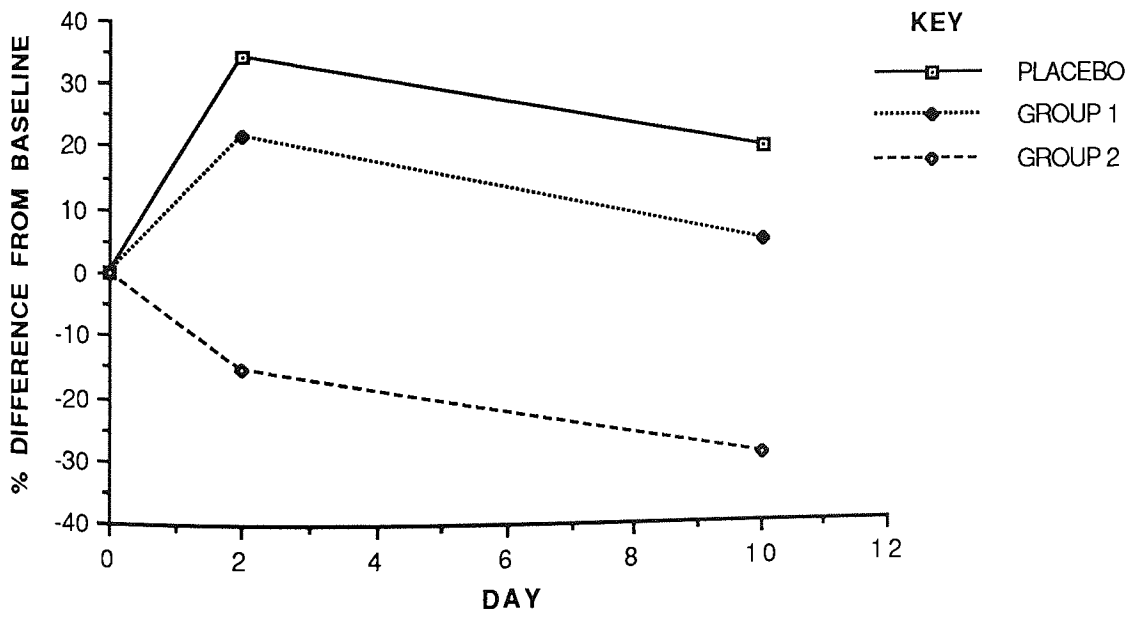


Table 7.6 Data for the pattern reversal N75-P100 amplitude for placebo, group 1 (0.5 mg/day SDZ 210-086) and group 2 (1.0 mg/day SDZ 210-086).

SUBJECTS	N75-P100 AMPLITUDE			STATISTICS	
PLACEBO	DAY 0	DAY 2/3	DAY 10/11		
GRey	2.81	5.16	4.77	F Ratio	4.09
KA	5.02	7.59	6.68	NS	
SCr	7.89	9.65	10.93		
DC	3.79	6.35	5.39	F' Ratio	
	141.07				
DR	5.89	7.17	5.92	P<0.005	
GM	13.55	16.29	12.94		
MEAN	6.49	8.70	7.77		
SD	3.88	4.00	3.35		
SE	1.58	1.63	1.37		
GROUP 1					
AD	7.89	9.53	9.04	F Ratio	1.67
MT	3.18	3.31	3.04	NS	
GRei	8.60	11.25	9.88		
KM	2.34	3.73	3.60	F' Ratio	
	104.05				
KB	6.32	6.63	4.17	P<0.005	
IM	5.22	6.27	5.45		
MEAN	5.59	6.79	5.86		
SD	2.51	3.14	2.91		
SE	1.02	1.28	1.19		
GROUP 2					
SCo	1.56	2.76	3.06	F Ratio	1.17
JA	13.09	10.05	10.54	NS	
BB	8.02	6.59	4.60		
BM	7.38	8.01	7.62	F' Ratio	24.72
BW	7.47	4.05	4.23	P<0.005	
HA	13.06	11.33	5.73		
MEAN	8.43	7.13	5.96	Fg ratio 0.37	NS
SD	4.30	3.34	2.72	Fd ratio 3.27	
	P<0.01				
SE	1.76	1.36	1.11	Fi ratio 4.79	P<0.01

KEY: F ratio- variation across the days and F' ratio- inter-individual variation from a two-way analysis of variance, Fg ratio- variance between groups; Fd ratio- variance between days and Fi ratio- interaction of the two, from a split-plot two-factorial analysis of variance.

7.4 Discussion

There were no statistically significant effects of the cholinergic drug SDZ 210-086 on the latencies of the flash or pattern reversal VEP. However, the amplitudes of these VEPs were significantly ($P<0.05$) reduced, particularly at the dose of 1.0 mg/day. Hence, SDZ 210-086 may reduce the flash VEP N2-P2 amplitude at doses as low as 0.5 mg/day and the pattern reversal N75-P100 amplitude at doses of 1.0 mg/day. This also suggests a greater sensitivity of the flash VEP for SDZ 210-086.

The present study examined very low doses of SDZ 210-086. The study had originally been designed to examine the tolerability, safety and plasma levels of SDZ 210-086 during multiple dosing. However, due to an increase in liver enzymes, indicating the potential hepatoxicity of SDZ 210-086, the study was discontinued, although further examination of the effects at higher doses i.e. 1.5 and 2.0 mg/day of SDZ 210-086 had been planned. Therefore, a full investigation of the effects of SDZ 210-086 on the VEPs could not be made.

There are several comments which need to be made about the design of the study reported. Firstly, the baseline or control VEP measurements had been carried out between 1400-2130 hours (appendix 1.5), whereas the "test" VEPs were recorded before 1200 hours, resulting in a time difference of up to 10 hours in some subjects. In chapter 5, there were no significant effect of the time of day on the flash and pattern reversal VEP, therefore this difference in recording time of the control and test VEPs is negligible. However, for investigations into the drug effects on VEPs, a better experimental design would have been to keep the VEP recordings within 1 hour over the recording days. This would allow a control from sleep and meal control and increase the validity of comparison between baseline and test recordings. The VEP was satisfactorily recorded only in subjects DC, SCo and JA, where the difference in time of baseline and test recordings was less than 1 hour. However, there was no sleep control over the subjects and many were awake until 3-4 am watching video tapes. Thus the control of the time from sleep was lost and an added factor of drowsiness was entered.

The results indicated great inter-individual variability in the latency and amplitude measurements, which was contributed by the different age ranges within each group. For instance, the placebo subjects were all aged within the 20-29 year age range, whereas the age of group 1 subjects spanned three decades (20-49 years) and group 2 subjects spanned over two decades (20-39 years). There are reports that with increasing age the flash N2 and P2 and the pattern reversal P100 latency increases with age (section 3.2.2 for flash VEP and section 3.3.1.1 for pattern reversal VEP).

Therefore, the subjects within each group should ideally have been distributed equally according to age.

A majority of the subjects were cigarette smokers and based on informal questioning, admitted to increasing the number of cigarettes smoked during the day due to a lack of any stimulating activity during the study. The non-smokers were also subjected to an increased exposure to cigarette smoke, since all the subjects were allowed use of a single room for recreation. Smoking has been reported to alter the VEP in healthy subjects. The amplitude of the flash VEP components including the N2-P2 amplitude has been reported to increase due to an increase in nicotine administration through cigarette smoking (Friedman and Meares 1980; Woodson et al. 1982). This may account for the increase in the flash N2-P2 amplitude in the placebo group. In addition, Fotzsch et al. (1986) reported an increase in the pattern reversal P100 latency in smokers as compared to non-smokers. However, Luria and McKay (1979) failed to find any significant change in the pattern reversal VEP P100 latency or amplitude. In the present study, since there was no change in the P100 latency, the extent of nicotinic effects through smoking remain unclear. A uniform selection of non-smokers would have reduced any added psychopharmacological actions of nicotine on the flash and pattern reversal VEPs.

At present there have been no reports on the effect of cholinergic agents on the human flash and pattern reversal VEPs. In the cat, physostigmine (an AChEi) has been reported to cause a reduction in the amplitude of the pattern reversal VEP (Harding et al. 1983). Further studies on the transient VEP at various spatial frequencies (0.1-1.6 cycles/degree) revealed that the VEP to low spatial frequencies were affected more than those to higher spatial frequencies (Kirby et al. 1986). These effects were demonstrated with both a reversible AChEi, physostigmine and an irreversible AChEi, diisopropylfluorophosphate (DFP). However, the VEP recovered to baseline levels after DFP eventhough the blood activity of AChE did not increase. Therefore the excess ACh levels which caused the initial reduction in the VEP lost its effectiveness over time. These workers proposed that the recovery of visual function in the presence of excessive ACh was due to either a change in the cholinergic receptors (through number or affinity), and/or compensation for excess ACh by another neurotransmitter system.

Recently, Arakawa et al. (1992) also reported that physostigmine increased the amplitude and induced a phase lag of the steady-state pattern reversal VEP (at 4 Hz and 0.1 cpd). At 6.6 Hz, the pattern reversal VEP amplitude was found to be spatial frequency-selective, with maximal effects at intermediate spatial frequencies (exact

spatial frequency values were not quoted). The transient pattern reversal VEP (at 2 Hz), displayed no changes in the latencies of the P1, N1 and P2 components, but the P1-N1 amplitude was diminished and the N1-P2 amplitude increased. These effects were blocked by the anticholinergic agent scopolamine. These workers suggested that the cholinergic system may be influencing the pattern reversal VEP in a spatial frequency-selective manner. Comparison of these results with the human pattern reversal VEP are limited as it is unclear which component of the cat VEP corresponds to the human VEP.

These studies in the cat suggest that there is a cholinergic influence on the VEP which is selective for spatial frequency. This may explain to an extent why only the flash (with ultimately the lowest spatial frequency) but not the pattern reversal VEP is affected in Alzheimer's disease (Harding et al. 1981, 1984, 1985; Wright et al. 1984a, 1986, 1987) and by scopolamine (Bajalan et al. 1986, Gilles et al. 1989).

Overall, had the study continued to investigate higher doses of SDZ 210-086, this would have provided an ideal opportunity to investigate the effects of a centrally-active cholinergic agent on the human transient flash and pattern reversal VEPs. As yet, there have been no reports on the cholinergic effects although workers have investigated anticholinergic effects on the VEP in humans (Bajalan et al. 1986, Gilles et al. 1989).

CHAPTER 8

The Effects of Cholinergic Modulation on the Flash and Pattern Reversal Visual Evoked Potential

8.1 Introduction

Chapter 4 highlighted that many clinical trials carried out in AD patients can result in inconclusive results. This was partly attributed to the heterogeneous nature of the disease and diagnostic difficulties associated with the condition (Drachman 1983). An alternative strategy would be to test potential therapeutic agents in healthy volunteers before the disease group. The use of healthy volunteers would then establish the efficacy of a therapeutic agent under more homogeneous and controlled conditions. In addition, many trials involve the use of cognitive tests (table 4.1), where the selection of tests are dependent on the investigator. This can lead to misinterpretation of the effects of some agents, e.g. from table 4.1 physostigmine displayed improvement in some psychometric tests but not others (Christie 1982; Peters and Levin 1982; Sullivan 1982; Beller et al. 1985; Gustafson et al. 1987; Stern et al. 1987 and 1988; Thal et al. 1989).

Substantial evidence indicates that the flash P2 and not the pattern reversal P100 latency is delayed in AD (section 4.10.7), the delay increasing with the duration of the disease. Therefore, the use of the flash and pattern reversal VEPs could provide a more uniform and quantifiable method of assessing drug efficacy in such clinical trials. Potential therapy could be expected to slow down, halt, or even reverse the flash P2 delay. Hence, testing efficacy of a therapeutic agent would be enhanced by incorporating the use of the flash and pattern reversal VEPs.

The cholinergic hypothesis (section 4.8.1) for the memory dysfunction in AD proposed that in healthy volunteers, anticholinergic agents such as scopolamine mimicked some of the cognitive deficits observed in the elderly (Bartus et al. 1982; Coyle et al. 1983), which could be reversed by cholinergic agents (Drachman 1977). Therefore, if scopolamine could also mimic the selective flash P2 delay, potential drug therapy could then be tested to reverse these effects in healthy volunteers. Therefore, a brief background of the actions of scopolamine has been reviewed.

8.2 Actions of scopolamine

The anticholinergic agent scopolamine is the (-)-isomer of hyoscine which blocks muscarinic receptors. Scopolamine has both central and peripheral actions (Meyers et

al. 1980; Reynolds 1989; Spero 1989). The peripheral actions of scopolamine are similar to those of atropine, including tachycardia (at some doses), but unlike atropine it can slow the heart. Other actions of scopolamine include smooth muscle relaxation, decreasing secretory activity of exocrine glands especially of the respiratory tract, the gastric, salivary and some sweat glands.

The central action of scopolamine differs from atropine, the former being 3-10 times more potent in its CNS effects (Reynolds 1989). Scopolamine produces depression of the cerebral cortex, especially of the motor areas and acts as a powerful hypnotic; the drowsiness sometimes being preceded by brief excitement. This sedatory or slowing action of scopolamine can also be accompanied by dizziness and fatigue, both common side-effects of the drug. Scopolamine with morphine has been used in cases of acute mania and delirium (Reynolds 1989), as well as for relief of withdrawal symptoms of morphine dependence. As a pre-anaesthetic, scopolamine calms the patient, reduces secretions, produces amnesia, diminishes some of the side-effects of the anaesthetic and assists the induction process. In obstetrics, scopolamine with morphine or pethidine produces a condition of amnesia and partial analgesia known as "twilight sleep". Scopolamine also acts on the vomiting centre in the brain and as an anti-emetic, is used in many vestibular disorders. It is effective as an anti-emetic in motion sickness, Meniere's disease, positional vertigo, labryrinthitis and operative manipulation of the otovestibular apparatus.

Scopolamine has also been used in symptomatic treatment of idiopathic and postencephalitic parkinsonism. The antiparkinsonian effect is achieved by correcting the relative central cholinergic excess thought to occur in parkinsonism as a result of dopamine deficiency. The effects of antimuscarinic agents in parkinsonism is moderate, reducing tremor and rigidity but without significant action on bradykinesia. Antimuscarinic agents such as scopolamine tend to be used as adjunctive therapy with levodopa (British National Formulary 1990).

Scopolamine in the eye is used as a 0.1-0.5% solution applied to the conjunctiva for mydriasis and cyclopegia. It is quicker acting with shorter duration than atropine, although it is more toxic. Following the blocking of muscarinic receptors in the eye, the ciliary muscles are relaxed and there is loss of ability to focus on near objects. The circular muscles in the iris relax and the pupil is dilate. However, mydriasis may precipitate acute closed-angle ("congestive") glaucoma in some patients, usually aged over 60 years, who are predisposed to the condition because of a small eyeball with a shallow anterior chamber and small diameter cornea.

8.2.1 Cognitive effects

Drachman and Leavitt (1974) reported that administration of scopolamine to young normal subjects produced memory and cognitive impairments similar to those seen in drug-free elderly subjects suffering from age-related memory loss. These workers found scopolamine had no effect on immediate memory (single-trial digit span test) but impaired performance on memory storage (supraspan digit storage test and free-recall word storage test), retrieval (retrieval by category test) and cognitive non-memory performance (WAIS- verbal IQ, performance IQ, full-scale IQ and a derived organic index) when compared to either drug free control subjects, subjects administered with physostigmine or those administered with methscopolamine (a peripherally acting scopolamine analogue). The elderly subjects displayed similar impairments as the scopolamine subjects, except the memory retrieval test was not impaired.

The effects of scopolamine were presumed to be specifically related to antimuscarinic activity and not its sedative action, since these memory and cognitive effects were not reversed by the subsequent administration of amphetamine, an attention enhancing catecholaminergic agent (Drachman 1977). The administration of scopolamine-amphetamine combination in young subjects, improved subjective and objective alertness and well-being, without improving performance on the memory and cognitive tests. The administration of scopolamine-physostigmine combination improved some (but without complete reversal) of the scopolamine induced impairments. This uneven improvement with physostigmine was probably due to the different modes and site of action of the two drugs (Drachman 1977).

The effects of cholinergic agents on human memory are thought to be specific. They influence the subject's ability to store information in long-term memory, with minimal effect on the retrieval of information from the long-term memory and have no effect on immediate short-term memory processes (Izquierdo 1989). Hence, the ability to encode new information into long-term memory (i.e. learning) is the aspect of memory most sensitive to cholinergic manipulation (Crow and Grove-White 1973; Drachman and Leavitt 1974; Drachman 1977; Sitaram et al. 1978). This deficit is also a frequent sign in AD (Dillman 1990). Since the scopolamine induced impairments are similar to those seen in AD, they have been widely accepted as a model for the memory and cognitive impairments occurring in AD, (Ostfeld and Aruguete 1962; Crow and Grove-White 1971 and 1973; Safer and Allen 1971; Sitaram et al. 1978; Bartus et al. 1985; Izquierdo 1989).

However, not all workers agree on the use of scopolamine induced amnesia as a model for AD (Fibiger 1991). There have been suggestions that the scopolamine

deficits differ greatly from those seen in AD (Beatty et al. 1986 and 1988; Troster et al. 1989), such that the "dissimilarities outweigh the similarities". For example, in young normal subjects the pattern of changes in regional cerebral blood flow produced by scopolamine differed from that seen in AD subjects (Honer et al. 1988). However, the main reason against the model was that muscarinic antagonists such as scopolamine produce an acute short-lasting blockade of cholinergic receptors, while AD is a chronic, slowly progressing, irreversible disorder, involving many neurotransmitters not just ACh (Gottfries 1990). But, the closest model in humans to the symptoms of the disease to date is that of the muscarinic antagonist (such as scopolamine) induced impairments and until another better model can be found, scopolamine will still be used to examine the memory and cognitive deficits occurring in AD.

8.2.2 Electrophysiological effects

There have been few reports on the effects of scopolamine on the EEG in humans. One of the earliest was by Ostfeld and Aruguete (1962) who reported that administration of scopolamine resulted in replacement of normal alpha activity by slower, low amplitude waves of 5 Hz and an inhibition of the EEG arousal response to single flashes.

Later, Sannita et al. (1987 and 1988) reported that scopolamine produced an overall decrease in the total power, an increase in the relative power of the 0.5-3.5 Hz and 24-40 Hz frequency bandwidths and a parallel reduction of the relative power in the 8-13 Hz frequency segment. These effects on the quantitative EEG occurred 90 minutes post scopolamine dosing and mainly on the posterior (central-occipital) electrode derivations with no significant change observed in the frontopolar electrode derivations.

Recently, Sloan et al. (1992) reported an increase in the drowsy or sleep activity (K complexes, sleep spindles, lambdoid waves) in the EEG of normal subjects administered with scopolamine. Quantitative analysis of the EEG revealed an overall bitemporal and symmetrical slowing of power spectrum in a pattern similar to that recorded from patients with AD. The changes in the EEG coherence spectrum were most marked in the left hemisphere. These scopolamine induced modifications were not present with methscopolamine, hence the effects on the EEG were indicative of the anticholinergic action at the cerebral level (Sannita et al. 1988; Sloan et al. 1992).

There are equally few reports on the effects of scopolamine on the auditory evoked potentials and event-related potentials (ERP). Buchwald et al. (1991) investigating the

effect of scopolamine on the midlatency auditory evoked potential reported a decrease in the P1 amplitude which eventually disappeared, but showed a marked recovery with physostigmine administration.

Hammond et al. (1987a) reported that scopolamine abolished the auditory P3 ERP but had no effect on the pattern reversal VEP or alpha rhythm. Meador et al. (1987) also reported scopolamine to increase the P3 latency and a reduction in P3 amplitude which was partially reversed by physostigmine but not by the adrenergic agent methylphenidate (Meador et al. 1988) or the antiserotonergic agent methysergide (Meador et al. 1989). The effect of scopolamine on the ERP to visual stimuli also showed a dose-dependent slowing of the P3 (Callaway 1984). The effect being greater on stimuli of high spatial frequency than on those of low spatial frequency (Callaway et al. 1985). These workers suggested that the scopolamine induced impairment was selective and only affected processing targets requiring attention (Brandeis et al. 1992).

Sannita et al. (1988) reported effects of scopolamine on the electroretinogram (ERG) included an increase in the amplitude of the a-wave but without any systematic trend related to dose. These workers also reported an increase in the a-wave latency in the placebo group compared to baseline measurements, and a reduction in the b-wave amplitude with cyclopentolate in the same study, hence the authors suggested further work with a more systematic approach was required.

There have been several reports of the effects of scopolamine on the VEPs. Bajalan et al (1986) investigated the effects 0.6 mg of scopolamine administered to 10 subjects. They reported significant ($P<0.05$) increases in the mean flash P2 and N3 latencies accompanied by an increase in the N2-P2 amplitude ($P<0.01$), but no effect on the pattern reversal VEP or the BAEP components. Gilles et al (1989) using 0.5 mg of scopolamine, also reported a significant ($P<0.03$) increase in the flash P2 latency in 16 healthy young male subjects, without any significant effect on the pattern reversal VEP.

Ray et al. (1991) compared the effects of scopolamine in the young, middle-age, elderly and patients with AD, on the latencies of the flash P2 and pattern reversal P100 components. There was a significant increase in the flash P2 latency in the young subjects by about 8 ms across all doses (0.02, 0.004 and 0.007 mg/kg). But there was also an increase in the pattern reversal P100 latency in all age-groups in response to scopolamine, in contrast to the findings of Bajalan et al. (1986) and Gilles et al. (1989). There were no significant effects of the peripherally acting anticholinergic agent glycopyrrolate on the VEPs. These workers also found no difference in the

flash P2 latency between the patients with AD and the elderly control in contrast to Harding's finding of a delayed P2 co-existing with a normal P100 (section 4.10.7). However, the patients were considered to be in the mild to moderate stage of the disease and inspection of the results presented revealed that the P2 latency in the AD group was around 20 ms greater than the elderly group although this did not reach significance.

In contrast to the above findings, Sloan et al. (1992) observed no effect of 0.6 mg scopolamine or 0.5 mg methscopolamine on the flash and pattern reversal VEPs in 10 healthy volunteers. The results published by these authors, showed that the flash P2 increased by 5.5 ms, whereas the P100 increased by 0.3 ms and the P2 and P100 with methscopolamine changed by less than 1 ms. Thus, there seemed to be an increase in the flash P2 with scopolamine but failed to reach significance.

Recently, Sannita et al. (1993) examined the effects of scopolamine at three doses (0.25, 0.5 and 0.75 mg) and topical application of cyclopentolate (a mydriatic) in young healthy male volunteers. There was no effect observed on the flash VEP latencies but the amplitude of wave IV-V (P2-N3) was reduced from 30 minutes post dosing. The pattern reversal VEP displayed a shortening of the N75 latency and an increase in N175 latency at the highest dose of scopolamine (0.75 mg), with no effect on the amplitudes. There was no effect on the flash or pattern reversal VEP with the cycloplegic drug cyclopentolate, suggesting that the effects were not due to any pupillary effects of scopolamine.

The effect of scopolamine to stimuli other than the flash or pattern reversal checkerboard have also been investigated. Smith et al. (1990) examined the effects of 1.2 mg scopolamine on 6 healthy young volunteers to the pattern onset-offset VEP. There was a significant increase in the CI latency and reduction in amplitude without any significant effects on the CII component. These workers did not examine the effect on the flash or pattern reversal VEP.

8.3 Investigation of the effect of scopolamine on the flash and pattern reversal VEPs

Scopolamine administered to healthy volunteers has been used as a model for the cognitive deficits observed in AD (section 8.2.1). Early studies of scopolamine indicated that it also mimicked the selective flash P2 delay observed in AD (Bajalan et al. 1986; Gilles et al. 1989), but recent studies have failed to observe such effects (Ray et al. 1991; Sannita et al. 1993 Sloan et al 1992). Therefore a study was carried

out in healthy volunteers to investigate whether the effects of scopolamine on the flash and pattern reversal VEPs mimic those observed in patients with AD.

8.3.1 Methods

8.3.1.1 Subjects

11 Young, healthy, normal, non-smoking male volunteers (age range 19-25 years; mean 22 ±2 years) were paid to participate in the study. All subjects underwent a full medical examination and fulfilled the inclusion criteria (appendix 1.-as for SDZ 210-086) before entering the study. All subjects had a visual acuity of 6/6 or better with correction where needed. Signed consent forms were obtained from each subject after being fully informed about the drug and the methods to be used in the study. The project was approved by the Human Science Ethics Committee at Aston University.

8.3.1.2 Instrumentation

The flash stimulus was delivered by a Grass PS22 stroboscope at intensity 2 (luminance of 1363 cd/m^2), placed 30 cm away from the eye subtending a field of 36^o and delivered at a rate of 1.7 flashes per second.

The pattern reversal stimulus consisted of a black and white checkerboard pattern of 56' checks in a field subtending 27^o 50' x 22^o 24' to the eye. The checks reversed at a rate of 1.7 Hz. The luminance of the white checks was 170 cd/m^2 and a contrast of 78% on a Bio-Logic television screen (with resolution of 700 lines horizontally and 350 lines vertically).

Both visual stimuli were generated from the Medelec OS 5 unit triggered by a 5V TTL pulse from the Bio-Logic Traveler™ electrodiagnostic testing system with twin disk drives. The bandpass filtering was set at 1-30 Hz (-3dB down point), and the gain at 30,000. Two runs each consisting of 50 averages were carried out for each eye, totalling an average of 100 responses per eye.

8.3.1.3 Procedure

The subjects had fasted overnight prior to the study day and given a standardised light breakfast 1 hour before dosing. Caffeine and alcohol beverages were prohibited both 12 hours prior to and during the study. Binocular flash and monocular right eye pattern reversal VEPs were recorded using silver-silver chloride electrodes placed on the scalp according to the international 10-20 system (Jasper 1958). The VEPs were recorded using the standard derivations used in our clinic, of O2 referred to C4 and O1

referred to C3 for both the flash and O2 and O1 both referred to Fz for pattern reversal stimulation. The interelectrode impedance was maintained below 5kΩ.

VEPs were recorded on arrival of the subjects (baseline run), then 1, 2, 4 and 6 hours post dosing (however, 5 subjects did not have their VEPs successfully recorded at 4 hours, thus the results for this time interval only refers to 6 subjects). 0.4 mg Scopolamine (hyoscine hydrobromide) was injected intramuscularly by a medical examiner who remained present throughout the duration of the study. In addition to VEPs, safety and tolerability examinations of vital signs such as supine and standing blood pressure and pulse were also monitored by the clinician at 1, 2, 3, 4, 6, 8 and 24 hours post dosing. An ECG was also recorded at 2.5 hours post dosing. The subjects were kept in a single room under supervision in case of any adverse reactions. In between testing, the subjects would read, work or watch television to overcome boredom and minimise the effects of drowsiness.

8.3.2 Results

8.3.2.1 Data analysis

Analysis of the flash VEP components were confined to measurement of N2 and P2 together with the late N3 and P3 latencies. The amplitude measurements included the N2-P2, P2-N3 and N3-P3 peak-to-peak amplitudes. The early P1 and N1 components were not present in all subjects due to the relatively young age of the subjects (Wright et al. 1985). For the pattern reversal VEP, the N75 and P100 and the later N140 and P160 latencies and the N75-P100, P100-N140 and N140-P160 peak-to-peak amplitudes were also analysed. For clarity, only results from the right hemisphere were considered as there was no difference in results between hemispheres.

Statistical analysis was carried out using the one factor analysis of variance with repeated measures to examine the effect of scopolamine on the VEPs. The least squares difference (Fischer's LSD test) was then applied to examine the difference between group mean values.

8.3.2.2 Flash VEP

The group mean results for the flash VEP have been presented in table 8.1. In order to magnify any changes, the percentage difference between the means obtained at baseline and the 1, 2, 4 and 6 hour intervals were calculated and plotted in fig. 8.1 for the latencies and fig. 8.2 for the amplitudes of the flash VEP.

There was a significant ($F=3.45$, $df=3$, $P<0.05$) increase in the mean P2 latency over 1, 2 and 4 hours after which the P2 latency returned towards baseline levels (fig. 8.1). Comparison of the group means revealed a significant increase in P2 latency at the 1, 2, 4 and 6 hour intervals compared to baseline (LSD $t=3.67$, $P<0.05$). There was very little change (within 3 ms) in the mean N2 latency. However, the later mean N3 latency displayed a small increase over the 1, 2, an 4 hours followed by a dramatic decrease at 6 hours but failed to reach significance. The P3 displayed an increase in the mean latency at the 1 and 2 hours similarly to P2, followed by a return towards baseline levels at 4 hours and a shortening at 6 hours. However, no significance was reached, probably due to the large standard deviation values (table 8.1) obtained for this P3 component.

Amplitude trends included a steady increase in the mean N2-P2 amplitude reaching a maximum at 4 hours, but failing to reach statistical significance (fig. 8.2). The mean P2-N3 amplitude displayed an increase up to 2 hours followed by a return towards baseline levels at 4 and 6 hours. In contrast, the mean N3-P3 amplitude displayed a slight increase at 1 hour followed by a steady decrease over 2, 4 and 6 hours. Generally, the P2-N3 and N3-P3 amplitudes displayed very similar trends (fig. 8.2), suggesting that these changes reflected the effect of scopolamine on the N3 component which was common to both of these measurements.

The standard deviation values for the mean N2 latency were high when compared to normative data obtained previously for our laboratory by Wright et al. (1985), who obtained N2 standard deviation values of around 12 ms for the 20-29 year age range. A contributing factor for the higher values obtained in the present study could be the difficulty in identifying some of the VEP components in some subjects. For example, subject 009 TJ displayed a P-N-P or W type response to the flash stimulation, making identification of both the N2 and P2 components difficult (fig. 8.3). The first negative component occurred around 40 ms, followed by a positive around 75 ms, a second negative around 90 ms and a second positive around 116 ms. Since the P2 rarely occurs before 90 ms, the second positive was identified as the P2 component and the preceding negative as the N2 component, accounting for its apparently "late" occurrence. In contrast, subject 001 RR displayed a N2 occurring around 45 ms. Figure 8.4 shows the major positive component for this subject, was broad, beginning around 60 ms, reaching a trough around 100 ms and ending by 145 ms, hence accounting for the "early" N2 component. Interestingly, these waveforms displayed different effects on the flash VEP on administration of scopolamine. Subject 009 TJ (fig. 8.3) displayed no change in the P2 latency over the day. However, subject 001 RR (fig. 8.4) displayed a P2 component which became broader after scopolamine

Table 8.1 Results of the mean group latencies and amplitudes of the flash VEP following scopolamine administration. Data from channel O2-C4.

	BASELINE	1 HOUR	2 HOUR	4 HOUR*	6 HOUR~
N2 Latency					
Mean	69.69	69.34	69.07	67.66	66.70
SD	16.67	16.29	16.36	18.02	14.57
P2 Latency					
Mean	107.65	111.68	112.84	114.26	109.57
SD	8.85	7.38	9.49	5.83	9.03
N3 Latency					
Mean	157.40	159.45	159.09	159.04	150.98
SD	18.80	21.52	21.96	20.97	12.29
P3 Latency					
Mean	197.35	200.11	202.24	200.61	188.97
SD	28.99	28.50	36.77	30.36	27.11
N2-P2 Amplitude					
Mean	9.78	9.91	11.03	12.42	11.87
SD	5.94	5.03	5.37	5.11	6.03
P2-N3 Amplitude					
Mean	12.60	13.80	14.27	13.32	13.06
SD	5.94	5.29	5.53	7.50	5.35
N3-P3 Amplitude					
Mean	7.78	8.37	7.42	7.18	6.75
SD	3.51	3.52	3.54	4.94	3.8

* Data from 7 subjects only. ~ Data from 10 subjects. See appendix 3 for full results.

Figure 8.1 Plot of the percent difference from baseline in the group mean latency of the flash VEP components before (at time 0 hour) and after scopolamine administration. Data from channel O2-C4.

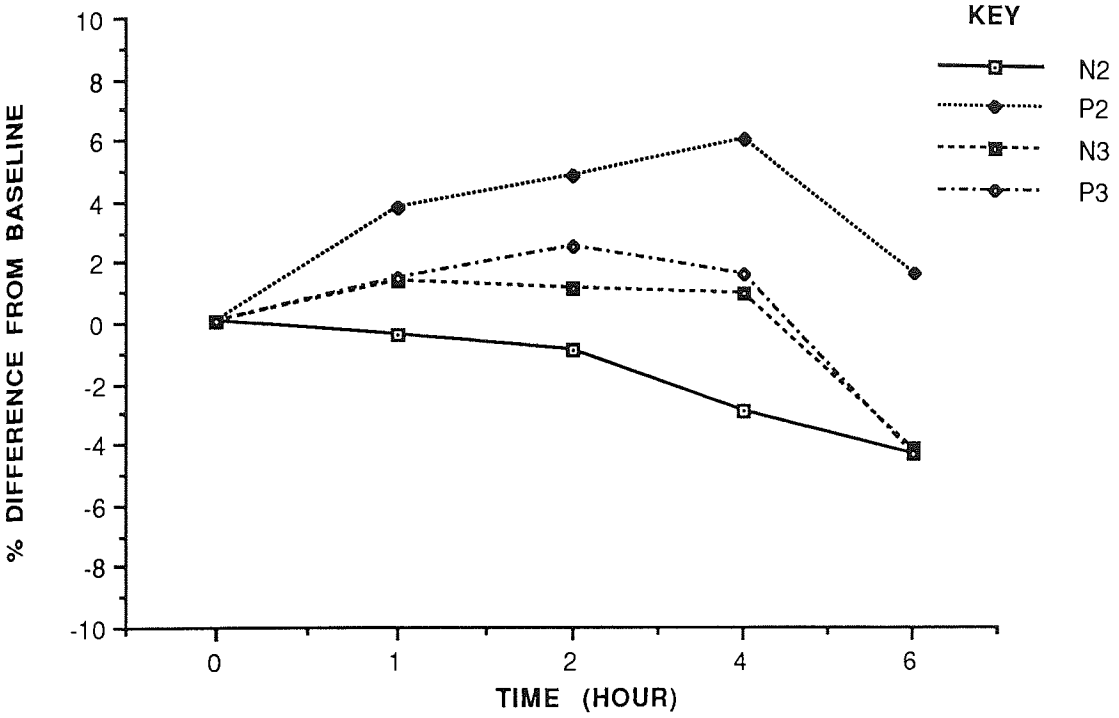


Figure 8.2 Plot of the percent difference from baseline in group mean amplitudes of the flash VEP components before (time 0 hour) and after scopolamine administration. Data from channel O2-C4.

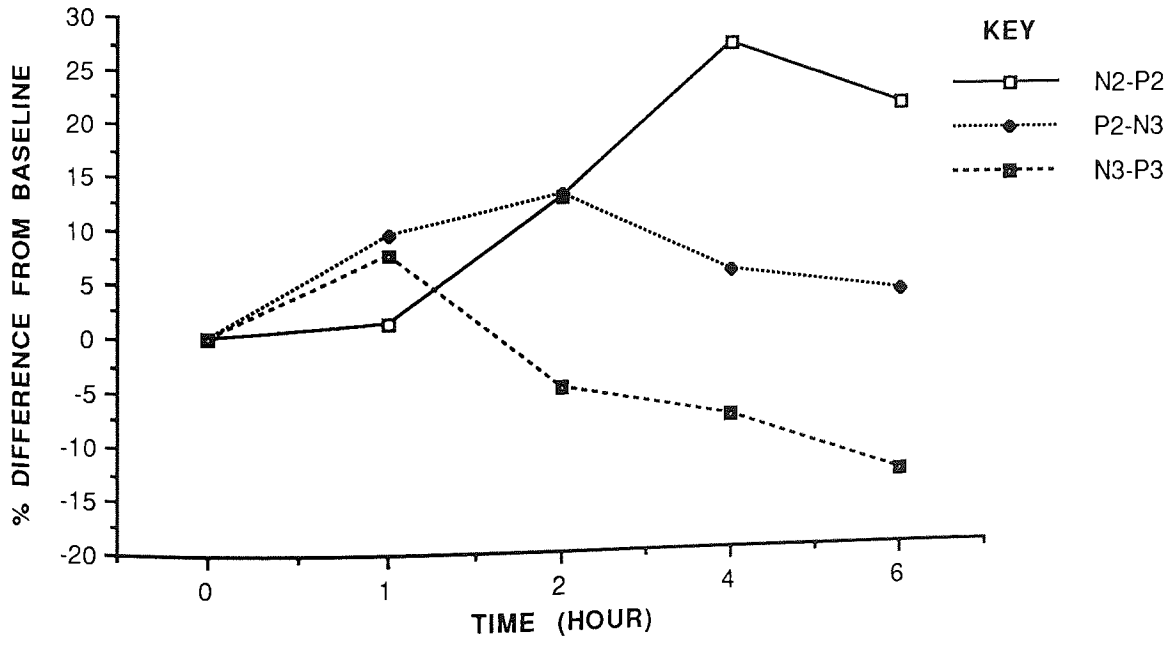


Figure 8.3 Flash VEP waveforms recorded from subject 009 TJ showing a P-N-P type response. The P2 was identified as wave "c" of this W response and N2 was taken as the preceding "b" wave. Recorded at (from top to bottom) baseline, 1, 2 and 6 hours. Data recorded from channel O2-C4.

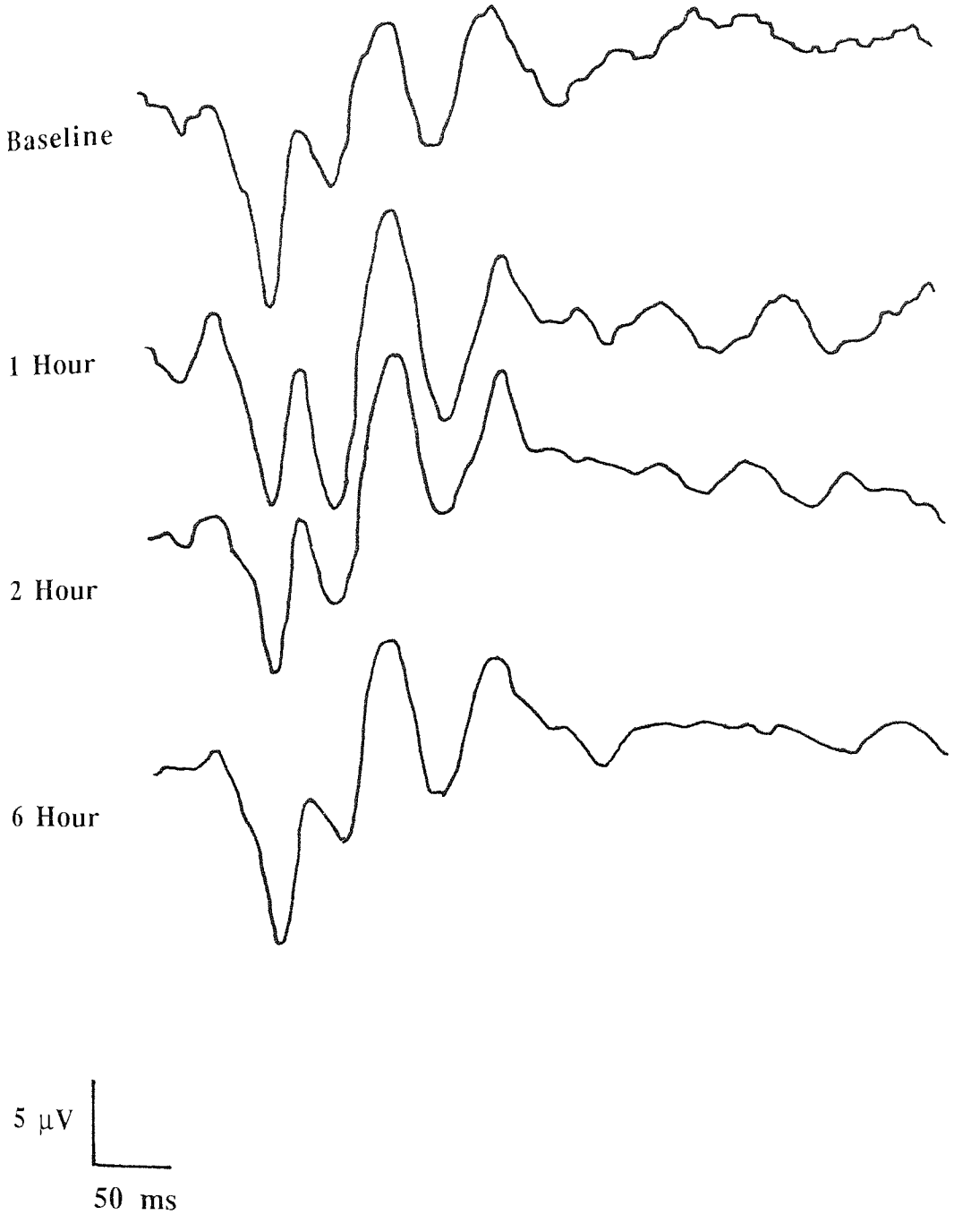
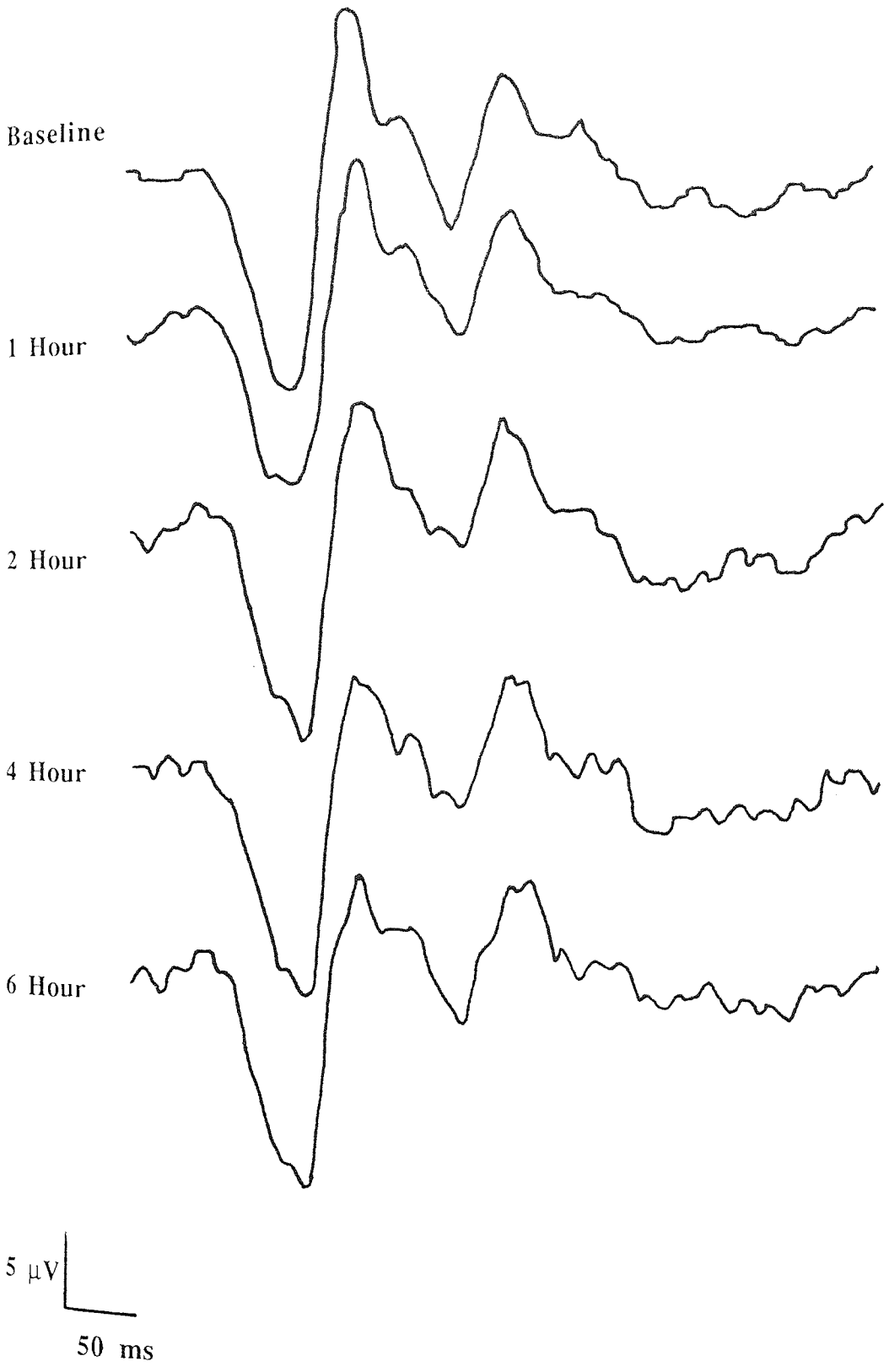


Figure 8.4 Flash VEP waveforms from subject 001 RR. The N2 occurs early around 45 ms followed by P2 at 105 ms. There is also a broadening of the P2 at 1 hour post dosing which begins to disappear around 6 hours. Recorded at (from top to bottom) baseline, 1, 2, 4 and 6 hours. Records from channel O2-C4.



administration thereby increasing its latency value. This effect was also observed by Bajalan et al. (1986).

8.3.2.3 Pattern reversal VEP

The group mean and standard deviation results for the pattern reversal VEP have been presented in table 8.2. The percentage difference between the means obtained at baseline and the 1, 2, 4 and 6 hour intervals were calculated and plotted in fig. 8.5 for the latencies and fig. 8.6 for the amplitudes of the pattern reversal VEP.

Similarly to the flash N2 latency, the mean pattern reversal N75 latency showed very little change (within 3%) post scopolamine dosing. The mean P100 latency also displayed very little change except at the 4 hour interval where there was an increase. However, this was due to the small sample (n=7) in this group, as the mean P100 latencies for these 7 subjects displayed a maximum change of only 1.4 ms overall from baseline to 6 hours. The mean N140 latency also showed very little change, with a maximum of a 1.1% decrease at 6 hours. In contrast, the P160 displayed an increase at 1 and 2 hours followed by a decrease at the 4 and 6 hours.

Figure 8.5 Plot of the percent difference from baseline in the group mean latency of the pattern reversal VEP components before (time 0 hour) and after scopolamine administration. Data from O2-Fz.

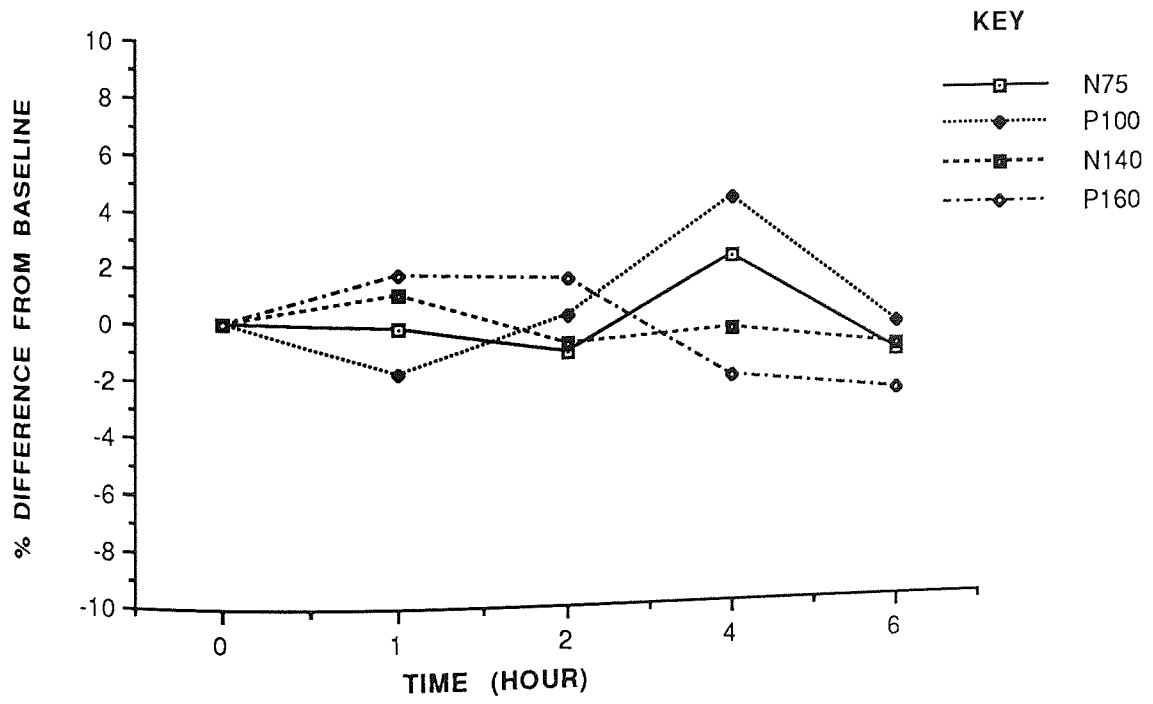


Table 8.2 Results of the mean group latencies and amplitudes of the pattern reversal VEP following scopolamine administration. Data from channel O2-C4.

	BASELINE	1 HOUR	2 HOUR	4 HOUR*	6 HOUR~
N75 Latency					
Mean	70.67	70.85	70.05	72.41	69.92
SD	8.09	7.15	7.12	8.65	8.48
P100 Latency					
Mean	112.22	110.26	112.48	117.05	111.92
SD	13.63	11.21	12.43	7.41	12.89
N140 Latency					
Mean	178.45	180.22	176.94	177.73	176.56
SD	21.08	20.24	17.39	22.78	24.94
P160 Latency					
Mean	229.49	233.49	232.89	224.75	223.34
SD	19.19	18.73	21.29	21.70	18.62
N75-P100 Amplitude					
Mean	7.46	7.95	9.57	11.16	9.73
SD	3.61	3.64	4.37	3.88	4.04
P100-N140 Amplitude					
Mean	11.35	12.14	13.57	14.22	14.01
SD	4.23	4.64	5.44	4.88	4.44
N140-P160 Amplitude					
Mean	5.35	4.83	6.17	5.60	5.88
SD	3.03	3.69	3.35	3.76	4.29

* Data from 7 subjects only. ~ Data from 10 subjects. See appendix 3 for full results.

The amplitudes of the pattern reversal VEP components all showed similar trends (fig. 8.6). Both the N75-P100 and P100-N140 amplitudes showed a steady increase up to 4 hours before starting to return towards baseline levels at 6 hours. However, only the N75-P100 amplitude changes were significant ($F=5.309$, $df=3$, $P<0.05$). Comparison of the mean N75-P100 amplitude values, showed an increase at 2, 4 and 6 hours when compared to both baseline and 1 hour measurements. The P100-N140 amplitude also displayed an increase between 2, 4 and 6 hours compared to baseline, but failed to reach statistical significance. There was also no statistically significant change in the N140-P160 amplitude which displayed change in mean amplitude below 1 μV over the day.

The standard deviation values of the major pattern reversal P100 component were slightly high compared to normative data of Wright et al. (1985). This was largely due subject 009 TJ displaying an "early" P100 latency (fig. 8.7). In contrast, subject 006 JS displayed a P100 occurring beyond 135 ms which was considered clinically abnormal for the age (fig. 8.8). The pattern reversal VEP of this subject was generally of poor quality with a low amplitude and broad P100 and P160 components, particularly at baseline level.

Figure 8.6 Plot of the percent difference from baseline in the group mean amplitudes of the pattern reversal VEP components before (time 0 hour) and after scopolamine administration. Data from channel O2-Fz.

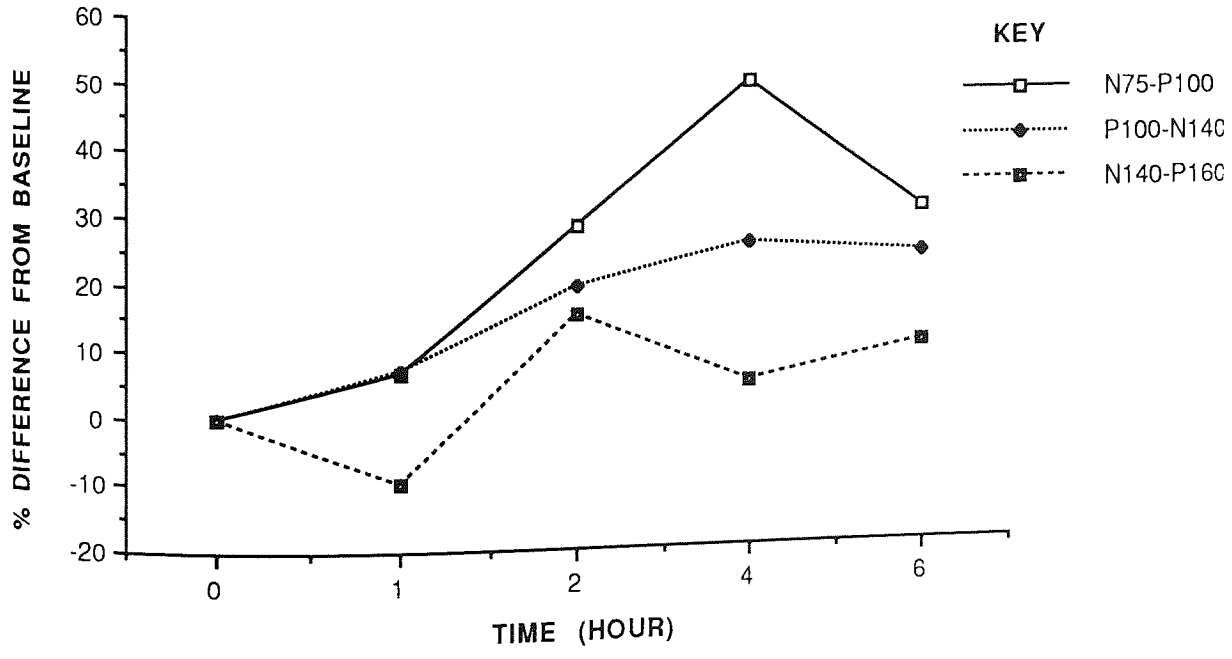


Figure 8.7 Pattern reversal VEP waveforms from subject 009 TJ showing an early P100 component. Recorded at (from top to bottom) baseline, 1, 2 and 6 hours. Records from channel O2-C4.

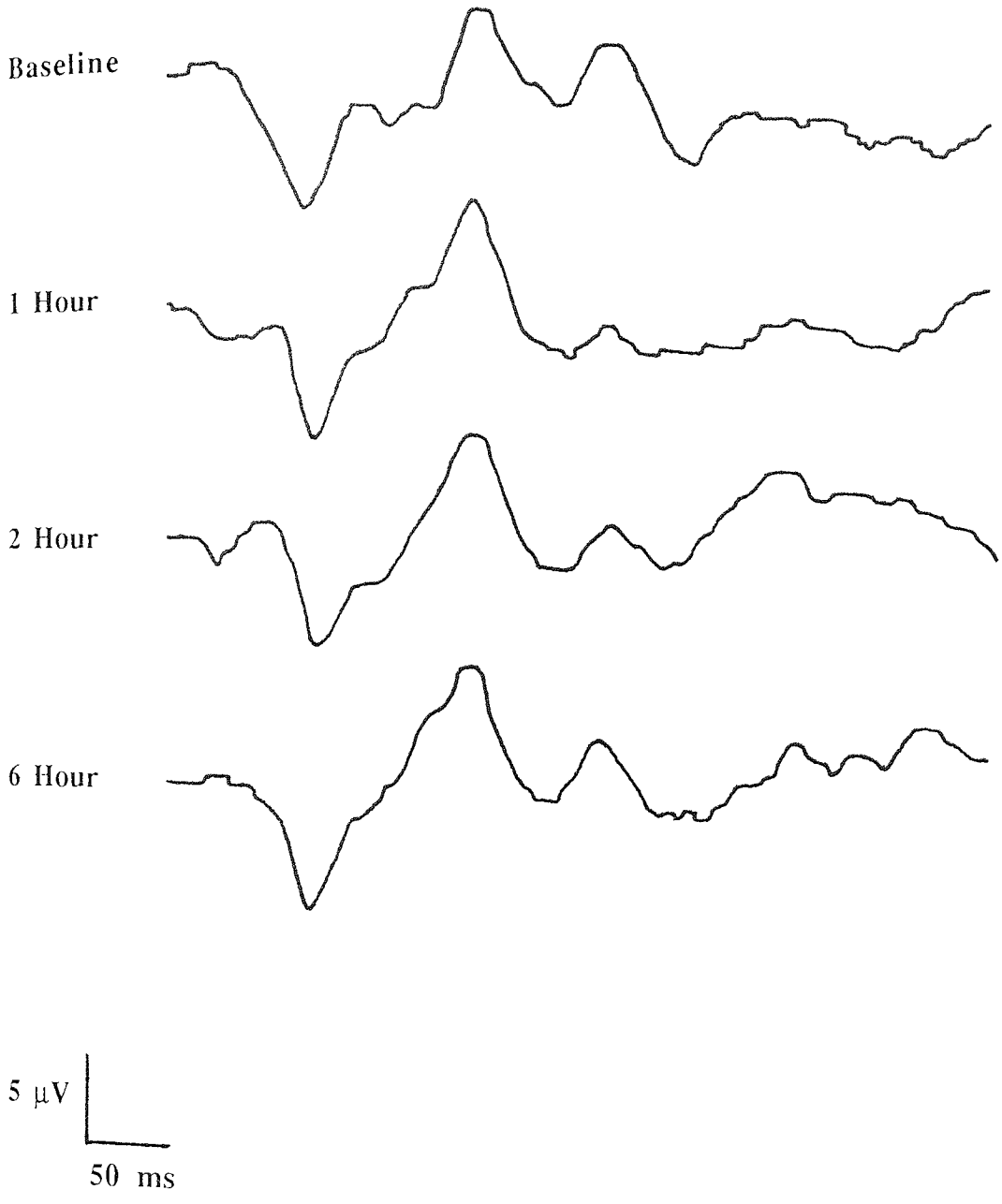


Figure 8.8 Pattern reversal VEP waveforms from subject 006 JS showing a broad and delayed P100 component. Recorded at (from top to bottom) baseline, 1, 2, 4 and 6 hours. Records from channel O2-C4.

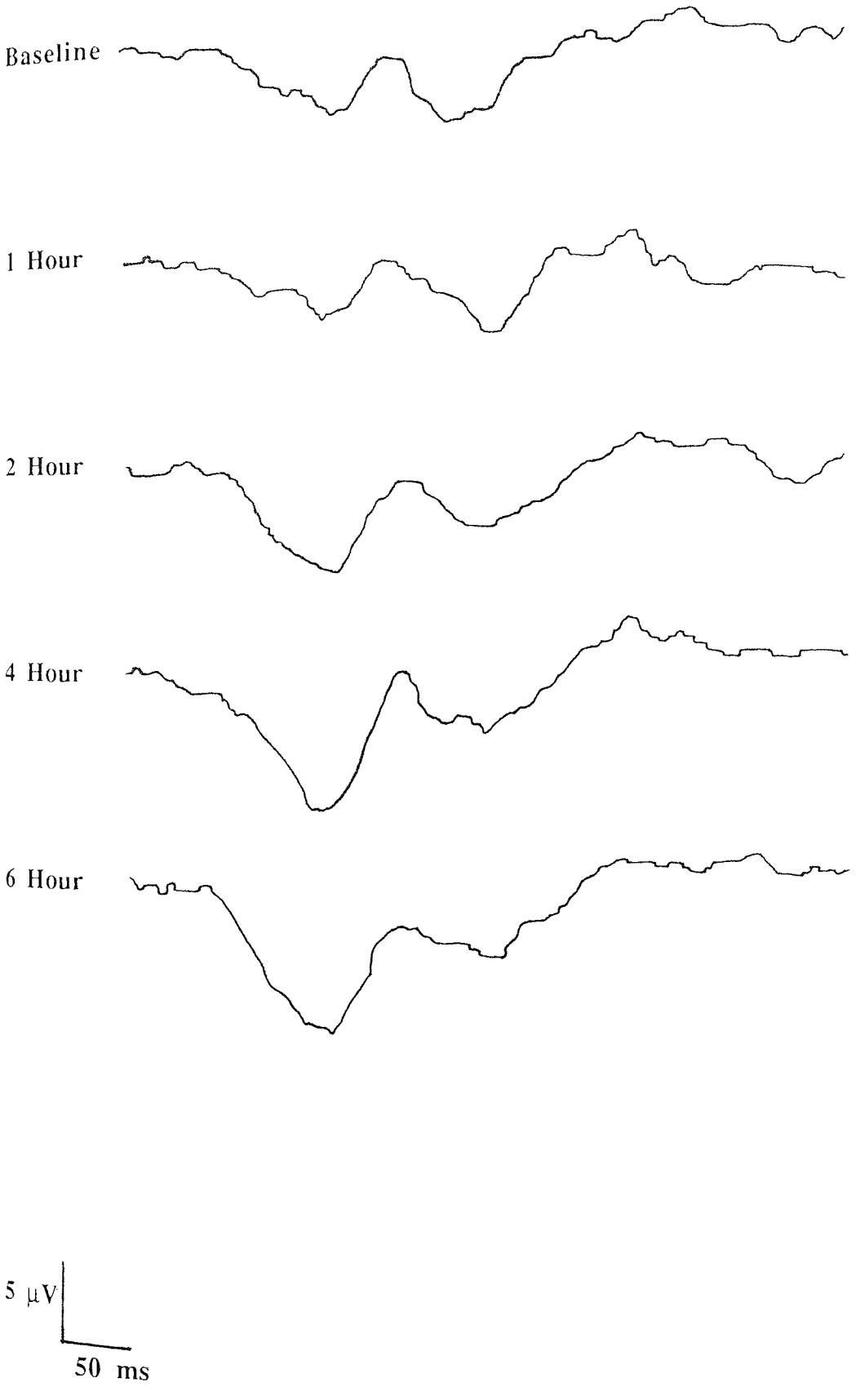
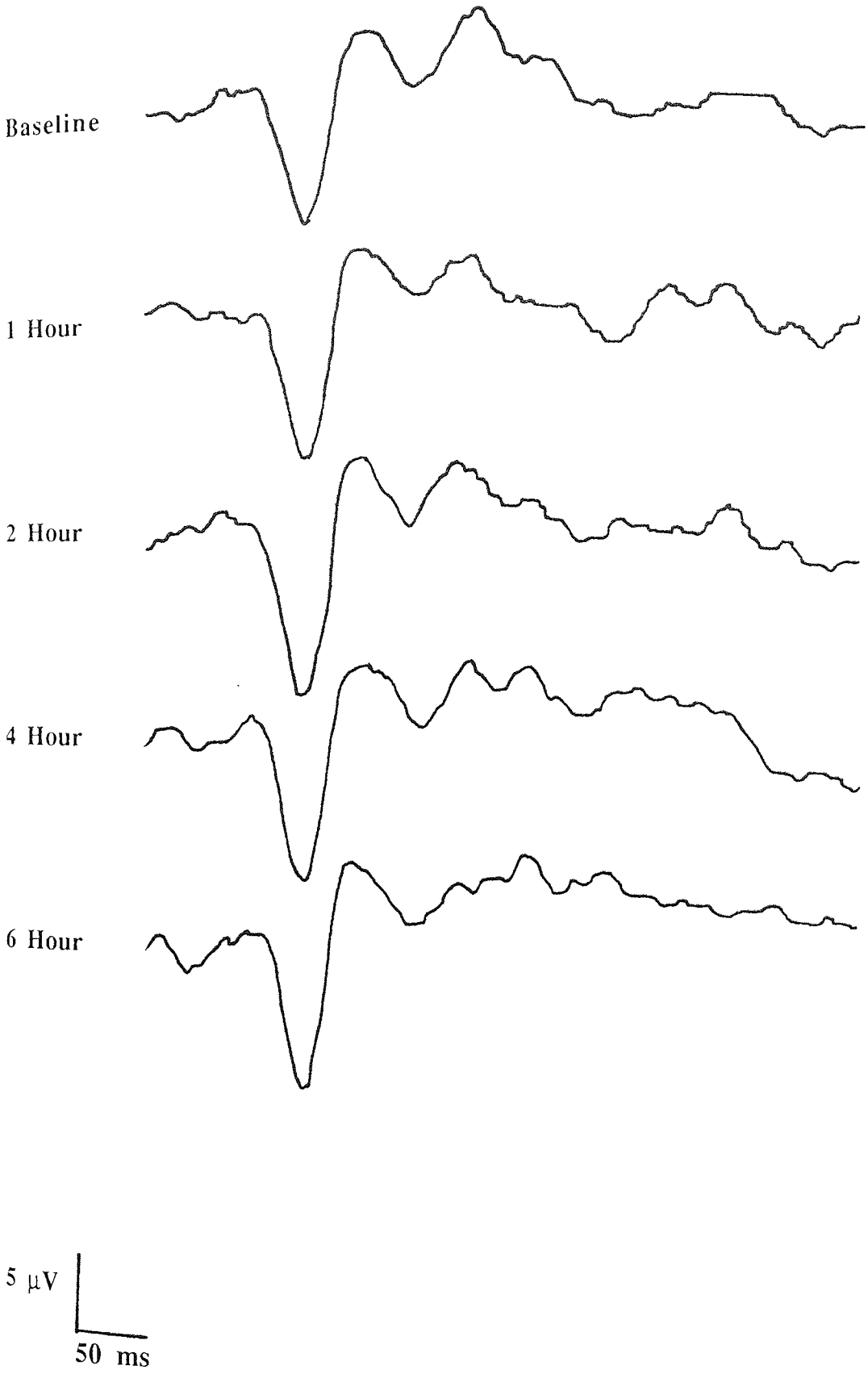


Figure 8.9 Pattern reversal VEP waveforms from subject 001 RR showing little change in the P100 latency. Recorded at (from top to bottom) baseline, 1, 2, 4 and 6 hours. Records from channel O2-C4.



In general, the later N140 and P160 components of the pattern reversal VEP (like the flash VEP), showed greater standard deviation values than the earlier N75 and P100 components, reflecting the clinical importance of these earlier components. Interestingly, subject 001 RR who displayed a broadening flash P2 component (fig. 8.3), displayed a pattern reversal VEP which was remarkably unchanged in P100 latency (fig. 8.9), although there was a steady increase in amplitude over the day compared to baseline. These effects were similar to those observed by Bajalan et al. 1986).

8.3.2.4 Side-effects

The main complaints from the subjects included a feeling of light-headedness or dizziness between 5-20 minutes after scopolamine injection which subsided within an hour in most subjects. Most subjects suffered from an increased feeling of thirst and dry mouth, and only 3 experienced a slight blurring of vision but still attained a visual acuity of 6/6 on the Snellen chart. These effects were all expected (section 8.2) and classed as mild by the subjects.

8.3.2.5 Vital signs

The following results were made available by Sandoz Ltd. and recorded by the medical examiner Dr. T. Betts.

The reduction in the pulse was reduced post-dosing (fig. 8.10) as expected from scopolamine administration. The lowest pulse was recorded around 4 hours post dosing after which the pulse returned towards pre-dosing levels by the next day. The fall in the blood pressure with scopolamine administration (fig. 8.11) also reached lowest values around 4 hours post dosing. As before, baseline levels were reached by 24 hours. The subjects were always under medical supervision and were allowed to leave with permission from the medical officer.

Figure 8.10 The effect of scopolamine on the supine and standing pulse. The graph represents group mean values as individual values were not available.

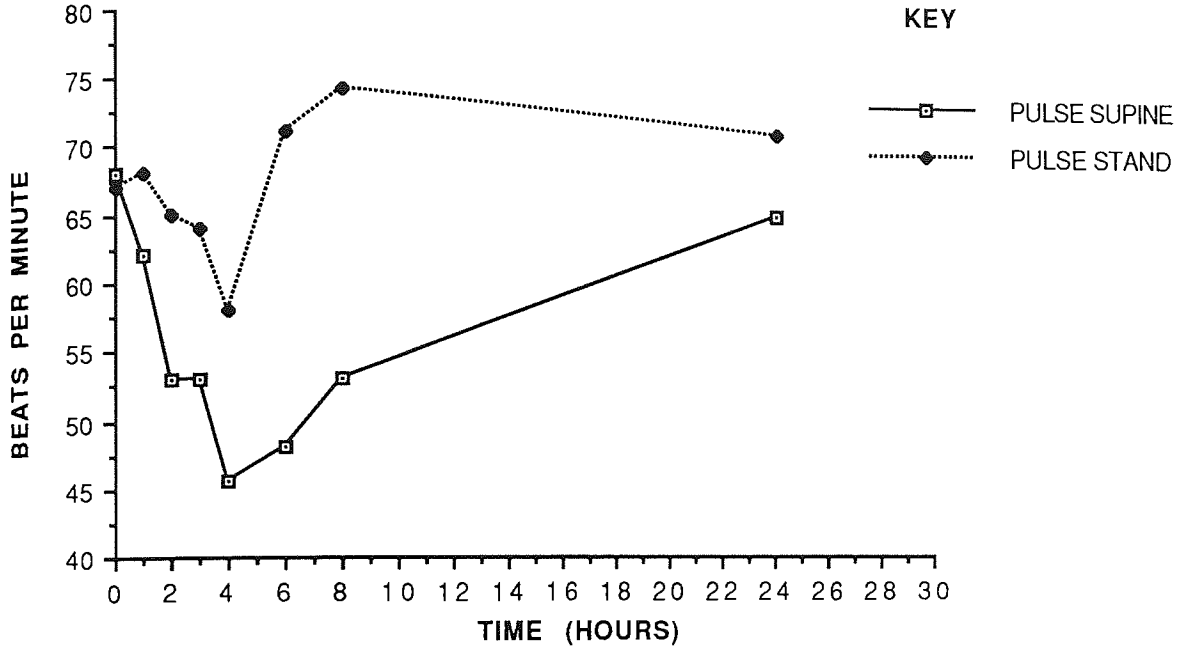
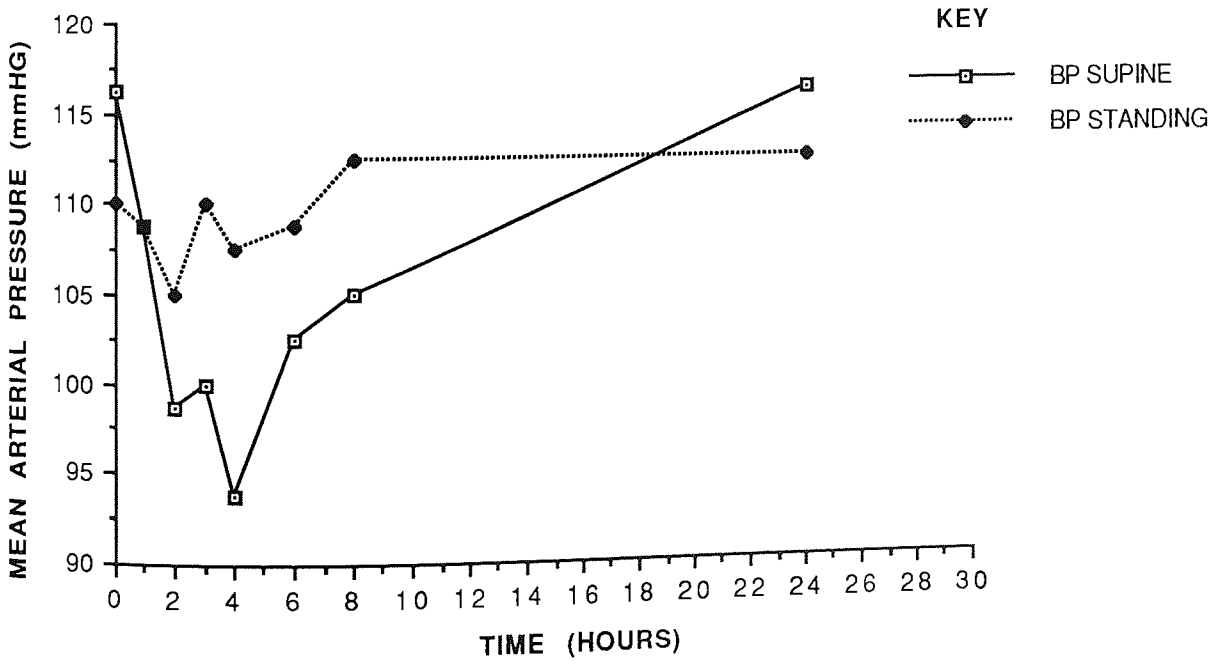


Figure 8.11 The effect of scopolamine on the supine and standing systolic blood pressure. The graph represent group mean values.



8.3.3 Discussion

There was a discrepancy in the effects of scopolamine on the flash and pattern reversal VEP reported in the literature. The most consistent effect reported was a flash P2 delay (Bajalan et al. 1986; Gilles et al. 1989; Ray et al. 1991) with no effect on the pattern reversal P100 latency (Bajalan et al. 1986; Gilles et al. 1989; Sannita et al. 1993; Sloan et al. 1992). The present study was in agreement with these observations. However, many workers also reported additional effects observed in their study which were not observed by others. For example, Bajalan et al. (1986) reported an increase in the flash N3 latency and N2-P2 amplitude, which was not observed by other workers. In the present study, the flash N3 latency and the N2-P2 amplitude displayed an increase over the day but failed to reach statistical significance. Ray et al. (1991) reported an increase in the pattern reversal P100 latency which was not observed by any of the other workers. Sannita et al. (1993) reported an increase in the flash P2-N3 amplitude, which was observed in the present study but failed to reach statistical significance. These workers also reported a decrease in the pattern reversal N75 latency and an increase in the N175 latency, both of which were not observed by any other workers or in the present study. The present study however, observed a significant increase in the pattern reversal N75-P100 amplitude which was not observed by other workers. Also in the present study, the later flash P3 and pattern reversal P160 components were examined, but the effects of scopolamine on these components have not been reported by other workers.

Therefore there seems to be a difference in opinion on the effects of scopolamine on the flash VEP. Although the most consistent observation has been a delayed flash P2 latency, this was not observed by Sannita et al. (1993) or Sloan et al. (1992). Interestingly, Sloan et al. (1992) reported a 5.5 ms increase in P2 latency 1 hour post dosing which failed to reach significance, although smaller increases reported by other workers reached statistical significance at 1 hour post dosing, e.g. Bajalan et al. (1986) reported an increase of 5.3 ms; Gilles et al. (1989) reported an increase of 3.5 ms and the present study observed an increase of 4.03 ms. The increase in the P2 latency in the present study, although small was observed consistently in 8 out of the 11 subjects. The failure of Sannita et al. (1993) and Sloan et al. (1992) to observe a significant increase in P2 latency thus remains unclear since both groups studied volunteers of similar age groups and similar doses of scopolamine as those above.

The effects of scopolamine on the VEPs may have been due to peripheral effects such as drowsiness and pupil dilatation. However, pupil size has been reported not have a significant effect on the flash VEP (Kooi and Bagchi 1964; Spehlman 1985). In

addition, Ray et al. (1991) observed no significant increase in the pupil diameter (<0.5 mm) or a change in the visual acuity with scopolamine administration. Bajalan et al. (1986) carried out refractive checks and reported on a few subjects experiencing a high degree of heterophoria and difficulty in maintaining binocular vision, but did not affect the flash VEP and monocular stimulation was used for the pattern reversal VEP. Blurring has been reported to produce an increase in latency and attenuation of the pattern VEP components, especially to check sizes smaller than 20' (Regan and Richards 1971; Spekreijse et al. 1973), but in the present study there was no effect on the pattern reversal VEP latency and the N75-P100 amplitude was increased post dosing rather than attenuated. Since Sannita et al. (1993) failed to observe any changes in the flash and pattern reversal VEPs with cyclopentolate, it can be concluded that the effects of scopolamine on the VEPs were not due to any pupillary effects.

The effect of fatigue and drowsiness on the VEP can generally result in the appearance of alpha activity dominating the VEP, affecting latency and amplitudes measurements (Spehlmann 1985). However no such effect observed in the waveforms in the current study. In addition, Sloan et al. (1992) observed that drowsiness on administration with scopolamine, did not play a part in the VEPs recorded. Therefore, the effects on the VEPs cannot be attributed to any sedatory effects of scopolamine.

Therefore, in view of the findings of the present study and those previously reported in the literature (Bajalan et al. 1986; Gilles et al. 1989), scopolamine delays the flash P2 latency without affecting the pattern reversal P100 latency, an effect similar to that observed in patients with AD by Harding's group and confirmed by others (section 4.10.7). However, the magnitude of the impairment due to scopolamine was not as great as that seen with the disease. This can be attributed to the low dose of scopolamine used and also the disease includes dysfunction in many neurotransmitter systems not just in the cholinergic muscarinic system, as provided by scopolamine administration. Hence to a limited extent, scopolamine mimics the effects of AD on the flash and pattern reversal VEPs and therefore provides a model for the disease-induced impairments seen on the flash VEP.

There is evidence that the rat visual cortex receives cholinergic input (Houser et al. 1983) and that the cholinergic innervation of the striate cortex originates from cholinergic neurones of the septum, diagonal band of Broca, nucleus basalis of Meynert and substantia innominata (Coyle et al. 1983; McKinney et al. 1982; Mesulam 1990; Singer 1990; see also section 2.4.3). The cells in the diagonal band of Broca and nucleus basalis of Meynert are also grossly reduced in AD (McKinney et al. 1982; Perry and Perry 1982; Coyle et al. 1983) and related to recent memory and

learning, the latter being consistent with the dysfunction in recent memory and learning in the early stages of AD (Coyle et al. 1983). Based on these observations, Bajalan et al. (1986) suggested that the VEP abnormalities were associated with reductions in recent memory and learning as the cholinergic innervation to the visual cortex also arises from these areas.

Bajalan et al. (1986) also suggested that the scopolamine-induced VEP abnormalities reflected changes in the diffuse ascending arousal system. There is evidence, that EEG rhythms originate from subcortical areas involved in the ascending reticular arousal system which are cholinergic (Ostfeld and Aruguete 1962; Shute and Lewis 1967; Spehlmann 1971; Rossor 1982). There is also a reduction in CAT levels in Brodman's area 17 (Davies 1979; Rossor et al. 1982) and area 19 (Perry et al. 1977) of the visual cortex. In addition, the fibres of the geniculostriate visual pathway are not thought to be under a cholinergic influence unlike those of the tectal pathway (Shute and Lewis 1967). Hence, since the pattern reversal VEP is thought to be transmitted via the geniculostriate pathway and the flash VEP via the tectal pathway, the latter is more likely to be affected by cholinergic manipulation than the former. This would account for the delayed flash P2 co-existing with an unaffected pattern reversal P100 component in young healthy subjects administered with scopolamine, as well as in patients with AD.

8.4 The effect of SDZ ENA 713 and scopolamine combination on the flash and pattern reversal VEPs.

A common approach to the treatment of AD has been through the use of agents which increase ACh transmission (section 4.7.3). Of the several methods to improve cholinergic function, the use of acetylcholinesterase inhibitors (AChEis) has met with the greatest success (table 4.1). However, the usefulness of AChEis has been undermined by their short half-life and potentially serious side-effects (section 4.7.3). Hence there is a need for AChEis which can overcome these limitations. SDZ ENA 713 ((-)N-methyl-(3'-(1"-dimethylamino)-ethyl)-phenyl-carbamate hydrogentartrate) developed by Sandoz Ltd., was found to be centrally active, with a relatively prolonged duration of action and low activity on the cardiovascular system at centrally active doses. Data about SDZ ENA 713 as provided by Sandoz Ltd. (Sandoz 1989) has been presented in appendix 4.

The previous study (section 8.3) illustrated that scopolamine induced a selective flash P2 delay in healthy volunteers. Therefore, a study was designed to investigate the

effects of the AChEi SDZ ENA 713 on the electrophysiological deficits induced by scopolamine. The proposed study was to be carried out in 16 healthy male volunteers. Four treatment strategies were designed to investigate the baseline effect of scopolamine as well as the effect of two well-tolerated doses of SDZ ENA 713 on the scopolamine induced impairments. Each subject was to receive the following treatments in a randomised, double-blind crossover design with a one week interval between treatments:

Treatment A: Placebo (oral) / Placebo (i.m)
Treatment B: Placebo (oral) / Scopolamine 0.4 mg (i.m)
Treatment C: SDZ ENA 713 1.5 mg (oral) / Scopolamine 0.4 mg (i.m)
Treatment D: SDZ ENA 713 3.0 mg (oral) / Scopolamine 0.4 mg (i.m)

The study proposed to investigate the effect of the treatments on a selection of psychometric tests designed by Professor D. M. Warburton (University of Reading). These included verbal and non-verbal memory (pattern recognition, memory for spatial location and visuospatial memory) testing, a rapid visual information processing task, the Baddeley logic test, and mood assessment. Electrophysiological investigations included flash and pattern reversal VEPs.

Since a combination of both SDZ ENA 713 and scopolamine was to be administered, a pre-study investigation into the safety and tolerability of the proposed combinations was carried out. This pre-study provided an ideal opportunity to time-table and examine the potential effects on the VEP recordings proposed for the main study. The following describes the results obtained during the first of the pre-study investigations.

8.4.1 Methods

The study was carried out under conditions identical to those described in 8.3 unless stated otherwise.

8.4.1.1 Subjects

4 Healthy normal young non-smoking male volunteers (age range 20-25 years, mean 22.5 years) were paid to participate in the study. All subjects underwent a full medical examination and fulfilled the inclusion criteria (detailed in appendix 1.-as for SDZ 210-086) before entering the study. All subjects had a visual acuity of 6/6 or better with correction where needed. Signed consent forms were obtained from each subject after being fully informed about the drugs and the methods to be used in the study.

The project was approved by the Human Science Ethics Committee at Aston University.

8.4.1.2 Instrumentation

Identical to that described in above study (see section 8.3.1.2).

8.4.1.3 Procedure

The procedure was identical to that described in section 8.4.1.3, with the following exceptions. Binocular and monocular right eye flash and pattern reversal VEPs were recorded using the standard derivations of O2 referred to C4 and O1 referred to C3 for the flash stimulation and O2 and O1 both referred to Fz for the pattern reversal stimulation. Previous studies in the literature have used either monocular (Bajalan et al. 1986; Sannita et al. 1988) or binocular (Ray et al. 1991; Sloan et al. 1992) fixation. Hence both monocular right eye, and binocular fixation methods were used in the present study to investigate the difference in responses between the two types of stimulation. The interelectrode impedance was maintained below 5kΩ.

VEPs were recorded on arrival of the subjects (baseline run), then 1, 2 and 4 hours post dosing for the binocular recordings with an additional recording at 6 hours post-dosing for the monocular VEPs. An oral dose of 1.5 mg SDZ ENA 713 was given to each subject followed by an intramuscular injection of 0.4 mg scopolamine (hyoscine hydrobromide) administered by a medical examiner who remained present throughout the day.

8.4.2 Results

8.4.2.1 Data analysis

Analysis of the flash and pattern reversal VEP components were as before (section 8.3.2.1). For clarity, only results from the right eye and hemisphere will be considered as there was no significant difference in results between the two eyes or hemispheres. Statistical analysis was carried out using one factor analysis of variance with repeated measures to examine the effect of SDZ ENA 713 and scopolamine on the VEPs. The least squares difference (Fischer's LSD test) was then applied to examine the difference between group mean values.

8.4.2.2 Flash VEP

The mean and standard deviation values of the flash VEP latencies and amplitudes have been presented in table 8.3. In order to magnify the changes in the VEP

components and compare changes on the same scale, the percentage difference from baseline values were calculated and presented graphically (figs. 8.12-8.15).

There was little change in the N2 latency to both binocular and monocular fixation (figs. 8.12 and 8.13 respectively). The N2 latency increased slightly at 1 hour followed by a gradual decrease thereafter. The maximum change in N2 latency was below 3% and not statistically significant.

The flash P2 latency displayed a gradual increase over the day. Flash VEP to binocular fixation showed a slight increase (2.55%) in P2 latency whereas monocular fixation showed a slight decrease (1.82%) 1 hour post-dosing, after which both fixation methods showed an increase up to the 4 hour period (figs. 8.12 and 8.13), but again failed to reach statistical significance.

The N3 component displayed little change at the 1 hour period with monocular fixation, followed by a gradual increase in latency over the day reaching a maximum at 6 hours (4.31%). The flash VEP to binocular fixation displayed a gradual increase in N3 latency from 1 hour onwards, reaching a maximum at 4 hours (2.73%).

The P3 component displayed the change of the greatest magnitude in percentage change in the latency from baseline of all the flash VEP components considered. There was a decrease in P3 latency reaching a maximum at 1 hour for binocular (fig. 8.12) and 2 hour for monocular fixation (fig. 8.13), followed by a return towards baseline levels. However, again this effect of SDZ ENA 713-scopolamine on the P3 latency failed to reach significance, probably due to the high standard deviation values associated with this component (table 8.3).

The flash VEP N2-P2, P2-N3 and N3-P3 amplitudes displayed very similar changes with the binocular condition (fig. 8.14), with a decrease at 1 hour followed by an increase towards baseline levels. The degree of change was greater the later the components occurred, such that the N3-P3 amplitude displayed change of the greatest magnitude (reduction of 58.23% at 1 hour) and the N2-P2 the least (with an increase of 8.71% at 4 hours). Monocular fixation displayed a slightly different effect (fig. 8.15), here all three amplitude measures showed a slight increase at 1 hour. However, the N2-P2 with monocular fixation, showed little change at 2 hours followed by a gradual increase at 4 and 6 hours by 6.56% and 8.71% respectively. The P2-N3 amplitude with monocular fixation, showed a decrease at 2 hours (19.18%) followed by a gradual return towards baseline. The N3-P3 amplitude with monocular fixation, displayed the most dramatic change, with a decrease at 2 (29.83%) and 4 hours (35.80%) followed by an increase of 17.9% above baseline at 6

Table 8.3 Effect of scopolamine-SDZ ENA 713 combination treatment on the mean and standard deviation values for the flash VEP. Data from O2-C4.

	BASELINE	1 HOUR	2 HOUR	4 HOUR	6 HOUR
N2 LATENCY					
Mean (binocular)	64.70	65.92	65.19	63.97	
SD	6.50	6.69	8.34	6.88	
Mean (monocular)	67.87	68.61	68.61	67.87	66.17
SD	6.30	5.19	7.11	6.60	6.35
P2 LATENCY					
Mean (binocular)	105.47	108.16	106.94	110.11	
SD	6.95	9.62	8.46	8.27	
Mean (monocular)	107.42	105.47	111.09	112.79	111.57
SD	6.33	7.93	8.11	4.82	6.74
N3 LATENCY					
Mean (binocular)	160.89	164.31	165.04	165.29	
SD	18.68	19.70	18.10	16.71	
Mean (monocular)	147.46	146.73	149.88	149.66	153.81
SD	13.15	13.75	14.23	15.53	15.08
P3 LATENCY					
Mean (binocular)	199.22	187.99	193.61	200.92	
SD	24.92	33.95	35.99	33.94	
Mean (monocular)	194.73	192.13	187.89*	188.68	194.63
SD	9.68	12.07	18.17	13.19	15.56
N2-P2 AMPLITUDE					
Mean (binocular)	14.08	13.24	13.18	14.28	
SD	4.93	4.25	4.69	2.49	
Mean (monocular)	8.38	8.53	8.49	8.93	9.11
SD	2.05	2.71	2.14	3.32	2.17
P2-N3 AMPLITUDE					
Mean (binocular)	17.39	14.05	15.17	17.24	
SD	7.54	6.41	8.27	5.86	
Mean (monocular)	10.01	10.88	8.09	8.82	9.27
SD	3.69	3.75	2.65	2.35	5.16
N3-P3 AMPLITUDE					
Mean (binocular)	5.77	2.41	2.81	3.60	
SD	4.01	1.56	2.12	2.43	
Mean (monocular)	3.52	3.84	2.47	2.26	4.15
SD	1.53	1.14	1.58	0.39	2.03

* N=3

Figure 8.12 The percentage change in group mean latencies from baseline of the flash VEP to binocular fixation on administration of scopolamine and SDZ ENA 713.

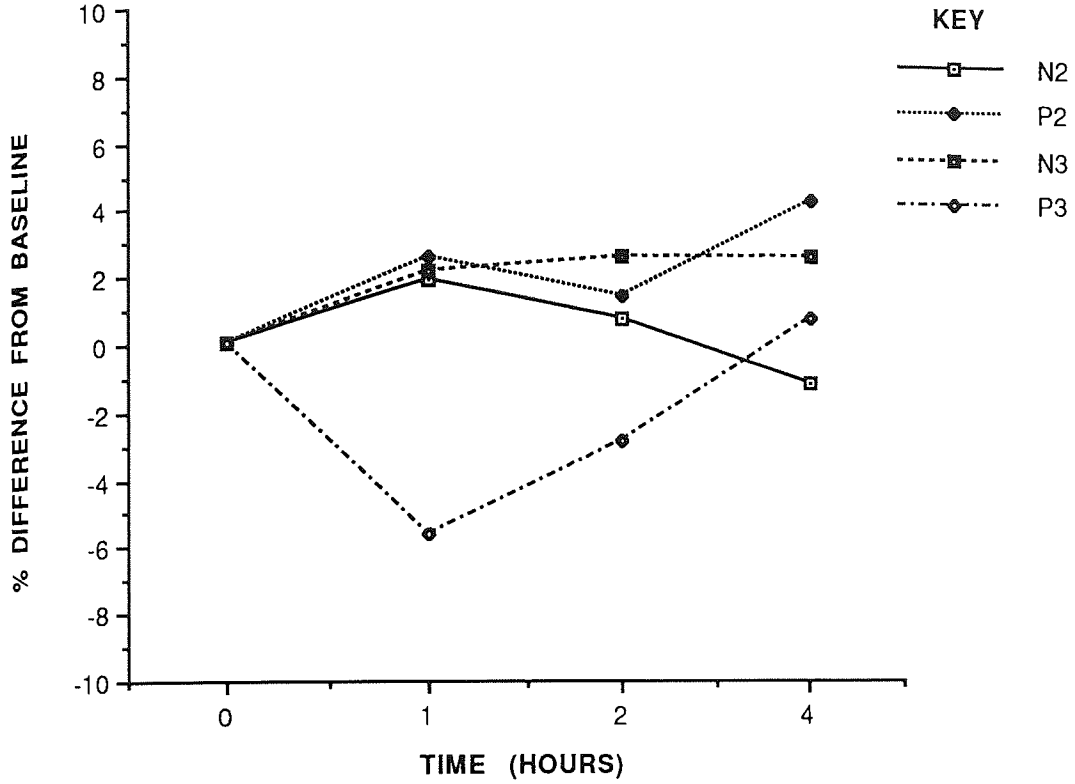
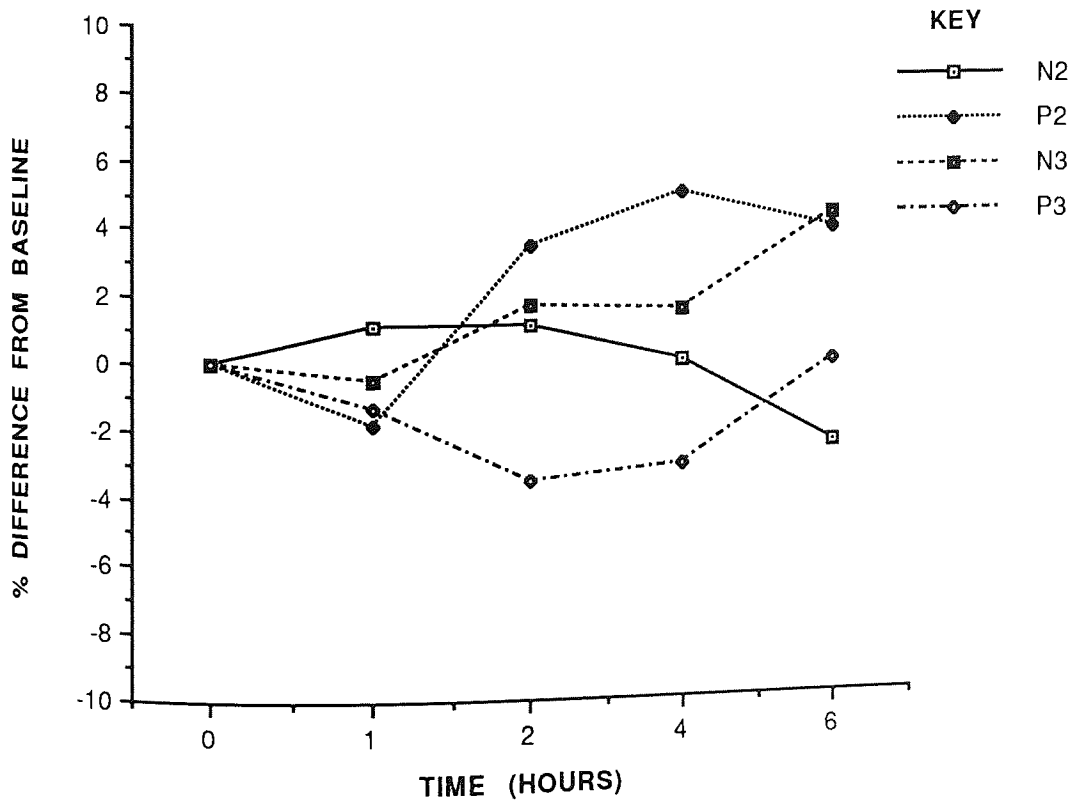


Figure 8.13 The percentage difference in group mean latencies from baseline of the flash VEP to monocular right eye stimulation on administration of scopolamine and SDZ ENA 713.



hours post dosing. Statistical analysis revealed no significant change in the amplitude values post-dosing.

Overall, the changes in the flash VEP discussed seem large, but they did not reach statistical significance and the graphs deliberately show an exaggerated effect. In general, the latencies of the later N3 and P3 components displayed greater inter-individual variations than the earlier N2 and P2 components, as indicated by the large standard deviation values (table 8.3). There was no statistically significant difference in latencies obtained with binocular or monocular fixation. However as expected, the amplitude measures with the binocular fixation were significantly ($P<0.01$) greater than those obtained with the monocular fixation.

Figure 8.14 Graph of the percentage difference in group mean amplitudes from baseline of the flash VEP to binocular fixation on administration of scopolamine and SDZ ENA 713.

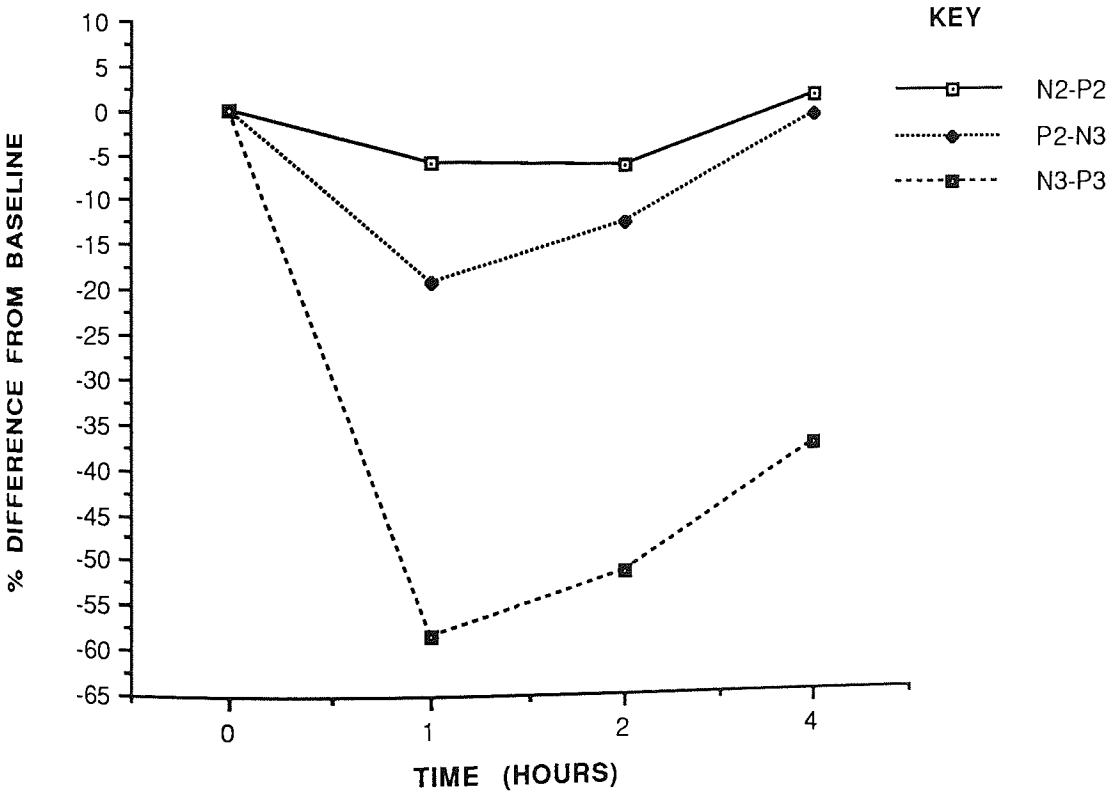
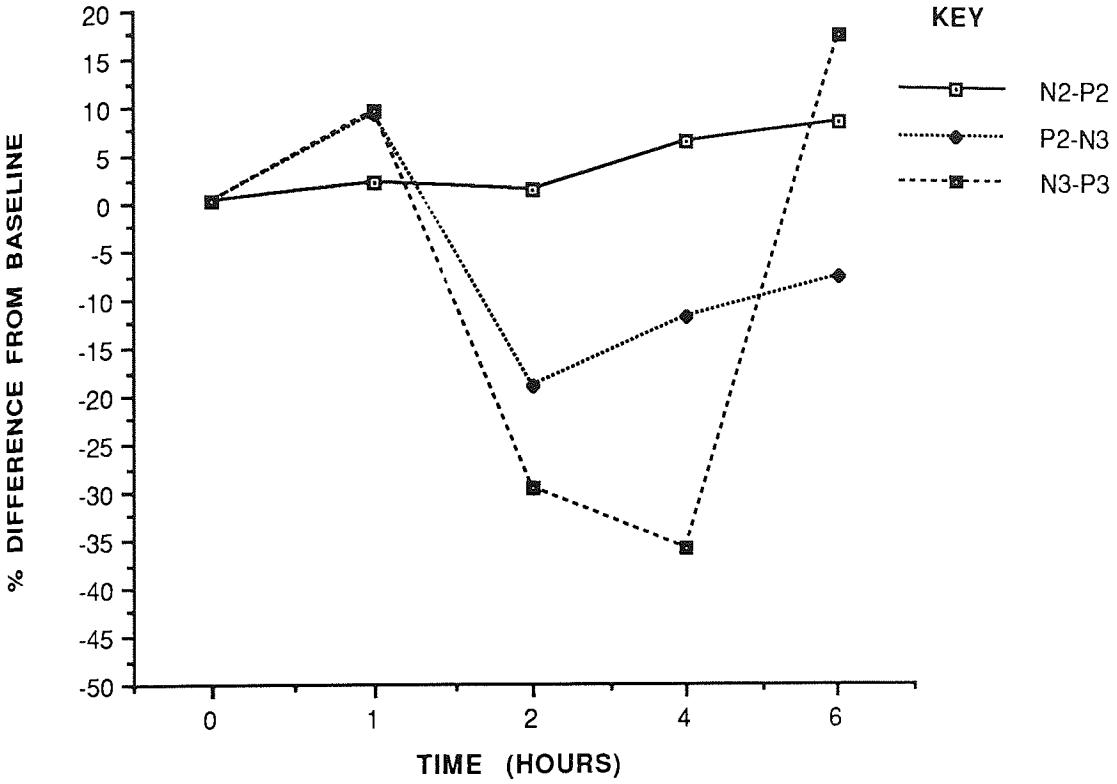


Figure 8.15 Graph of the percentage difference in mean amplitudes from baseline of the flash VEP to monocular fixation on administration of scopolamine and SDZ ENA 713.



8.4.2.3 Pattern reversal VEP

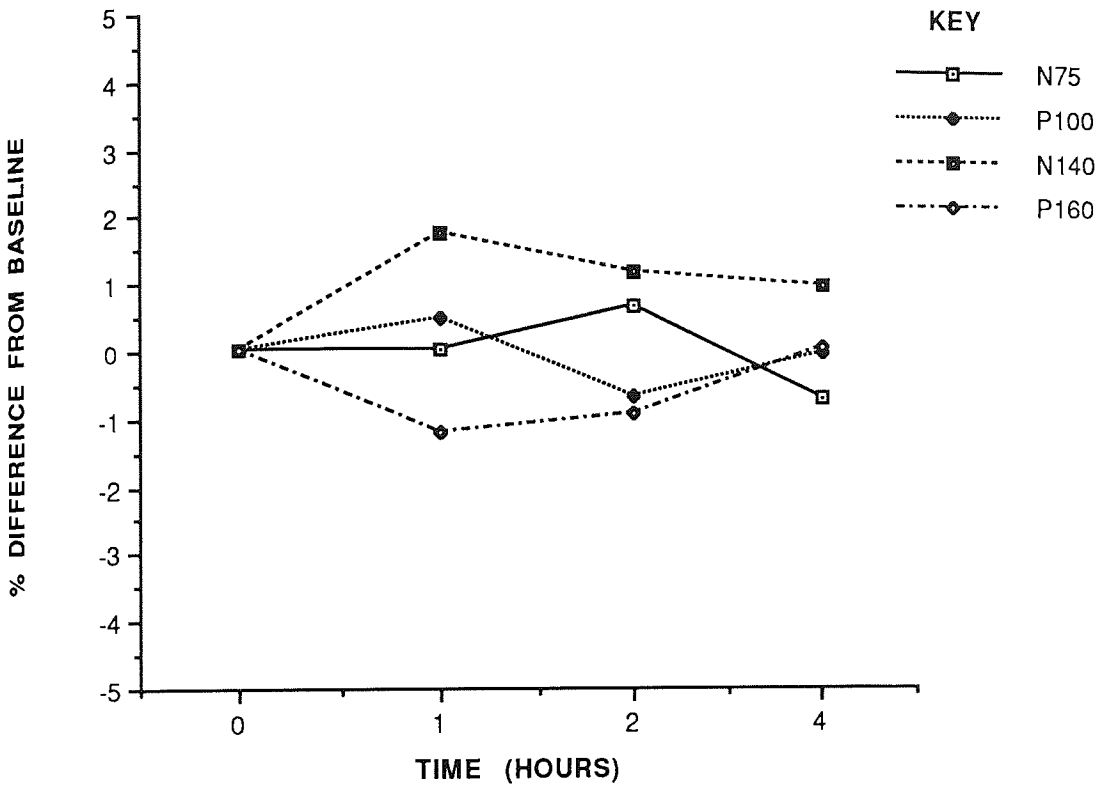
The results of the group mean values and standard deviations for the pattern reversal VEP have been presented in table 8.4. As with the flash VEP, the differences between baseline and post-dosing values have been exaggerated by calculating the percentage difference from baseline of the group means.

The pattern reversal VEP components displayed very little change with binocular fixation (fig. 8.16). The maximum effect was an increase of 1.74% in the N140 latency 1 hour post-dosing. However, the monocular condition displayed slightly more variable results (fig. 8.17). The N75 latency effects to monocular fixation, were similar to those obtained with binocular fixation although the former was of slightly greater magnitude, but the difference did not reach statistical significance. The P100 to monocular fixation however, showed a decrease in latency reaching a shortest latency values at the 4 hour period before returning towards baseline levels. There was a significant difference in the P100 latency ($F=4.926$, $df=3,7$, $P=0.002$), for binocular compared to monocular fixation, with the P100 latency occurring earlier with binocular fixation. The later occurring N140 latency displayed little change with a maximum effect of a 1.76% increase at 4 hours. The P160 displayed a gradual

Table 8.4 Effect of scopolamine and SDZ ENA 713 on the mean and standard deviation values for the pattern reversal VEP. Data from O2-C4.

	BASELINE	1 HOUR	2 HOUR	4 HOUR	6 HOUR
N75 LATENCY					
Mean (binocular)	72.51	72.51	73.00	72.02	
SD	10.71	10.10	9.97	9.84	
Mean (monocular)	74.95	75.44	76.66	75.93	73.00
SD	5.77	5.25	1.87	4.54	5.60
P100 LATENCY					
Mean (binocular)	109.37	109.87	108.64	109.38	
SD	3.74	3.34	2.91	2.76	
Mean (monocular)	117.43	116.70	116.21	112.06	114.26
SD	7.62	6.97	4.29	1.46	6.67
N140 LATENCY					
Mean (binocular)	168.46	171.39	170.41	170.17	
SD	5.55	4.54	3.04	2.92	
Mean (monocular)	166.75	166.75	167.97	169.68	167.97
SD	12.54	12.44	17.34	18.31	17.51
P160 LATENCY					
Mean (binocular)	239.50	236.57	237.31	239.75	
SD	12.64	10.83	13.46	15.60	
Mean (monocular)	224.12	226.56	232.91	224.12	219.24
SD	34.58	30.96	38.94	35.52	38.48
N75-P100 AMPLITUDE					
Mean (binocular)	9.48	10.50	12.79	13.19	
SD	3.02	3.31	4.86	3.79	
Mean (monocular)	7.92	7.31	8.74	9.34	9.56
SD	2.62	2.90	3.25	3.41	2.62
P100-N140 AMPLITUDE					
Mean (binocular)	15.27	14.14	16.54	16.18	
SD	7.80	4.72	6.38	6.53	
Mean (monocular)	12.34	10.84	12.08	13.45	13.82
SD	5.45	4.62	4.17	4.81	4.71
N140-P160 AMPLITUDE					
Mean (binocular)	8.71	6.60	7.39	7.44	
SD	5.14	3.01	4.64	5.39	
Mean (monocular)	6.23	5.26	5.73	4.64	5.13
SD	5.21	3.24	2.90	3.49	2.63

Figure 8.16 The percentage difference in group mean latencies from baseline of the pattern reversal VEP to binocular fixation on administration of scopolamine and SDZ ENA 713.



increase reaching a maximum at 2 hours (3.92%), followed by a return to baseline levels at 4 hours and a slight shortening at 6 hours (2.18%).

The N75-P100 amplitude with binocular fixation displayed a significant increase (fig. 8.18) post-dosing ($F=9.848$, $df=3$, $P<0.05$). Both the P100-N140 and N140-P160 amplitudes with binocular fixation, were reduced at 1 hour followed by an increase at 2 and 4 hours, but failed to reach statistical significance. Graphically all three amplitude displayed a parallel effects post-dosing (fig. 8.18). The N75-P100 and P100-N140 amplitudes under monocular conditions displayed similar effects with a reduction at 1 hour followed by a gradual increase over the day (fig. 8.19). In contrast, to binocular fixation, the change in the N75-P100 amplitude to the monocular fixation failed to reach statistical significance. The N140-P160 amplitude showed an effect similar to that seen with binocular fixation except there was a further reduction of 25.52% at 4 hours post dosing.

In general, the effects of scopolamine on the pattern reversal VEP amplitudes were much greater than on the latencies. The change in latencies occurred within 5% of their baseline values, whereas amplitude changed by 15.2% on average. The amplitudes to binocular fixation were again significantly ($P<0.01$) larger than those obtained with monocular fixation, except for the N140-P160 amplitude.

Figure 8.17 Graph of the percentage difference in group mean latencies from baseline of the pattern reversal VEP to monocular right eye stimulation on administration of scopolamine and SDZ ENA 713.

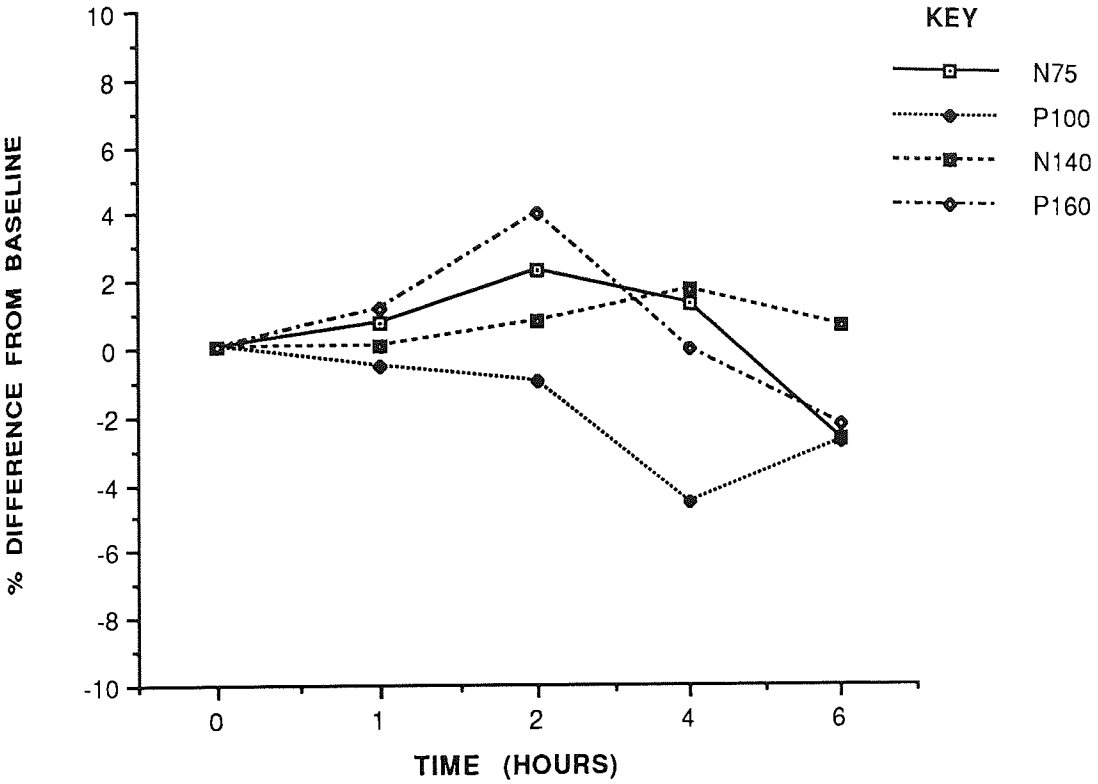


Figure 8.18 Graph of the percentage difference in group mean amplitudes from baseline of the pattern reversal VEP to binocular fixation on administration of scopolamine and SDZ ENA 713.

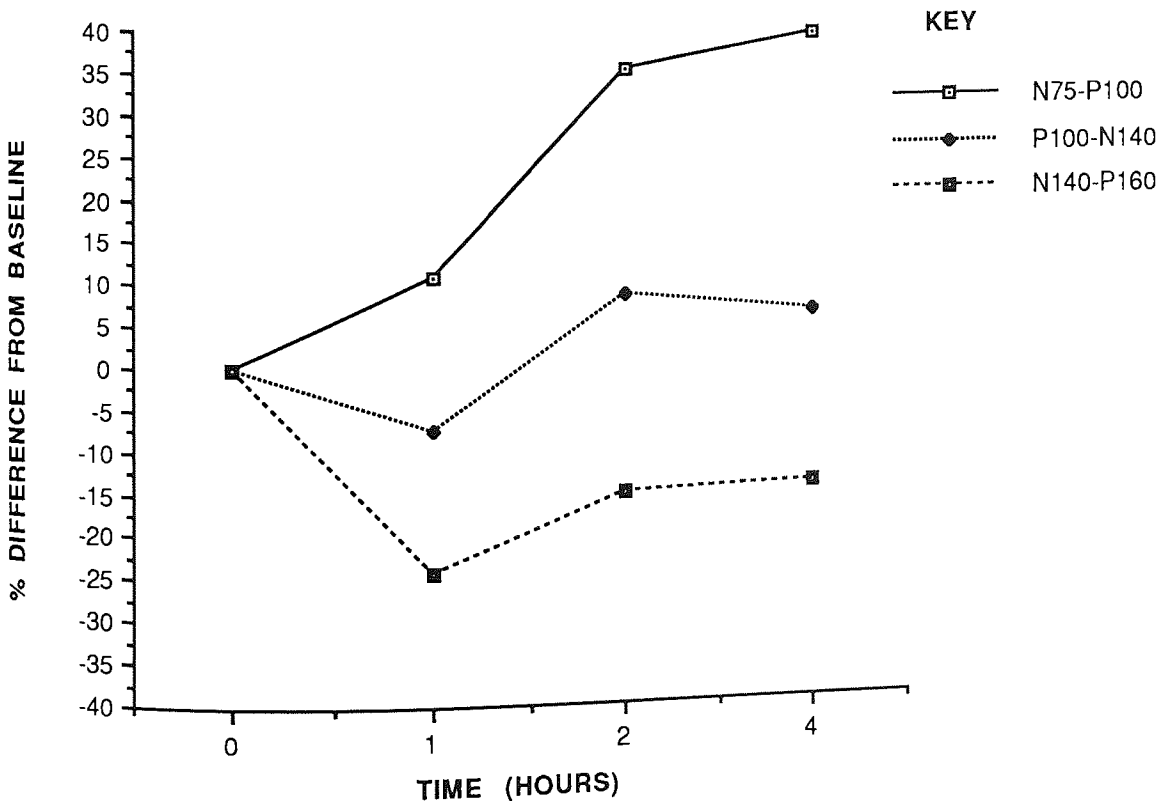
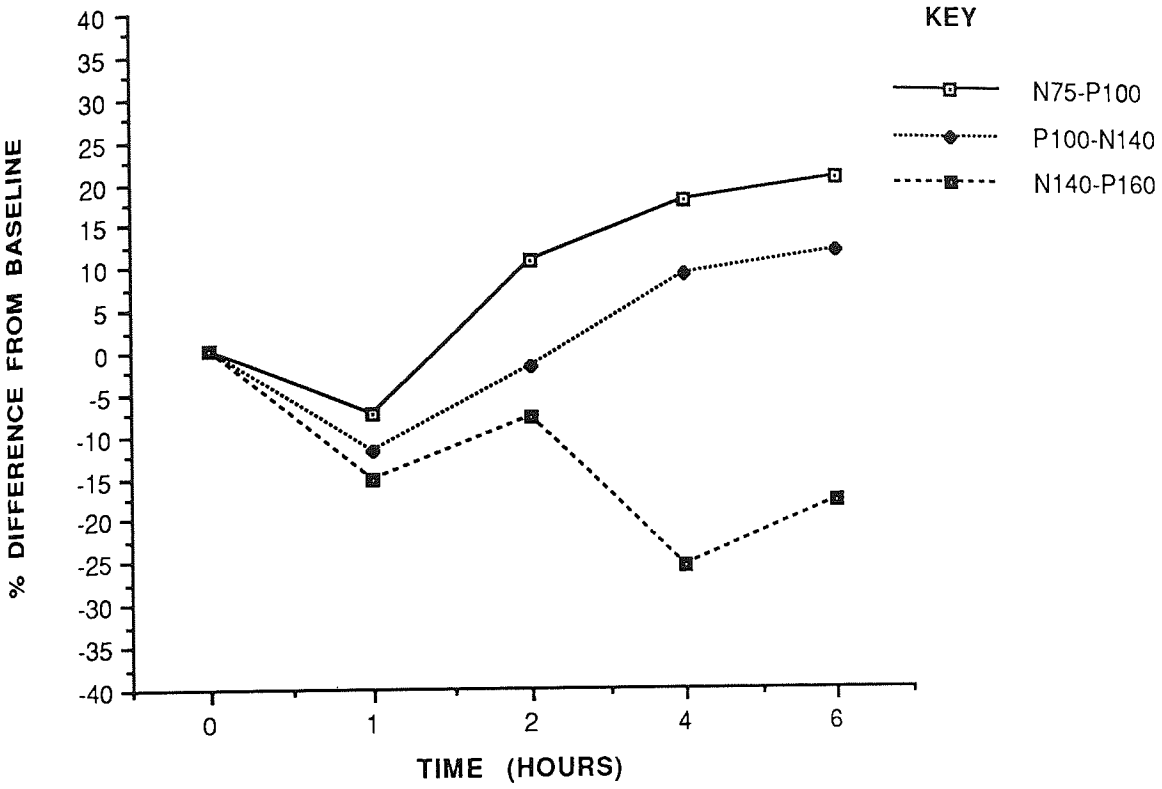


Figure 8.19 Graph of the percentage difference in group mean amplitudes from baseline of the pattern reversal VEP to monocular right eye stimulation on administration of scopolamine and SDZ ENA 713.



8.4.2.4 Side effects

The subjects complained of a general feeling of tiredness post scopolamine dosing for about 2-4 hours. Dry mouth and increased thirst was experienced between 0.5-1 hours post-scopolamine dosing and lasted for around 1-3 hours. There was a feeling of dizziness experienced by some subjects between 4-20 minutes after the scopolamine injection for between 5 minutes to about 1 hour. These effects were all expected (section 8.2). None of the subjects thought the side-effects were severe enough to interfere with their performance in the experiment. Only two subjects experienced a slight blurring effect but could still see the pattern stimulus clearly.

8.4.2.5 Vital signs

The following results were made available by Sandoz Ltd. and recorded by the medical examiner Dr. T. Betts.

The results of the mean arterial pressure (standing) have been presented in fig. 8.20. Only subject 002 AP showed an increase post-dosing, the others all showed a fall in pressure by 4 hours before returning to baseline by 8 hours. The biggest fall in pressure occurred in subject 004 TM with a fall of 20 mmHg at 6 hours.

The results of the mean arterial pressure (supine) showed a reduction in all 4 subjects post-dosing, from 1-4 hours before returning to baseline levels by 8 hours (fig. 8.21). The greatest reduction occurred in subject 004 TM again with a fall of 30 mmHg by 4 hours compared to baseline. The blood pressure of subject 003 JM was unrecordable at 2 hours eventhough the standing pressure was normal.

The effect of SDZ ENA 713-scopolamine was quite marked on the heart rate as monitored by the pulse. The pulse in the standing position was reduced in all subjects by 4 hours (fig. 8.22). Subject 002 AP displayed a slight increase in pulse (standing) over the 1-3 hours (from 76 bpm baseline to 84 bpm at 3 hours) before showing a dramatic decrease at 4 hours to 56 beats per minute (bpm), (a reduction of 28 bpm within an hour). Apart from subject 004 TM, the pulse (standing) of all subjects returned to baseline levels, with that of subject 004 TM also returning at baseline by 24 hours.

SDZ ENA 713 and scopolamine had a greater effect on the supine pulse. Subject 002 AP again displayed a slight increase in pulse at 1 hour (of 8 bpm) before showing a reduction well below baseline for up to 4 hours post-dosing, when the pulse was reduced by 24 bpm. Subject 003 JM displayed an extremely low pulse of 38 bpm at 4 hours, which was unexpected. However, all subjects were closely monitored by a medical examiner throughout the day and none complained of any major discomforts.

Figure 8.20 The effect of SDZ ENA 713 and scopolamine on the standing mean arterial pressure. Time 0 hour indicates time of SDZ ENA 713 administration.

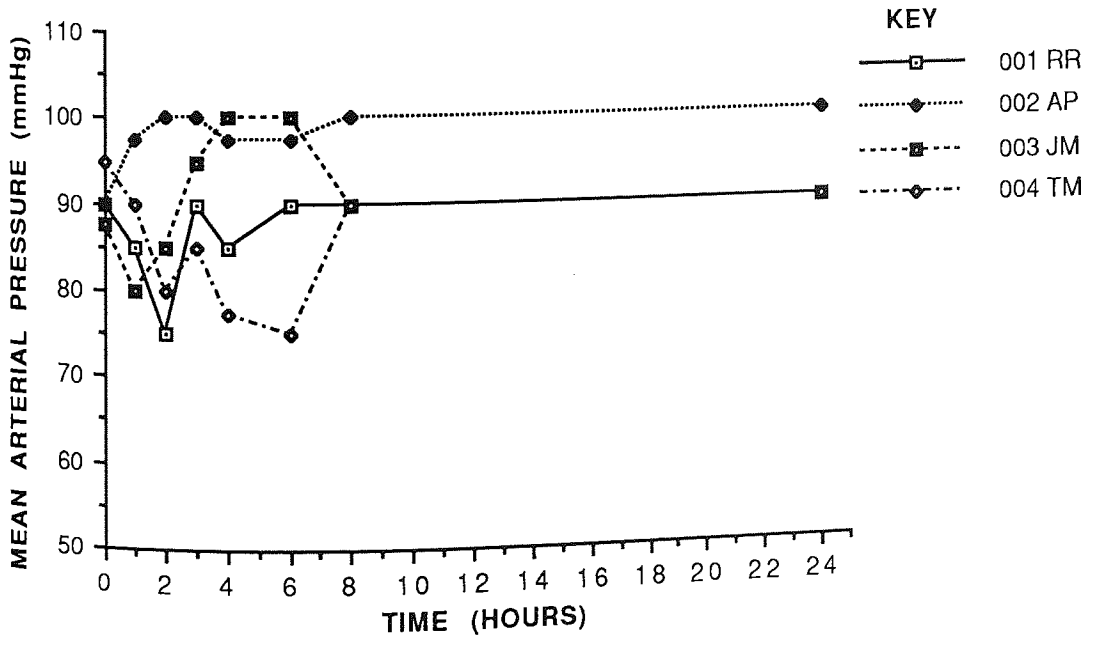


Figure 8.21 The effect of SDZ ENA 713 and scopolamine on the supine mean arterial pressure. Time 0 hour indicates time of SDZ ENA 713 administration.

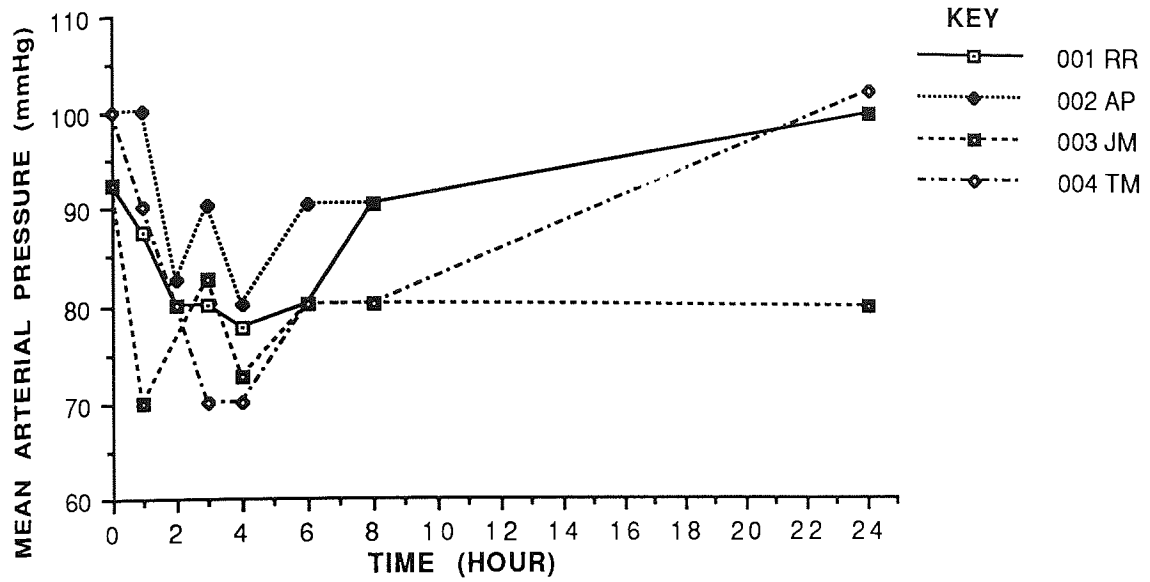


Figure 8.22 The effect of SDZ ENA 713 and scopolamine on the pulse (standing). Time 0 hour indicates time of SDZ ENA 713 administration.

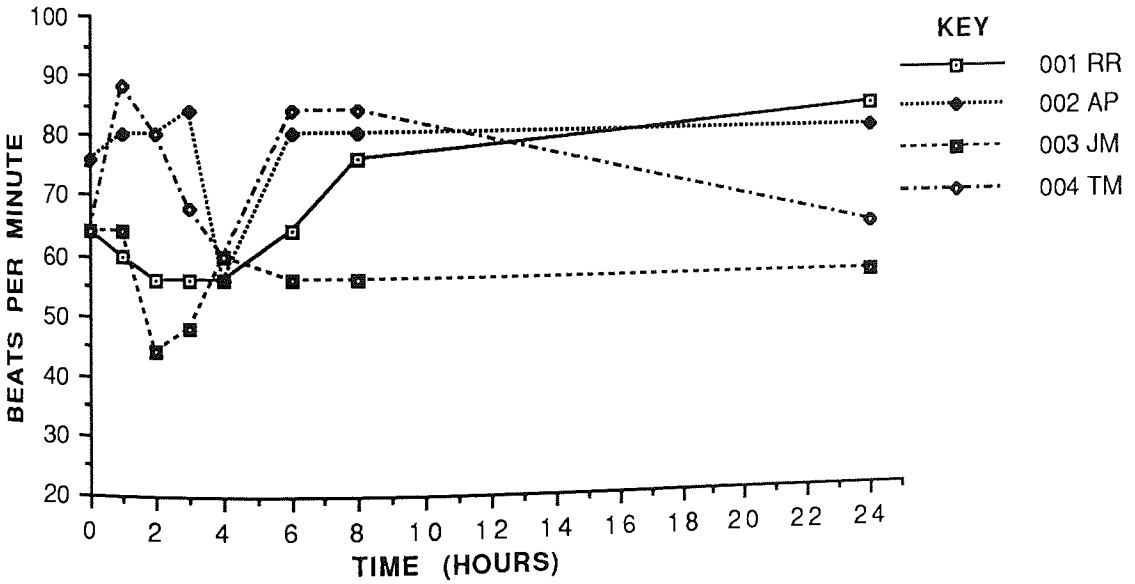
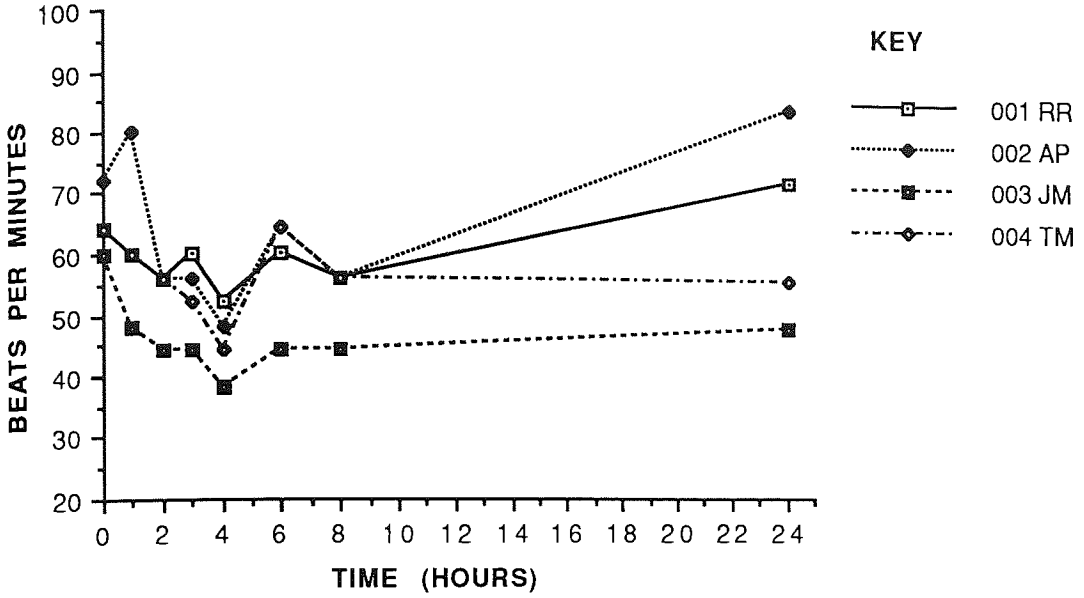


Figure 8.23 The effect of SDZ ENA 713 and scopolamine on the pulse (supine). Time 0 hour indicates time of SDZ ENA 713 administration.



8.4.3 Discussion

The tolerability study provided an opportunity to examine the potential effect of the SDZ ENA 713-scopolamine combination on the flash and pattern reversal VEPs for the main study.

There was no statistically significant effect of the SDZ ENA 713-scopolamine combination treatment on the flash VEP. The major effect was a shortening of the late flash P3 latency around 1-2 hours post-dosing (but failed to reach statistical significance), with little effect on the earlier N2, P2 and N3 components. The flash amplitudes also failed to display a statistically significant change post-dosing. Generally, the amplitude values of the later components were reduced within 1-2 hours post-dosing. Overall, the later components (N3 and P3) were affected more than the earlier ones (N2 and P2). However clinically, the flash N3 and P3 are rarely used (Halliday 1982; Spehlmann 1985), mainly due to the high inter-individual variation associated with components occurring later than P2, as observed in the present study. Since the majority of the studies on the effects of scopolamine alone on the flash VEP observed an increase in the P2 latency (Bajalan et al. 1986; Gilles et al. 1989; Sloan et al. 1992), the failure of the present study to display such an effect suggests that SDZ ENA 713 may have counteracted the effects of scopolamine on the P2 latency.

There was greater effect of the SDZ ENA 713-scopolamine combination treatment on the pattern reversal VEP. However, as with the flash VEP, the latencies of the pattern reversal components failed to display any statistically significant change post-dosing. There was a shortening of the P100 latency at 4 hours to monocular right eye stimulation, otherwise there was little change in the pattern reversal latencies. Ray et al. (1991) reported a significant increase in the P100 latency after scopolamine administration, an effect opposite to that observed in the present study. In addition, Sannita et al. (1988) reported a decrease in the N75 and increase in the N175 latency, but no significant effect on these components were observed in the present study. These observations suggest that SDZ ENA 713 may have reversed the scopolamine-induced increase in P100 latency as observed by Ray et al. (1991) and the decrease in N75 and increase in N175 latencies observed by Sannita et al. (1988). However, other workers have failed to observe any effect of scopolamine alone on the pattern reversal VEP (Bajalan et al. 1986; Gilles et al. 1989; Sloan et al. 1992). In addition, based on the VEP studies in patients with AD (Doggett et al. 1981; Harding et al. 1981 and 1985; Wright et al.1984a and 1986) and the mimicking of these effects by scopolamine (Bajalan et al. 1986), Harding and Wright (1986) proposed that the flash P2 but not the pattern reversal VEP was under a cholinergic influence (section 4.11). It has been proposed that the flash VEP was transmitted via the tectal pathway whereas the pattern reversal VEP is transmitted via the geniculostriate pathway (section 3.5 and 4.11), with the former likely to be under cholinergic influence but not the latter (Shute and Lewis 1967). Therefore, no effects on the latency of the pattern reversal VEP were expected.

The N75-P100 amplitude post-dosing displayed a significant ($P<0.05$) increase with the binocular fixation but not with monocular fixation. The effect of the scopolamine-SDZ ENA 713 treatment was similar in these stimulating conditions, although the magnitude of the amplitude values and the percentage change were much greater with the binocular fixation. This was expected (Spehlmann 1985), since responses from two eyes involve stimulation of a greater number of neurones compared to just one eye, resulting in a larger response.

In chapter 7, the cholinergic agent SDZ 210-086 resulted in a decrease of both the flash and pattern reversal VEP amplitudes. In addition, the flash N2-P2 amplitude showed greater sensitivity to the increase in ACh by SDZ 210-086. Studies in the literature support this observation, with the effect of increasing the cholinergic transmission through the use of AChE-inhibiting agents, result in a reduction in amplitude of the VEP to stimuli of low but not high spatial frequency (Harding et al. 1983; Kirby et al. 1986; Arakawa et al. 1992). Since the flash stimulus represents the

ultimate low spatial frequency, the effect of cholinergic agents was expected to be greater on the flash than the pattern reversal VEP. The present study did display an overall decrease in the flash VEP amplitudes (figs. 8.14 and 8.15), but failed to reach statistical significance. Therefore, it remains unclear why the N75-P100 amplitude was affected in the present study without any significant effects on the flash VEP.

The amplitude measurements of the flash and pattern reversal VEPs displayed greater effects to binocular than to monocular fixation. These observations would suggest the use of binocular fixation for future studies. However, potential side-effects of scopolamine on the eye resulting in diplopia could cause inaccurate recording of the pattern VEP, as a clear division of the black and white edges is essential. Therefore, monocular fixation is recommended for the pattern reversal VEP and binocular fixation for the unstructured flash VEP. The studies by Bajalan et al. (1986) and Sannita et al. (1988) both investigated the effects of scopolamine administration on the flash and pattern reversal VEPs using monocular stimulation, whereas Ray et al. (1991) and Sloan et al. (1992) used binocular stimulation. However, the effect of using either binocular or monocular stimulation resulted in contradictory results from these workers. Bajalan et al. (1986) used monocular stimulation to avoid effects of diplopia, reported no effect on the pattern reversal VEP, whereas Sannita et al. (1988) reported a decrease in the N75 and increase in the N175 latencies post scopolamine dosing. Ray et al. (1991) reported an increase in the P100 latency with binocular stimulation, yet Sloan et al. (1992) reported no effects on either the flash or pattern reversal VEPs. In the the present study there was little difference between the binocular and monocular stimulating condition on the flash and pattern reversal VEP latencies overall, with the exception of a decrease in the P100 latency at 4 hours post dosing under monocular fixation. However as mentioned, any side-effects of blurring could result in erroneous results with the pattern reversal VEP, hence monocular stimulation is recommended.

The monitoring of the vital signs resulted in some unexpected findings. Subject 003 JM displayed a very low supine pulse at 4 hours (38 bpm) and an unrecordable supine blood pressure at 2 hours. According to Sandoz Ltd., previous experience with SDZ ENA 713 showed no such effects. Their experience with 0.5 mg scopolamine in 12 subjects did display a fall in supine values, but they were less marked, whereas the fall in standing pulse and blood pressure was more marked than in the present study. Scopolamine is known to lower the heart rate (section 8.2), as does SDZ ENA 713, but the degree of the slowing of the heart was greater than expected in the present study. There was a possibility that administration of the SDZ ENA 713-scopolamine at higher doses of SDZ ENA 713 (3.0 mg/day as proposed for the main study) may

result in potentially dangerous reductions of heart rate in volunteers. The potential risks to the subjects hence outweighed the benefits of the study. Therefore with safety being of primary importance, further study of the effects of the SDZ ENA 713-scopolamine combination in young healthy male volunteers was prematurely discontinued.

8.5 Comparison of the effects of scopolamine alone and combination of SDZ ENA 713-scopolamine on the flash and pattern reversal VEP.

8.5.1 Flash VEP

A comparison between the data obtained with scopolamine alone (section 8.3) and the combination of SDZ ENA 713-scopolamine (section 8.4) was carried out on the flash VEP and pattern reversal VEP (to monocular fixation).

There was little change in the latency of the flash N2 component after treatment with either scopolamine alone or scopolamine-SDZ ENA 713 combination (fig. 8.24). Both treatments showed a shortening of the N2 latency post-dosing. In chapter 6, the time of day effects showed a change in the N2 latency of about 5%, in the present studies, the N2 latency with both treatments varied below this value. Hence there was no real effect of either treatment on the flash N2 latency.

The flash P2 latency also displayed similar changes after treatment with scopolamine and scopolamine-SDZ ENA 713 (fig. 8.25). The major difference between the two treatments on the P2 latency was that at the 1 hour period, the combination treatment displayed a shortening in latency, whereas treatment with scopolamine alone displayed a gradual increase from the 1 hour period to the 4 hour period before returning towards baseline. The percentage deviation in the P2 latency values with the combination treatment was also less than that obtained with scopolamine alone. In addition, the effects with scopolamine alone treatment were significant ($P<0.05$), whereas those with scopolamine-SDZ ENA 713 failed to reach significance. Interestingly, from chapter 5, the P2 displayed a fluctuation in latency of 2.2%. In the present study, the P2 latency post scopolamine dosing showed an increase greater than this during the 1-4 hour period, hence accounting for these results reaching statistical significance. However, the P2 change also exceeded the 2.2% fluctuation with the scopolamine-SDZ ENA 713 treatment at 1-4 hours, yet failed to reach significance. This may have been due to the high standard deviation values associated with such a small number of subjects (n=4) studied. Overall, further studies need to be carried out in order to confidently suggest that SDZ ENA 713 reversed the scopolamine-induced effects on the flash P2 latency.

Figure 8.24. Comparison of the change in flash N2 latency after treatment with scopolamine alone and combination of scopolamine-SDZ ENA 713.

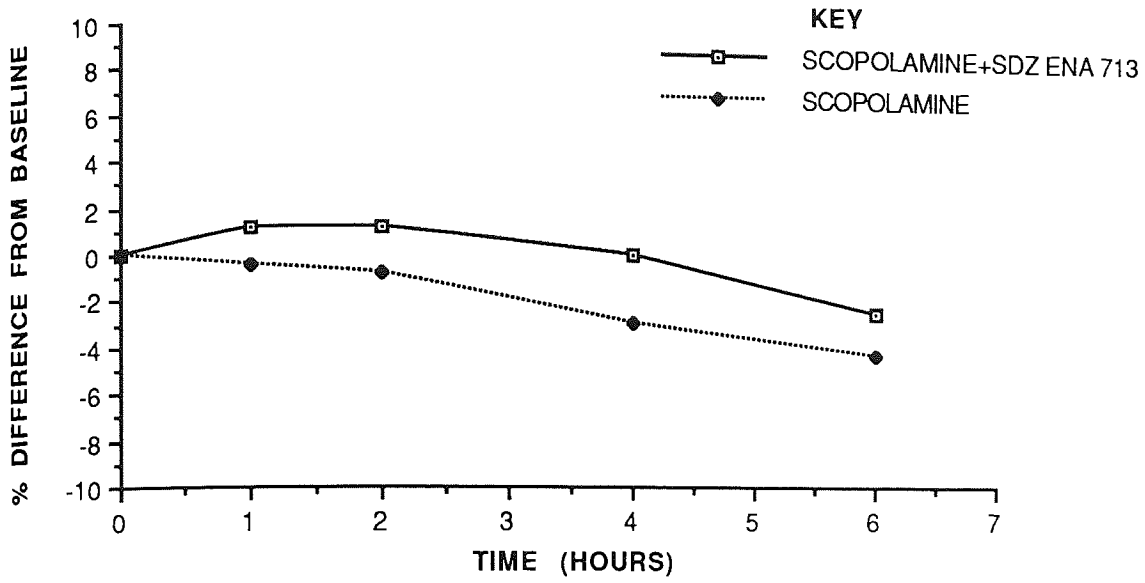
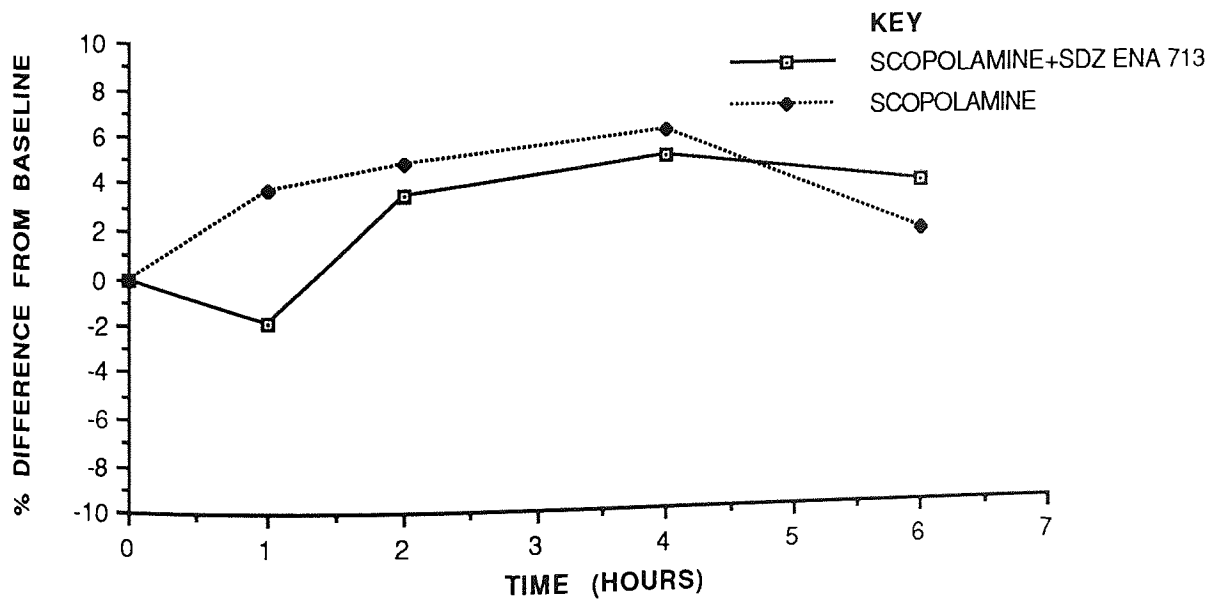


Figure 8.25 Comparison of the change in flash P2 latency after treatment with scopolamine alone and combination of scopolamine-SDZ ENA 713.



There was little difference between treatments in the N3 latency at the 1, 2 and 4 hour period (fig. 8.26). However, at the 6 hour period, the combination treatment displayed an increase in latency whereas scopolamine alone displayed a decrease in latency. Hence at the 6 hour period, there is a temptation to suggest that SDZ ENA 713 may have reversed the effects of scopolamine on the N3 latency. Comparison of these with those from chapter 6, reveal that the percentage deviation at the 6 hours with both the scopolamine alone and the scopolamine-SDZ ENA 713 combination treatment was less than the natural fluctuation levels of 4.6%. In addition, there was no statistically significant effect obtained on the N3 latency with either treatment, so overall, as with the flash N2 latency, the N3 latency was also not affected by the cholinergic manipulation.

The effects on the flash P3 latency of the two treatments revealed opposing effects (fig. 8.27). Whereas the scopolamine alone treatment displayed an increase in latency from 1-4 hours post-dosing, the scopolamine-SDZ ENA 713 treatment displayed a decrease in latency over the 1-4 hours. At the 6 hour period, the P3 latency was shortened with scopolamine alone, but lengthened with the scopolamine-SDZ ENA 713 treatment. Hence these effects suggest that SDZ ENA 713 may have reversed the effects of scopolamine on the P3 latency. However, neither treatment had displayed a statistically significant effect, probably because the degree of change was less than that due to natural variation (6.5%) as shown by results in chapter 6.

The difference between the two treatments was greater for the flash VEP amplitude measurements. The flash N2-P2 amplitude displayed a small increase over the day after treatment with scopolamine alone. However, with the scopolamine-SDZ ENA 713 treatment, there was a sharp increase in the N2-P2 amplitude post-dosing. However, there were no statistically significant effects of either treatment obtained with the flash N2-P2 amplitude. In addition, the natural fluctuation of this amplitude as examined in chapter 5 (36.5%) was greater than the change in amplitude obtained in the present study. Therefore, there was no effect of either scopolamine alone or scopolamine-SDZ ENA 713 treatments on the flash N2-P2 amplitude.

There was a similar effect of scopolamine alone and scopolamine-SDZ ENA 713 on the flash P2-N3 amplitude up to the 1 hour period (fig. 8.29). Thereafter, the scopolamine alone treatment showed a further increase in amplitude followed by a fall towards baseline. In contrast, with the combination treatment, there was a sharp fall in amplitude at the 2 hour period, followed by an increase towards baseline. Hence at the 2 hour period the two treatment strategies displayed opposing effects. The greatest magnitude of change in the P2-N3 amplitude was shown by the combination treatment

Figure 8.26 Comparison of the change in flash N3 latency after treatment with scopolamine alone and combination of scopolamine-SDZ ENA 713.

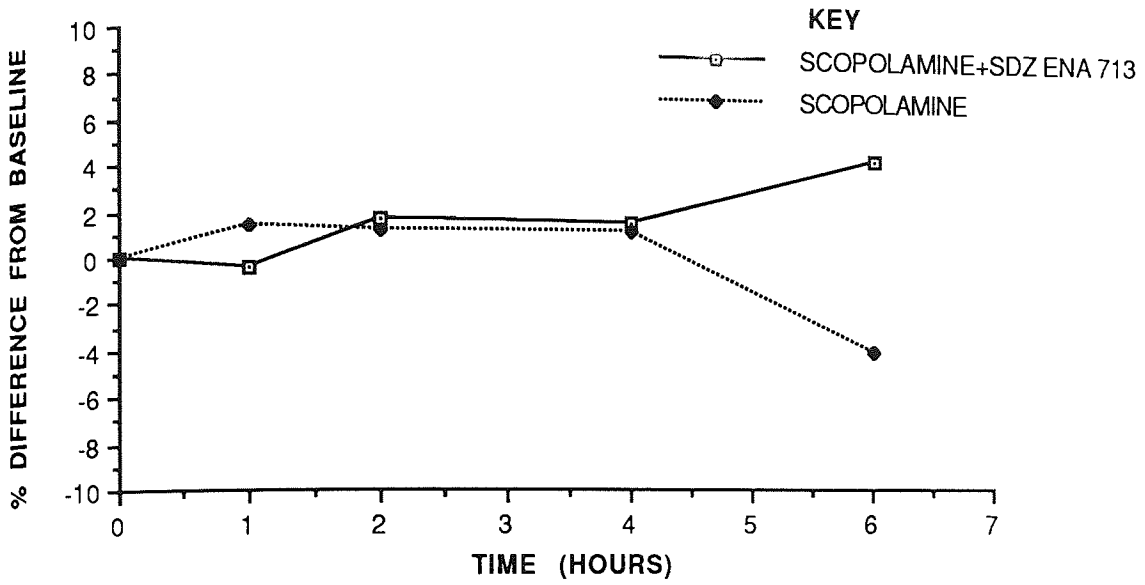
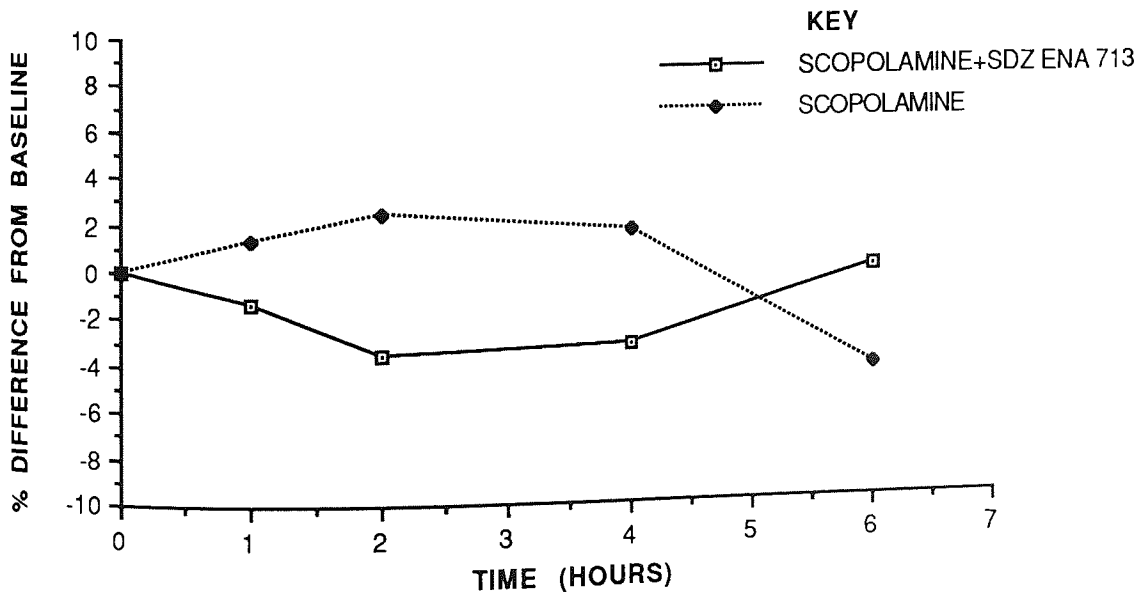


Figure 8.27 Comparison of the change in flash P3 latency after treatment with scopolamine alone and combination of scopolamine-SDZ ENA 713.



at 2 hours, but this was still within the variation displayed by the time of day effects (23.8%) from chapter 6.

The effect of the two treatments again displayed similar effects on the flash N3-P3 amplitude up to the 1 hour period (fig. 8.30). At the 2-4 hours, both treatments displayed a fall in amplitude with that to the combination treatment being of greater magnitude. However, at 6 hours, the combination treatment displayed a sharp increase in N3-P3 amplitude, whereas scopolamine continued to display a fall. As before, the magnitude of change with the treatments was below 48.6% obtained with time of day effects.

Overall, the combination of scopolamine-SDZ ENA 713 treatment displayed either decreases in amplitudes of a greater magnitude than with scopolamine alone (P2-N3 and N3-P3 amplitudes) or if both treatments displayed an increase in amplitude (N2-P2), then the magnitude of the increase was reduced with the combination treatment. In chapter 7, the effect of the cholinergic agent SDZ 210-086 displayed a fall in the flash VEP amplitude. Hence, the results of the present study indicate that scopolamine and SDZ ENA 713 were exhibiting opposite effects on the flash VEP amplitudes.

Figure 8.28 Comparison of the change in flash N2-P2 amplitude after treatment with scopolamine alone and combination of scopolamine-SDZ ENA 713.

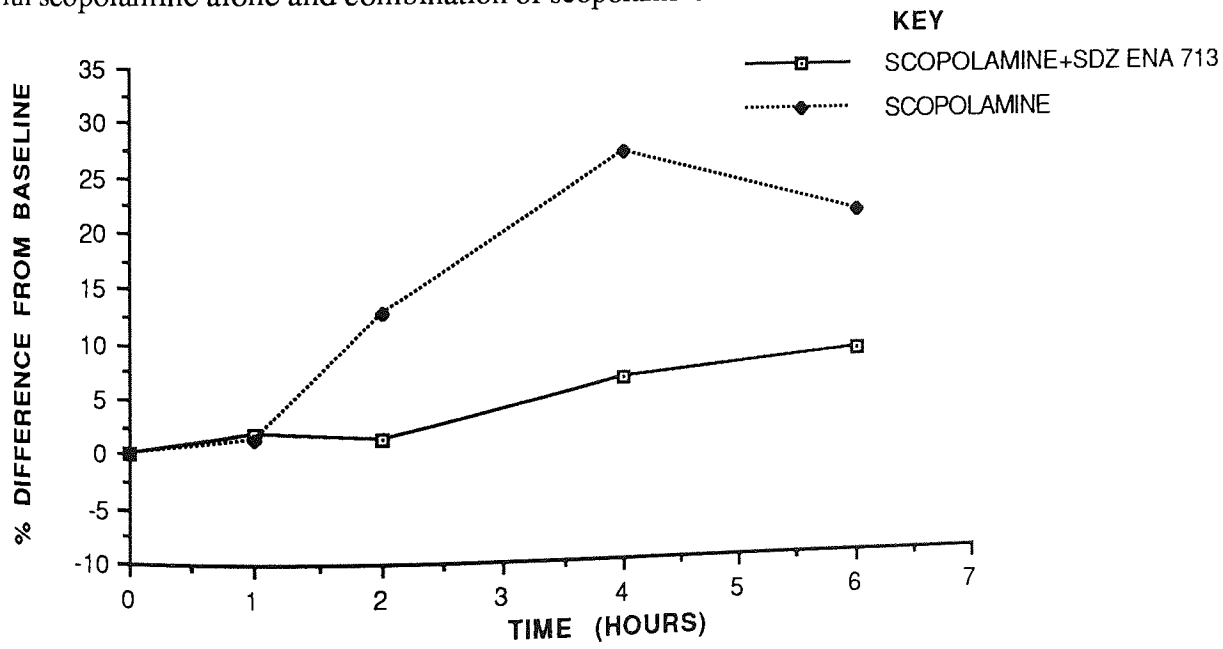


Figure 8.29 Comparison of the change in flash P2-N3 amplitude after treatment with scopolamine alone and combination of scopolamine-SDZ ENA 713.

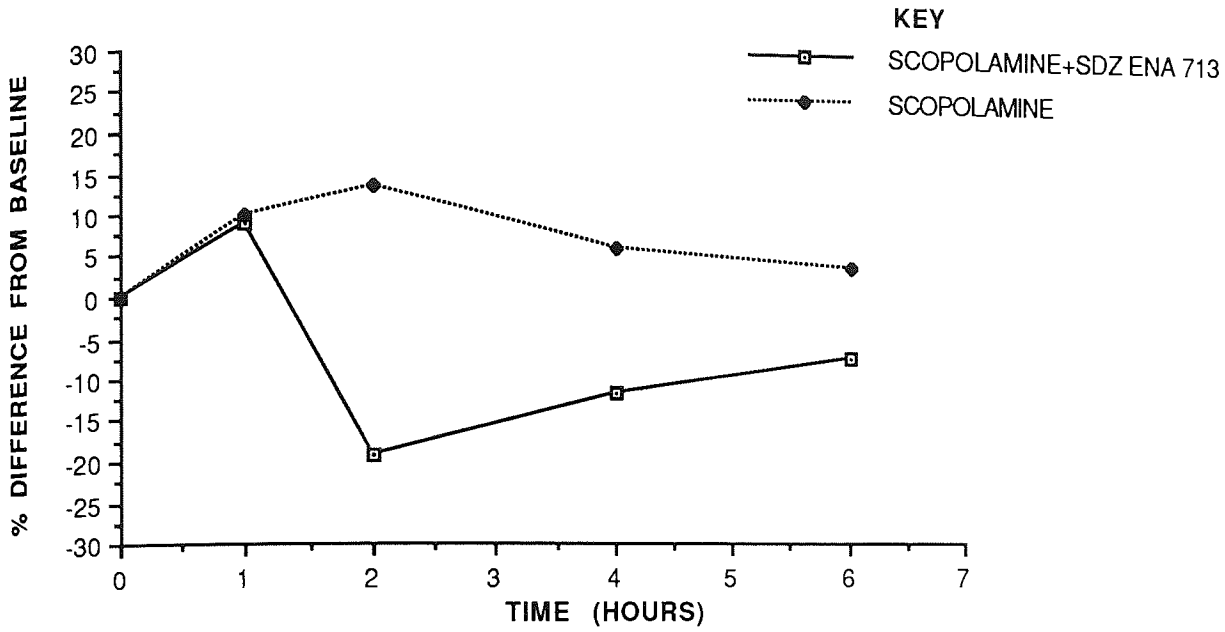
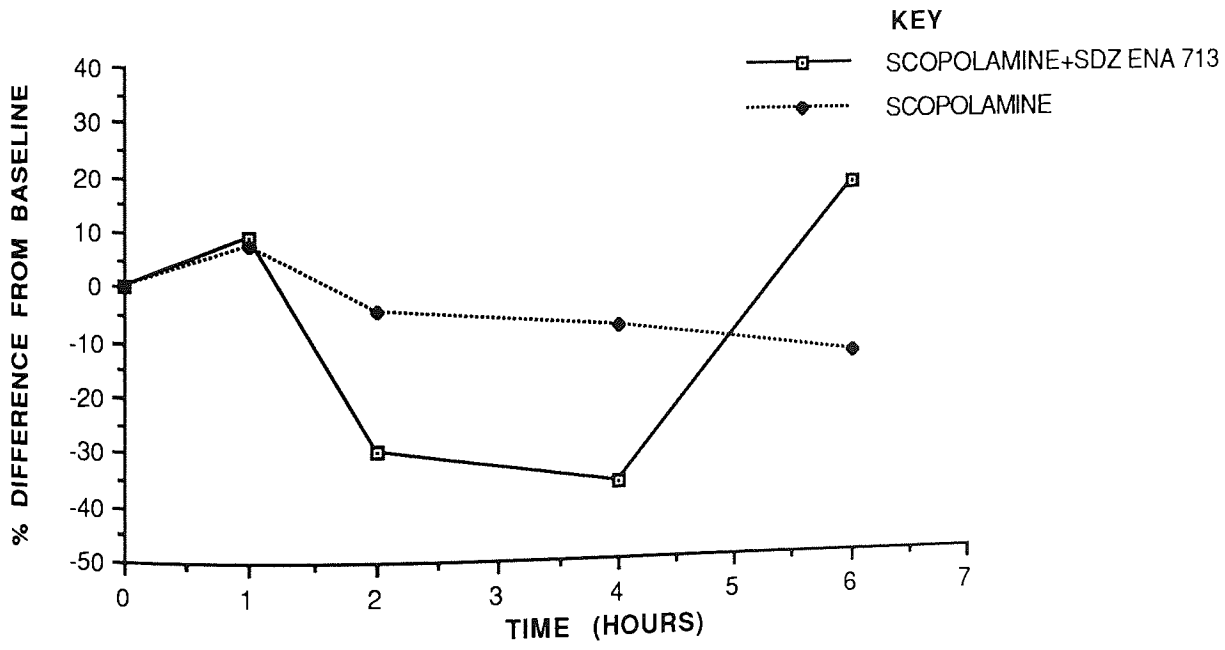


Figure 8.30 Comparison of the change in flash N3-P3 amplitude after treatment with scopolamine alone and combination of scopolamine-SDZ ENA 713.



8.5.2 Pattern reversal VEP

Similarly to the flash N2, the pattern reversal N75 latency displayed little difference between scopolamine alone and scopolamine-SDZ ENA 713 treatment (fig. 8.31). The only difference between the two treatments was at 2 hours when scopolamine alone showed a slight shortening in latency but the combination treatment showed a slight increase in N75 latency. Overall, the change in latency due to the treatments was less than that due to natural variation (chapter 6, N75 latency varied by 4.5%).

The P100 latency displayed similar changes to scopolamine and scopolamine-SDZ ENA 713 treatments throughout the day except at the 4 hour period (fig. 8.32). Here the two treatments displayed opposing effects. Scopolamine alone caused an increase in the P100 latency at 4 hours, whereas the scopolamine-SDZ ENA 713 combination resulted in a decrease in latency at the 4 hours. These changes at the 4 hours were greater than that observed by the P100 latency from the time of day study in chapter 5 (fluctuation of 2.7%). However, the statistical analysis did not reveal any significant effects of either treatment on the P100 latency. This was because the effect of the treatments over the whole day was considered not just the difference between the baseline and the 4 hour values. However, this opposite effect by the two treatments suggest that SDZ ENA 713 may have reversed the P100 latency delay occurring 4 hours after scopolamine administration.

Figure 8.31 Comparison of the change in pattern reversal N75 latency after treatment with scopolamine alone and combination of scopolamine-SDZ ENA 713.

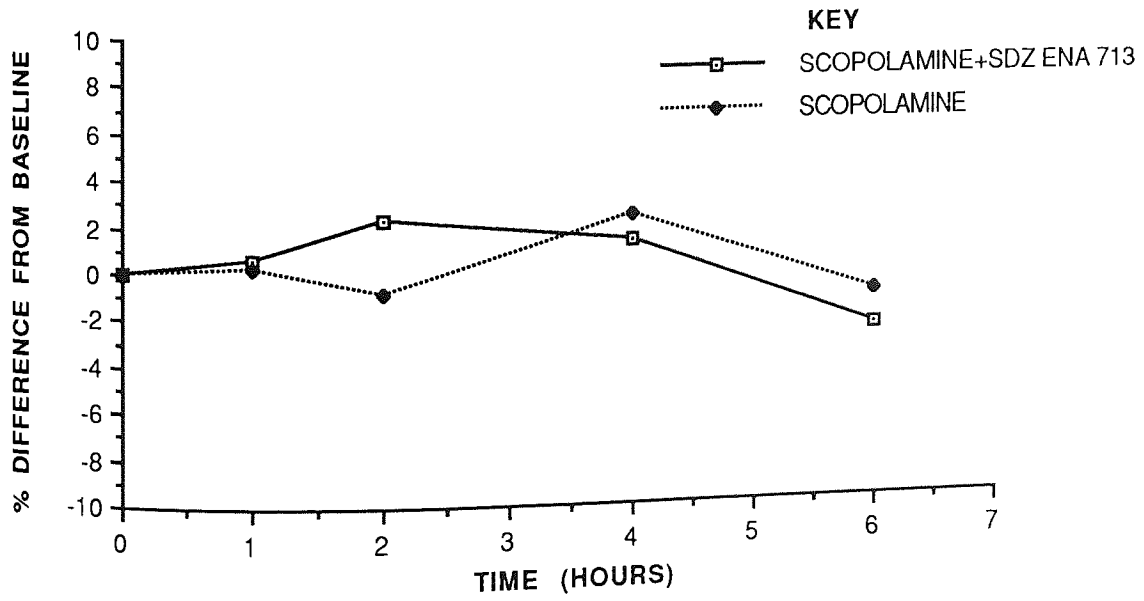
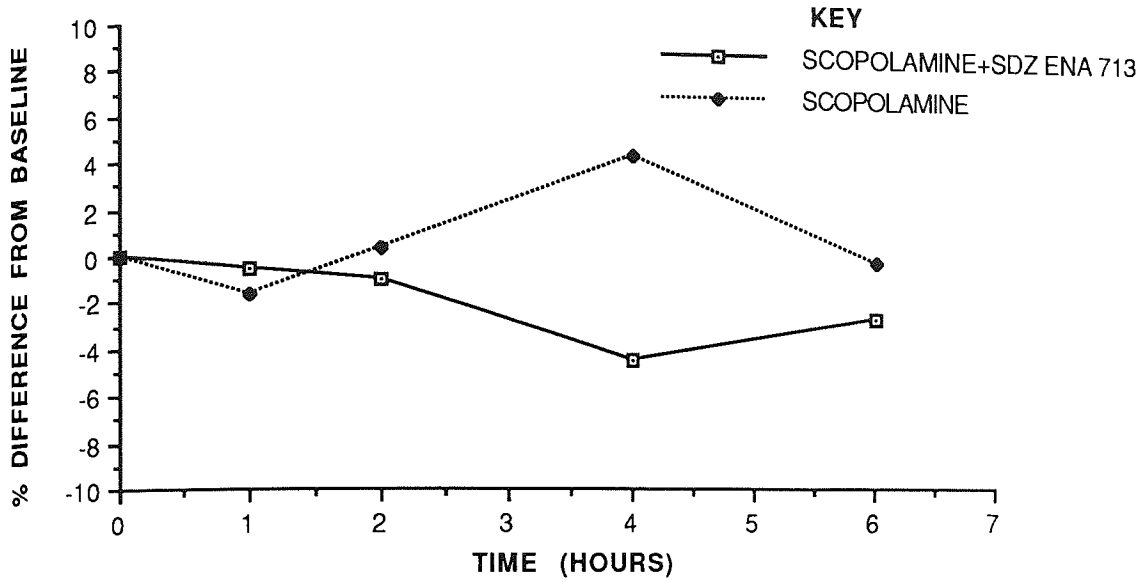


Figure 8.32 Comparison of the change in pattern reversal P100 latency after treatment with scopolamine alone and combination of scopolamine-SDZ ENA 713.



The N140 and P160 latencies both displayed similar effects to the two treatments (Figs 8.33 and 8.34 respectively). The N140 latency showed little change post scopolamine dosing. The scopolamine-SDZ ENA 713 treatment showed a small increase in the N140 latency post-dosing. Similarly, the P160 latency was increased at 1-2 hours with both treatments and decreased from 2-6 hours. However, as with the N140 latency, the P160 latency increase was greater with the combination treatment and the decrease thereafter was less than that with scopolamine alone. In both cases, there were no significant effects of the treatments on the latencies, and the magnitude of change was below that observed in chapter 6 when no treatment was administered.

The pattern reversal VEP amplitude measurements displayed similar effects to the two treatments, i.e. there was a general increase in the amplitudes with the scopolamine treatment. The N75-P100 amplitude with scopolamine-SDZ ENA 713, showed a fall in amplitude at 1 hour followed by a small increase thereafter (fig. 8.35). Whereas the N75-P100 amplitude with the scopolamine alone treatment displayed an increase up to 4 hours before falling towards baseline levels. The effects of the increase in amplitude with the scopolamine had reached statistical significance. Comparison of the percentage change due to time of day effects (chapter 6) showed that the percentage change displayed by scopolamine administration was greater in magnitude.

A similar effect was also seen with the P100-N140 amplitude (fig. 8.36). There was a small decrease in amplitude at the 1 hour followed by an increase thereafter with

scopolamine-SDZ ENA 713 treatment. Whereas with the scopolamine alone treatment, there was a gradual increase over the day. This indicates that the changes are attributable to those occurring in the P100 as this component is common to both measurements.

The N140-P160 amplitude with scopolamine alone also displayed an increase from 2-6 hours, whereas with the combination treatment, there was a fall in amplitude over the day (fig. 8.37). However, the magnitude of change did not exceed the fluctuation present in the N140-P160 amplitude when no treatment is given (chapter 6).

Hence the increase in the amplitudes seen with scopolamine alone seems to have been reduced with the combination of scopolamine-SDZ ENA 713.

Figure 8.33 Comparison of the change in pattern reversal N140 latency after treatment with scopolamine alone and combination of scopolamine-SDZ ENA 713.

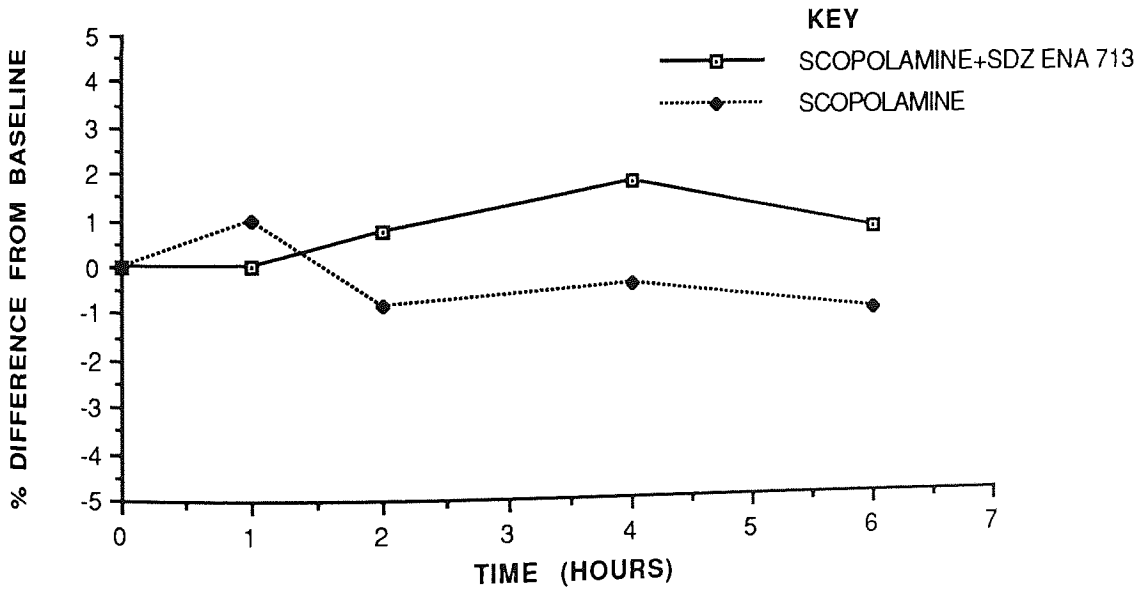


Figure 8.34 Comparison of the change in pattern reversal P160 latency after treatment with scopolamine alone and combination of scopolamine-SDZ ENA 713.

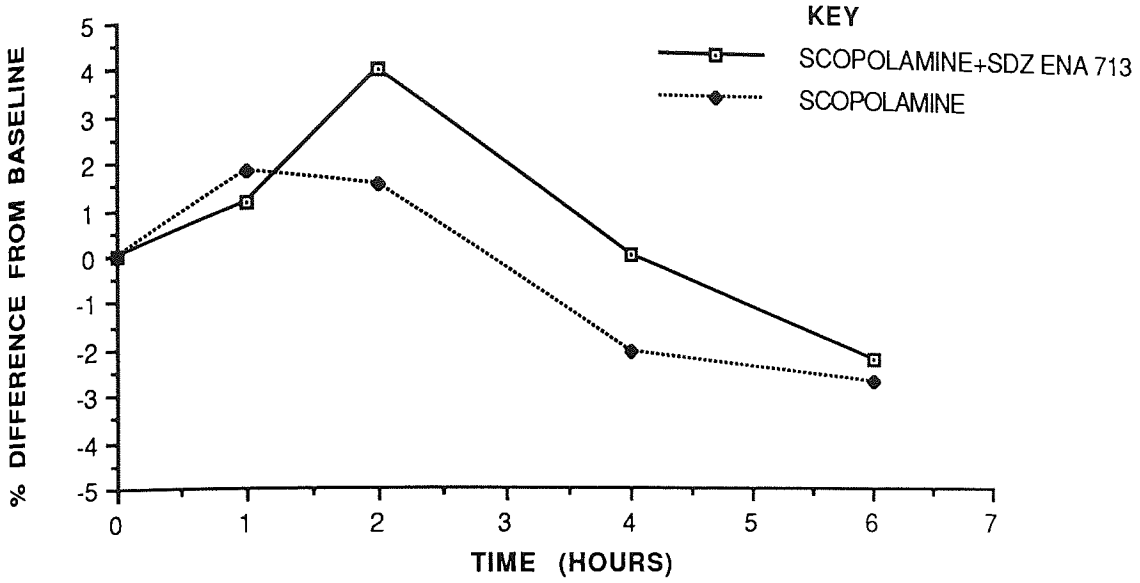


Figure 8.35 Comparison of the change in pattern reversal N75-P100 amplitude after treatment with scopolamine alone and combination of scopolamine-SDZ ENA 713.

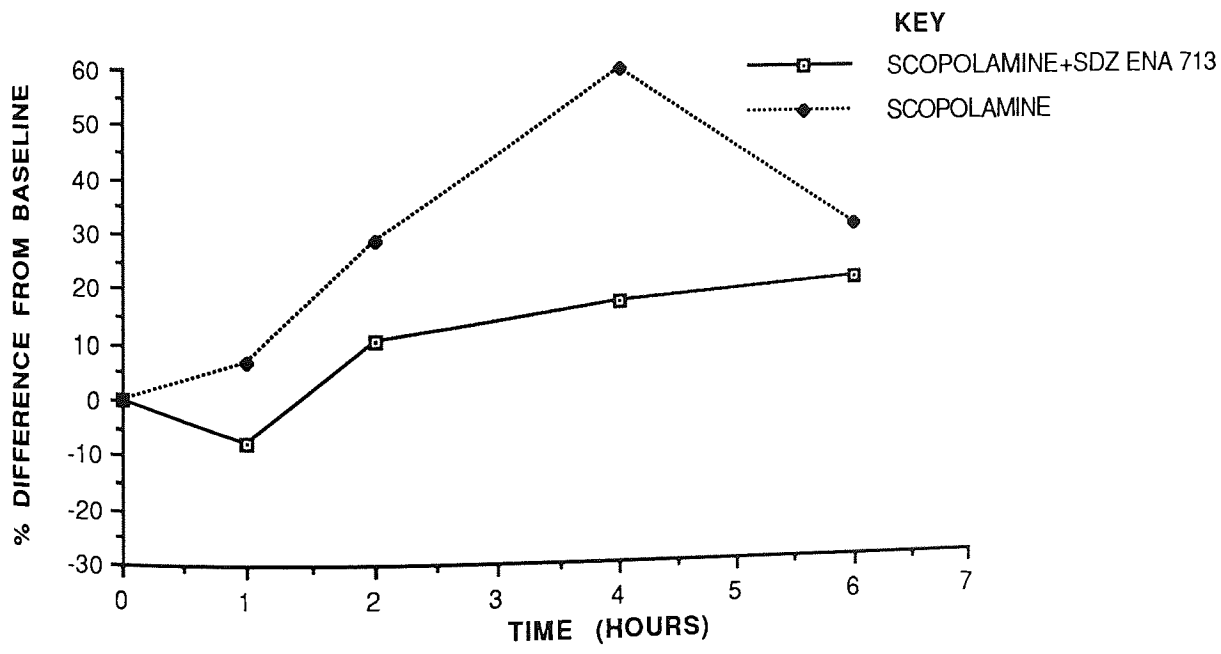


Figure 8.36 Comparison of the change in pattern reversal P100-N140 amplitude after treatment with scopolamine alone and combination of scopolamine-SDZ ENA 713.

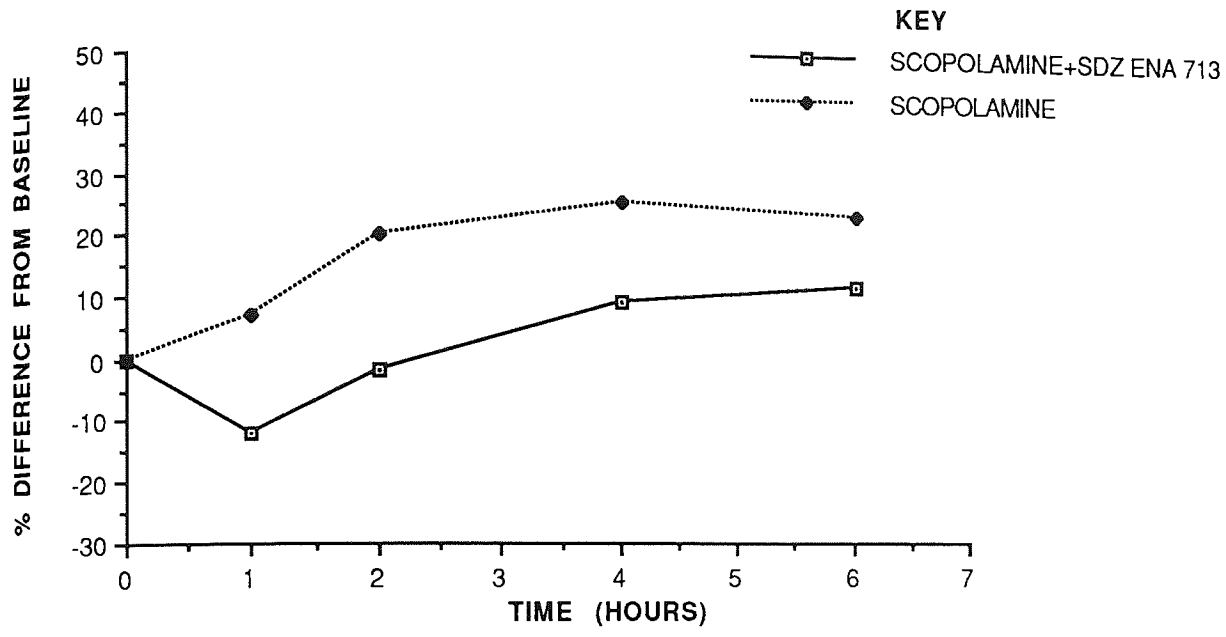
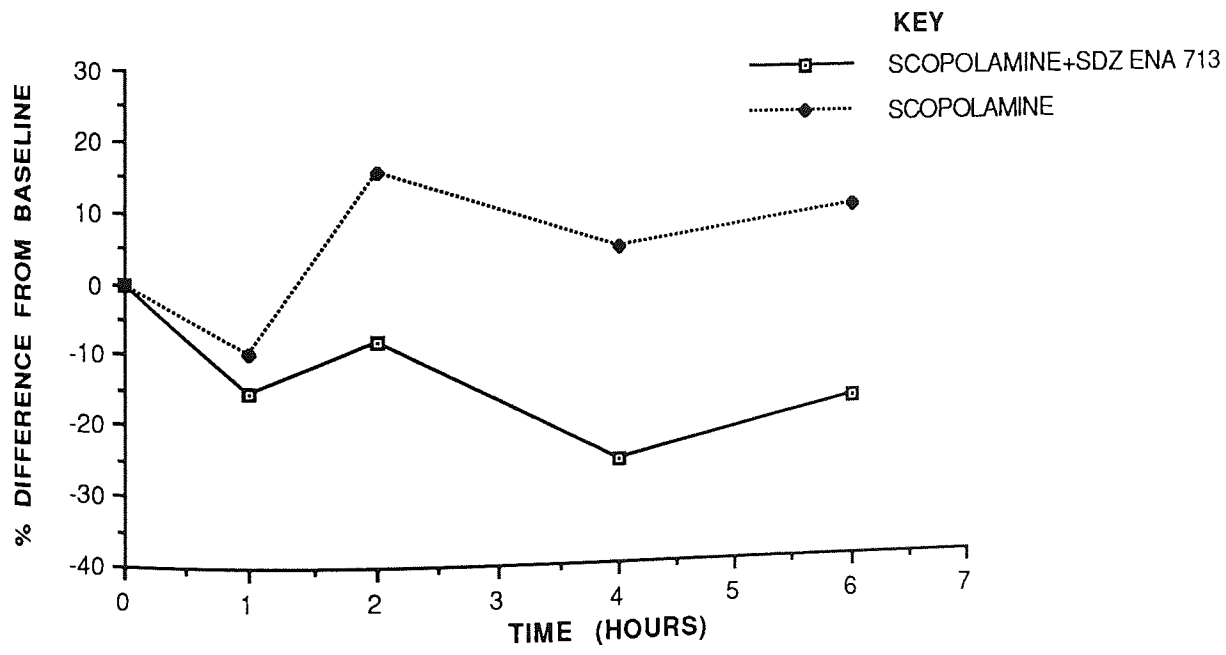


Figure 8.37 Comparison of the change in pattern reversal N140-P160 amplitude after treatment with scopolamine alone and combination of scopolamine-SDZ ENA 713.



8.6 Conclusions

The effect of scopolamine administration in young healthy male volunteers resulted in an increase in the flash P2 latency and the pattern reversal N75-P100 amplitude. There were no statistically significant effects of the combination treatment of scopolamine with the AChEi SDZ ENA 713 on the flash and pattern reversal VEPs. Comparison of the results obtained with the two treatments indicated that SDZ ENA 713 may reverse some of the scopolamine-induced effects on the VEPs. The effects of SDZ ENA 713 were not fully investigated as the combination of this agent with scopolamine resulted in dangerous reductions in the heart rate. However, if the planned studies (section 8.4) of investigating the effects of SDZ ENA 713 on psychometric tests and flash and pattern reversal VEPs had been carried out, there is an indication that any scopolamine-induced deficits may have been reversed or reduced.

The present study also provided evidence of a cholinergic influence on the VEPs, with the effect on the flash VEP being greater than on the pattern reversal VEP. This leads to the possible explanation of the selective delay on the flash P2 co-existing with a pattern reversal P100 of normal latency. It was proposed that the flash and pattern reversal VEPs are processed through different pathways (section 3.5 and 4.11) i.e. the flash is processes via the tectal pathway and the pattern reversal VEP via the geniculostriate pathway. In addition, there is evidence of a cholinergic influence on the tectal pathway but not on the geniculostriate pathway (Shute and Lewis 1963 and 1967).

The finding of an increase in the pattern reversal N75-P100 amplitude in the absence of a similar significant increase in the flash N2-P2 amplitude in the present study could not be explained fully. In chapter 7, the cholinergic agent SDZ 210-086 caused a reduction in both the N2-P2 and N75-P100 amplitudes with the effect on the flash amplitude occurring at the lower doses. In addition, studies on the flash VEP in patients with AD also show an increase in the N2-P2 amplitude (Wright et al. 1986) and administration of scopolamine in healthy volunteers also display an increase in the N2-P2 amplitude (Bajalan et al. 1986). The increase in the pattern reversal VEP amplitude has been reported in patients with AD (Coben et al. 1983; Huisman et al. 1987) but reached significance only for the later N140-P160 amplitudes. Hence the effect of scopolamine in the present study was expected to affect the flash N2-P2 amplitude in preference to the pattern reversal N75-P100 amplitude. The N75-P100 amplitude was also significantly increased ($P<0.05$) with the scopolamine-SDZ ENA 713 combination to binocular fixation, but the magnitude of increase was reduced

compared to scopolamine alone administration. Therefore, the effect of cholinergic modulation on the amplitude of the flash and pattern reversal VEPs remains unclear from the results in the present study.

CHAPTER 9

Effect of Scopolamine Eye Drops on the Flash and Pattern Reversal Visual Evoked Potential

9.1 Introduction

In primates there are two visual parallel processing pathways based on their projection to the dLGN and termed the magnocellular or M-cell and parvocellular or P-cell pathways (detailed in section 2.3). These pathways commence from the retina and are preserved throughout the visual pathway from the diencephalon to the striate and extrastriate corticies. The M-cell pathway is largely responsible for transmitting information of low spatial frequency whereas the P-cell pathway is responsible for transmitting information of high spatial frequency (Derrington and Lennie 1984; Kaplan and Shapley 1986; section 2.3.2.3). Therefore, it may be postulated that the flash which contains no spatial information would be transmitted via the M cell pathway and the pattern reversal response transmitted via the P cell pathway.

In patients with AD, there is selective degeneration of structures related to the M-cell pathway (section 4.8). For example, there is a loss of large diameter retinal ganglion cells (perimetry greater than 40 μm) and a selective depletion of large diameter axons from the optic nerve (Hinton et al. 1986; Sadun and Bassi 1990). Since the large retinal ganglion cells and axons belong to the M-cell pathway, these observations suggest that the M-cell pathway is affected greater than the P-cell pathway in AD.

ACh has been implicated as a neurotransmitter in the retina (section 2.2.4.1), especially in the layers containing amacrine cells (Brecha 1983; Puro 1985; Masland and Tauchi 1986). In addition, the activity of ganglion cells are thought to be modulated by a subclass of displaced amacrines cells called the "starburst" (Famiglietti 1983) or "coronate" (Vaney 1984) cells. The observation of a cholinergic influence at the level of the retina has been supported by the findings of Morrison and Reilly (1989) who reported that topically applied scopolamine (0.025%), transiently reduced the contrast sensitivity of sinusoidal gratings. Since the deficit occurred with low (2-5 cpd) but not high (10-20 cpd) spatial frequency gratings, the involvement of the M-cell pathway was further implicated.

Therefore, the delayed flash P2 coexisting with a pattern reversal P100 of normal latency, observed in patients with AD, may be due to either the selective loss of retinal ganglion cells and/or to a deficit in the transmission of the flash VEP via the cholinergic tectal pathway. In addition, the effects of systemically administered

scopolamine (chapter 8) may have been either due to a direct action on cholinergic cells in the retina or an action on the cholinergic tectal pathway.

In order to test whether the anticholinergic action of scopolamine occurs at the retinal level, the effects of topically administered scopolamine was examined on the flash and pattern reversal VEPs.

9.2.Methods

9.2.1 Subjects

Six normal healthy volunteers (3 male and 3 female; age range 20-36 years; mean 24.5 ±2.41 years) were paid to participate in the study. All subjects had a Snellen visual acuity of 6/6 or better with correction where needed and normal visual fields. Subjects were excluded if they were contact lens wearers, had any history of recent ocular pathology, migraine, epilepsy or if they were receiving any medication. The effectiveness of cycloplegics such as scopolamine is greatly reduced in highly pigmented eyes (Ward and Charman 1986; Edwards and Llewellyn 1988; Eskridge et al. 1991), hence all the subjects chosen fulfilled the grade 1 of the standardised iris colour classification system (Seddon et al. 1990). This restriction to subjects with blue irides allowed for a more standardised response to the low concentrations of the drug, with a more rapid recovery by avoiding the sustained release from melanin pigment within the eye. The use of mydriatic drops may lead to an uncontrolled rise in intraocular pressure (IOP), therefore subjects with an IOP over 20 mmHg (using the Pulsair non contact tonometer) were also excluded. In addition, the filtration angle (anterior chamber angle) was checked using Van Heriks technique, and only grades 3 or 4 (20-45^{o}) were included.

Measurements of pupil diameter, IOP and amplitude of accommodation and administration of scopolamine eye-drops were carried out by Miss Rebecca Daniels (optometrist).

The subjects were not restricted in terms of diet or caffeine intake, but as the effects of scopolamine are potentiated by alcohol, the subjects were advised to avoid alcohol one day prior to and during the study day. Subjects were further advised to avoid driving, any activity requiring dynamic spatial judgements or bright sunlight during the period when accommodation and pupil size were noticeably affected. Signed consent forms were obtained from each subject after being fully informed about the drug effects and the methods to be used in the study. The project was approved by the Human Science Ethics Committee at Aston University.

9.2.2 Instrumentation

The flash stimulus was delivered by a Grass PS22 stroboscope at intensity 4 (luminance of 1925 cd/m^2), placed 30 cm away from the eye subtending a field of 36^o and delivered at a rate of 1.7 flashes per second.

The pattern stimuli were generated from a Cambridge Research Systems VSG2/1 grating generator and displayed on a 16 inch colour TV monitor (E120 Flexscan 9080i). The monitor had a 14 bit luminance resolution, 70 Hz frame rate, 624 pixels and 878 lines, using a standard raster technique. The mean luminance of the screen was 50 cd/m2. The pattern reversal stimulus consisted of a checkerboard pattern of 60' checks in a field subtending 4^o 17' x 5^o 43' to the eye at 3m. The checks were reversed at a rate of 1Hz and contrast was 85%.

The evoked potentials were recorded, stored and analysed using the Nicolet Pathfinder II electrodiagnostic system. The bandpass filtering was set at 0.5-75 Hz (-3 dB down point) and the gain at 30,000.

9.2.3 Procedure

The amplitude of accommodation, pupil size and IOP were measured prior to and after drug administration to monitor the peripheral activity of the drug. Artificial pupils of 4 mm diameter were adjusted before the eyes in an optometric trial frame, and the subjects trained to maintain central fixation. Monocular VEPs were recorded using silver-silver chloride electrodes using the standard derivations of O2 referred to C4 and O1 referred to C3 for the flash VEP and O2 and O1 referred to Fz for the pattern reversal VEP. The interelectrode impedance was maintained below 5 kΩ. Each run consisted of 100 averaged responses.

Since topical anaesthetics cause a softening of the corneal epithelium, one drop of benoxinate hydrochloride 0.4% was instilled in each eye to increase the absorption and transmission of scopolamine across the cornea as well as to reduce tear fluid secretion. One minute later, a 20 µl drop of 0.125% of scopolamine (hyoscine hydrobromide) was instilled in the left eye only using a micropipette, whist occluding the puncta to prevent any systemic absorption of the drug through the nasal mucosa. The amplitude of accommodation, pupil diameter, IOP followed by the flash and pattern reversal VEPs were measured and again at hourly intervals for 3 hours thereafter for each eye. The refractive error was monitored throughout to maintain accurate correction for the pattern stimuli in both the control right eye and the test left eye.

9.3 Results

9.3.1 Data analysis

The peripheral effects of scopolamine eye drops were monitored by measuring pupil diameter and amplitude of accommodation. The IOP was also monitored since the use of mydriatic drops may lead to an uncontrolled rise in IOP. The effect of scopolamine eye drops on the VEP were confined to measuring the flash N2, P2, N3 and P3 latencies and N2-P2, P2-N3 and N3-P3 amplitudes and the pattern reversal N75, P100, N140 and P160 latencies and N75-P100, P100-N140 and N140-P160 amplitudes.

Statistical analysis was carried out with a two-factor analysis of variance using a split-plot (with repeated measures) in randomised blocks. This allowed a test of differences between the eyes, between the runs irrespective of eyes, between the runs with respect to each eye and between subjects. Significance level was determined at $P<0.05$.

9.3.2 Pupil diameter

There was an increase in the pupil diameter of the left eye from a mean of 6.5 mm to 8.5 mm about 1 hour after scopolamine eye drops (fig. 9.1), compared to the right eye. The increase in pupil diameter of the left eye was present in all six subjects although the magnitude varied (appendix 5, table 1). For example, subject JB showed the least and subject JG the greatest increase in the intraocular difference in pupil diameter after scopolamine administration. The pupil diameter of the right eye was reduced slightly (below 1 mm), 1 hour after scopolamine administration in the left eye, but this was not statistically significant.

9.3.3 Amplitude of accommodation

The mean amplitude of accommodation was 8.1 D in the right eye and 8.4 D in the left eye prior to scopolamine administration. This was reduced such that the subjects were off-scale (below 2 dioptres) in the left eye 1 hour after scopolamine administration (fig. 9.2). There was little change in the right eye. The effect on the left eye was consistent in all six subjects (appendix 5, table 2). The amplitude of accommodation remained off-scale for the rest of the day, returning to pre-scopolamine levels 4-5 days after administration.

Figure 9.1. The effect of scopolamine eye drops on the pupil diameter. The solid and broken lines represent the control (right) and test (left) eyes respectively.

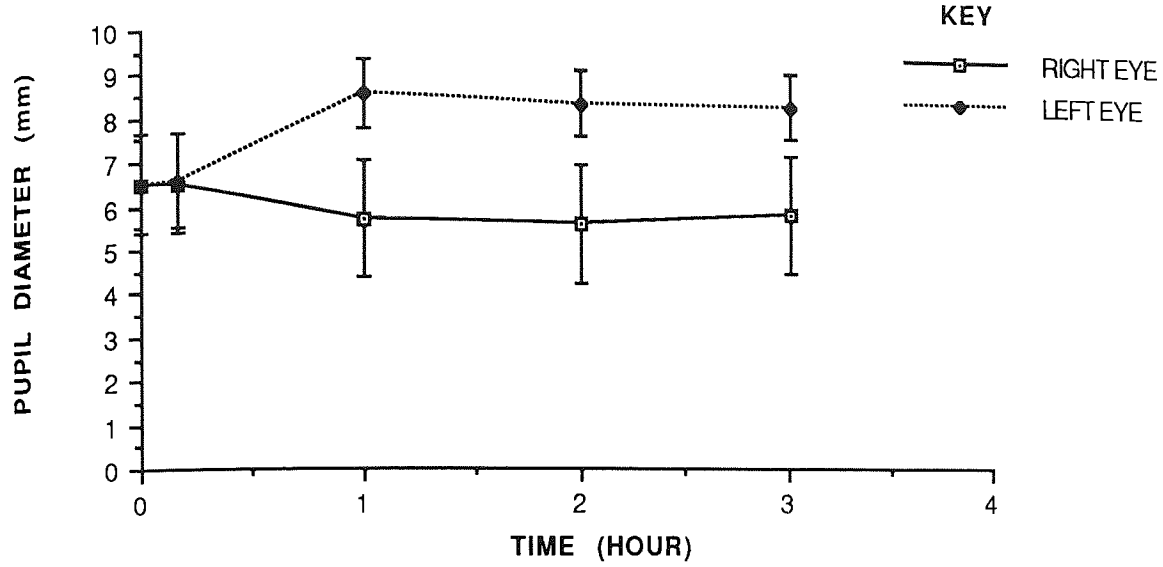
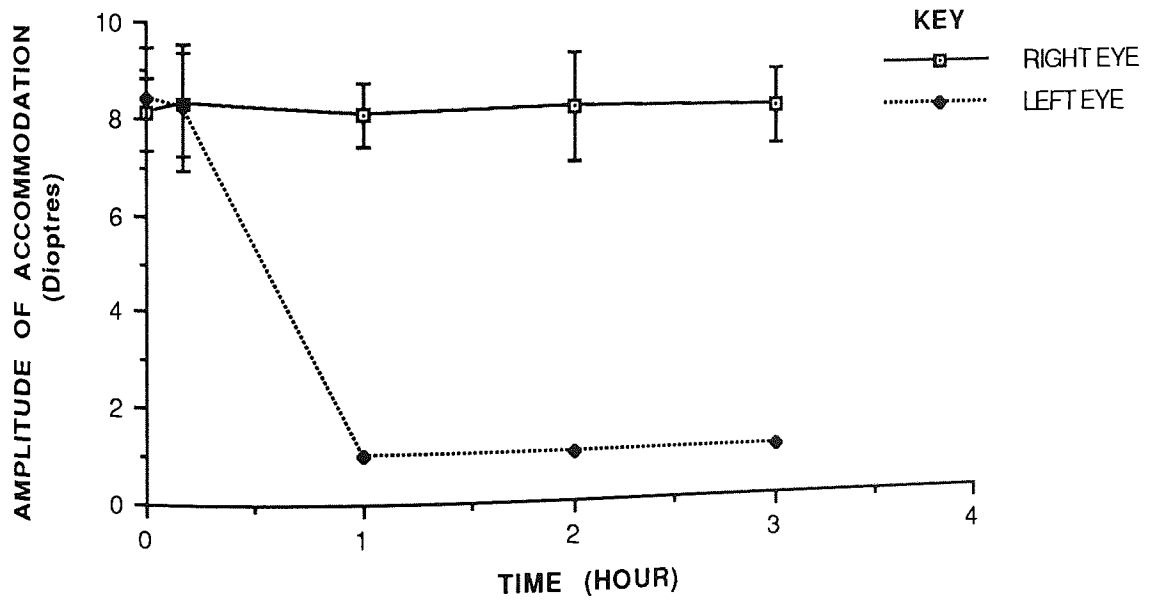


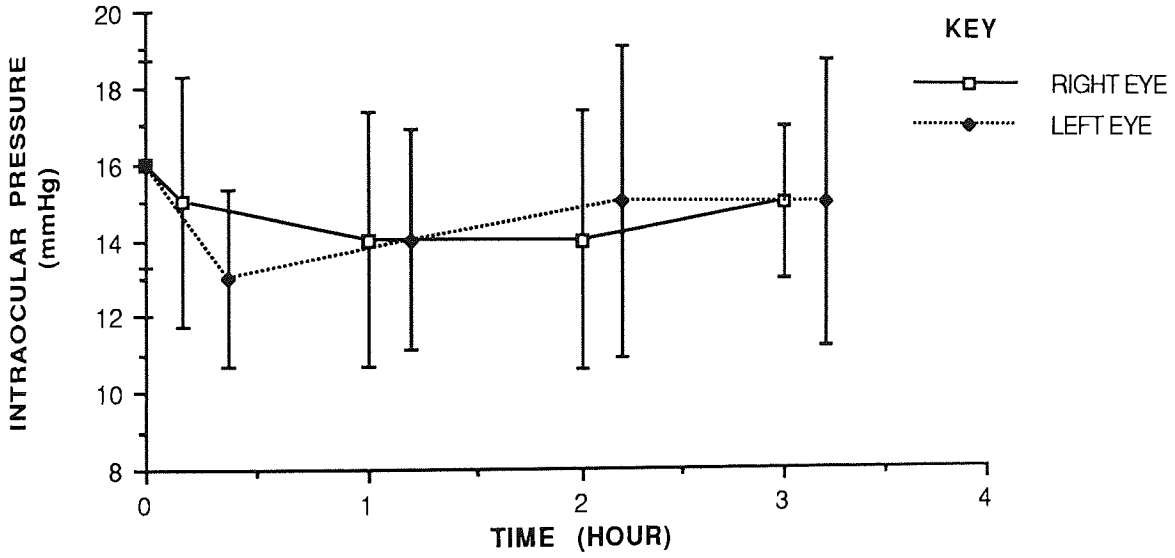
Figure 9.2 The effect of scopolamine eye drops on the amplitude of accommodation. The solid and broken lines represent the control (right) and test (left) eyes respectively.



9.3.4 Intraocular pressure

There was no overall change in the mean IOP in either the left or right eye (fig.9.3). Although the individual measures of IOP varied between subjects (appendix 5, table 3). The IOP in subject AD prior to scopolamine administration was 20 mm Hg which was on the borderline of the exclusion criteria and so monitored closely in this subject, but it did not rise above 20 mm Hg.

Figure 9.3 The effect of scopolamine eye drops on the intraocular pressure (mmHg). The values for the left eye have been staggered towards the right-hand side for clarity. The solid and broken lines represent the control (right) and test (left) eyes respectively.



9.3.5 Flash VEP

An example of the response to the flash stimulus is shown in fig. 9.4. There was little overall difference in the waveform between the eyes and the runs over the day. The results of the group mean latency and amplitude values of the flash VEP are shown in table 9.1. In order to compare, magnify and overcome any intraocular differences on the effects of scopolamine eye-drops over the day, the percentage change from the baseline values were calculated.

The effects of scopolamine eye drops on the flash N2, P2, N3 and P3 latencies are shown in figs. 9.5, 9.6, 9.7 and 9.8 respectively. There was very little change in latency of the flash VEP components. The greatest change was an increase of 4.14% in P3 latency in the control (right) eye. Statistical analysis revealed no significant change in the latency of the flash VEP components, although there was significant inter-individual variation ($P<0.05$), which was expected due to the small number of

Figure 9.4 An example of a flash VEP as recorded from subject AG. The solid and broken lines represent the right (control) and left (test) eyes respectively. There was little difference in waveform between the eye or runs. Waveform were recorded from channel O2-C4.

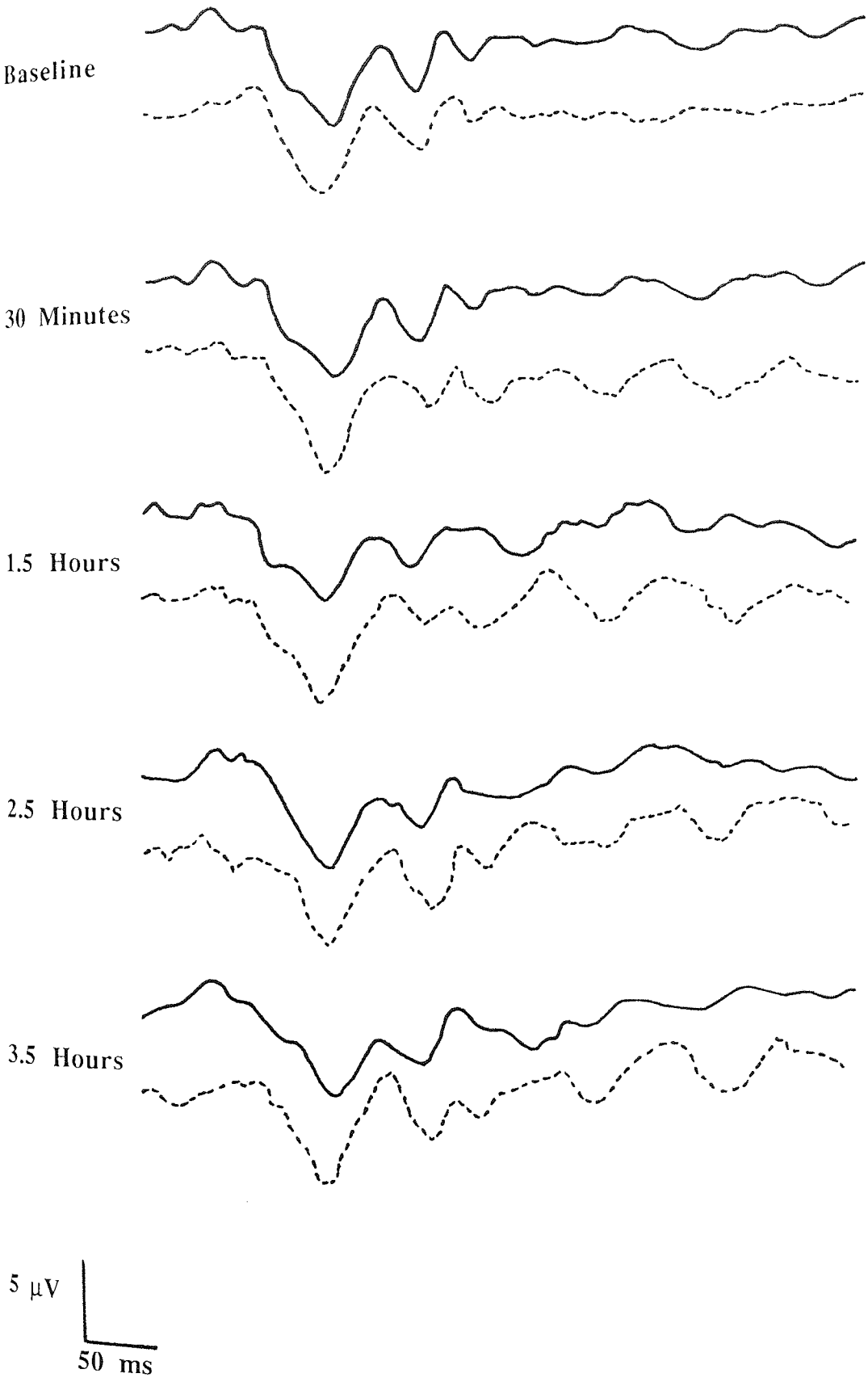


Table 9.1 The group mean values of the flash VEP latencies in the right (control) and left (test) eye. Data from channel O2-C4.

	BASELINE	30mins	1.5h	2.5h	3.5h
N2 LATENCY (ms)					
RE MEAN	58.67	59.00	63.14	56.67	60.00
SD	8.91	5.80	10.09	10.37	11.44
LE Mean	67.87	68.61	68.61	67.87	66.17
SD	6.30	5.19	7.11	6.60	6.35
P2 LATENCY (ms)					
RE Mean	111.33	108.80	110.50	108.67	108.17
SD	9.70	8.47	7.48	8.48	8.21
LE Mean	107.33	106.67	107.17	108.17	105.67
SD	8.59	7.58	7.25	9.09	8.24
N3 LATENCY (ms)					
RE Mean	150.50	148.67	149.33	150.00	150.33
SD	10.91	11.02	12.29	11.24	11.00
LE Mean	146.50	147.67	149.00	147.83	146.83
SD	11.15	12.71	12.68	12.25	12.70
P3 LATENCY (ms)					
RE Mean	180.83	183.67	182.83	187.67	183.50
SD	13.75	6.28	12.54	6.86	13.58
LE Mean	176.50	179.83	181.33	181.00	176.67
SD	17.16	20.43	19.75	18.70	20.07
N2-P2 AMPLITUDE (μV)					
RE Mean	10.62	9.29	9.87	8.33	8.08
SD	6.81	5.66	5.53	5.58	6.88
LE Mean	11.68	10.39	8.86	9.20	10.43
SD	7.79	7.16	4.71	5.25	6.05
P2-N3 AMPLITUDE (μV)					
RE Mean	8.78	8.91	9.04	8.00	7.86
SD	2.84	4.86	4.18	2.84	3.82
LE Mean	9.27	8.85	8.41	7.74	8.34
SD	5.32	4.27	3.33	3.95	4.17
N3-P3 AMPLITUDE (μV)					
RE Mean	5.31	6.90	9.22	7.84	7.27
SD	2.22	2.70	4.77	2.67	3.32
LE Mean	6.23	6.79	7.18	7.73	6.20
SD	3.02	3.92	2.83	2.88	3.66

KEY: RE -Right eye; LE -Left eye; SD -standard deviation.

Figure 9.5 Effect of topical administration of scopolamine on the flash N2 latency, in the control eye (solid line) and test eye (broken line). Data from channel O2-C4.

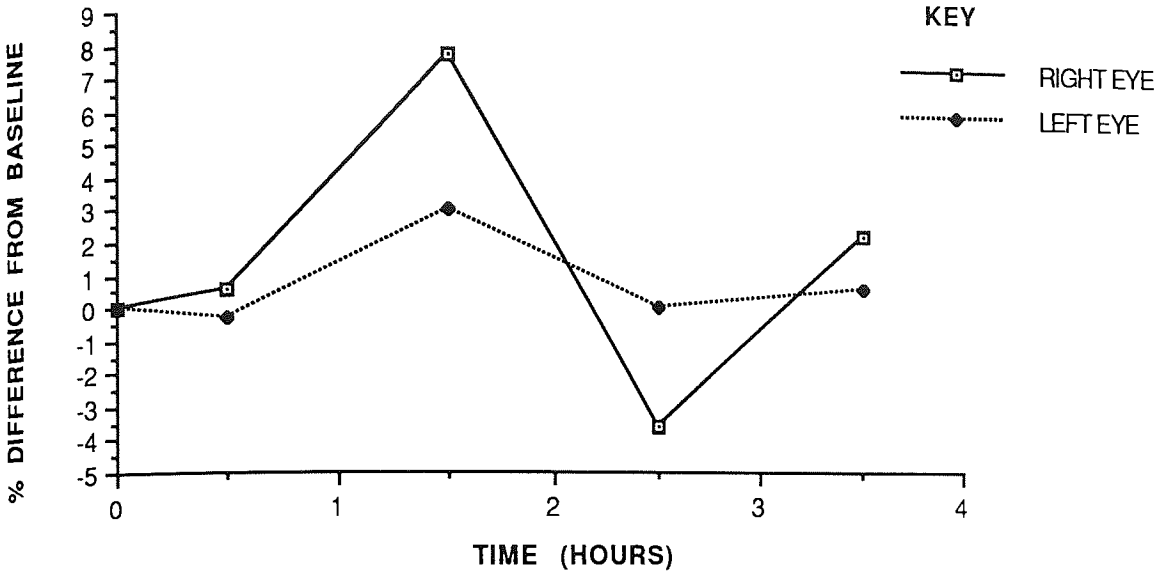


Figure 9.6 Effect of topical administration of scopolamine on the flash P2 latency, in the control eye (solid line) and test eye (broken line). Data from channel O2-C4.

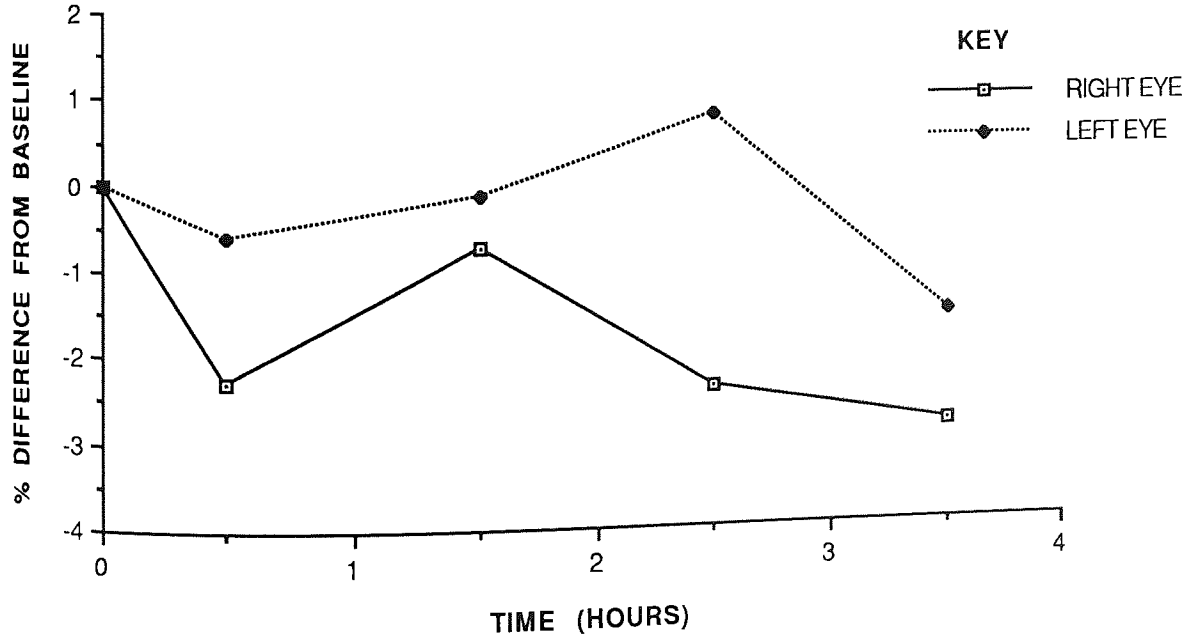


Figure 9.7 Effect of topical administration of scopolamine on the flash N3 latency, in the control eye (solid line) and test eye (broken line). Data from channel O2-C4.

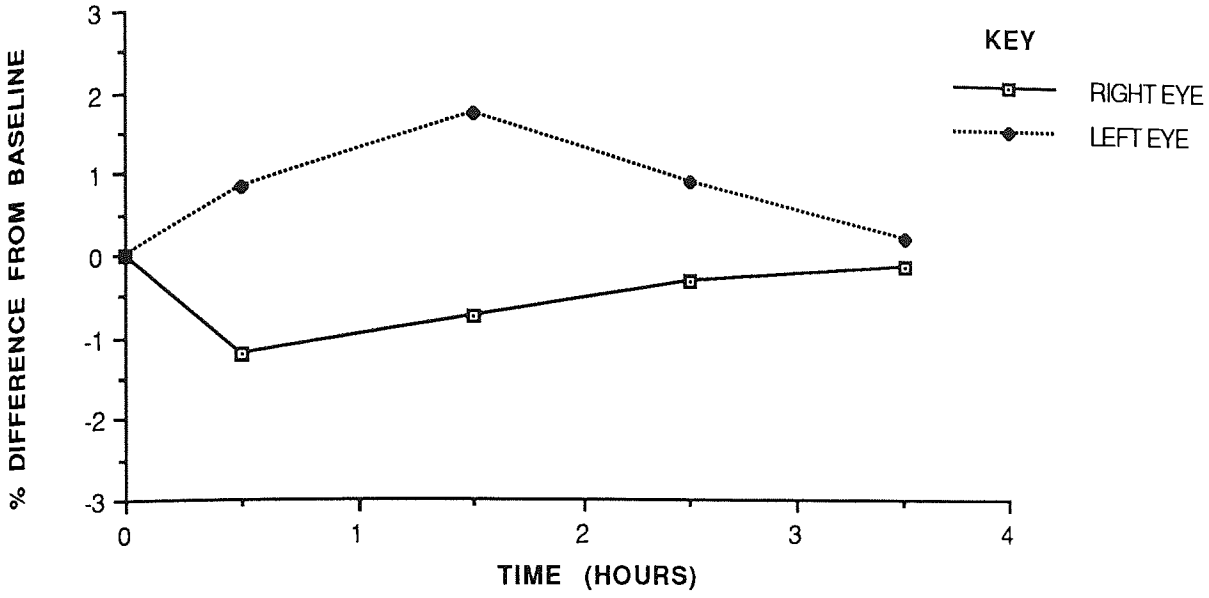
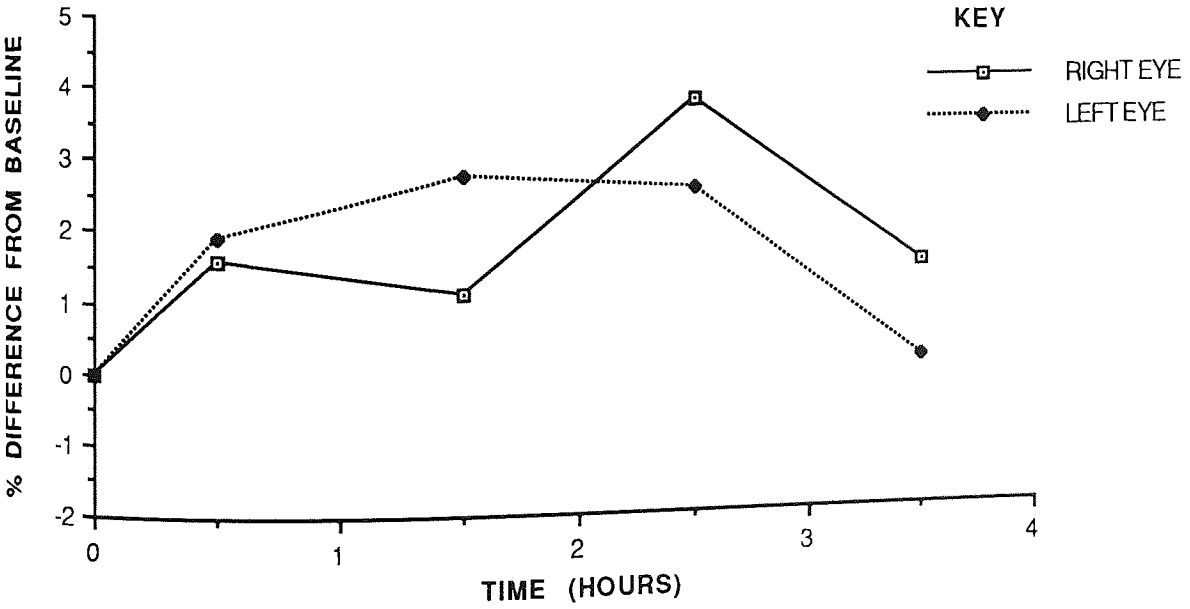


Figure 9.8 Effect of topical administration of scopolamine on the flash P3 latency, in the control eye (solid line) and test eye (broken line). Data from channel O2-C4.



subjects included in the study. Overall, there was no effect of topically administered scopolamine on the latency of flash VEP components.

The effects of scopolamine eye drops on the flash N2-P2, P2-N2 and N3-P3 inter-amplitudes are shown in figs. 9.9, 9.10 and 9.11 respectively. The N2-P2 amplitude was reduced in the test eye post scopolamine dosing, reaching a maximum at 1.5 hours (24.1%) before returning towards baseline at 3.5 hours (reduction of 10.7%). The N2-P2 amplitude in the control eye was also reduced but not in the same a manner, as there was a slight increase in amplitude at 1.5 hours but continued to decrease thereafter. The P2-N3 amplitude was also reduced in the test eye, a manner similar to that of the N2-P2 amplitude, but to a lesser degree. The control eye displayed very little change over 2.5 hours, but was reduced thereafter. In contrast to the changes in the N2-P2 and P2-N3 amplitudes, the N3-P3 amplitude was increased post scopolamine. The effect was greater in the control compared to the test eye. However, despite these changes in the flash VEP amplitudes, statistical analysis revealed no significant difference between the control and test eye. However there was significant inter-individual variation ($P<0.05$) as seen with the flash latency standard deviation values. Therefore, topical administration of scopolamine also had no effect on the flash VEP amplitudes.

Figure 9.9 Effect of topical administration of scopolamine on the flash N2-P2 amplitude, in the control eye (solid line) and test eye (broken line). Data from channel O2-C4.

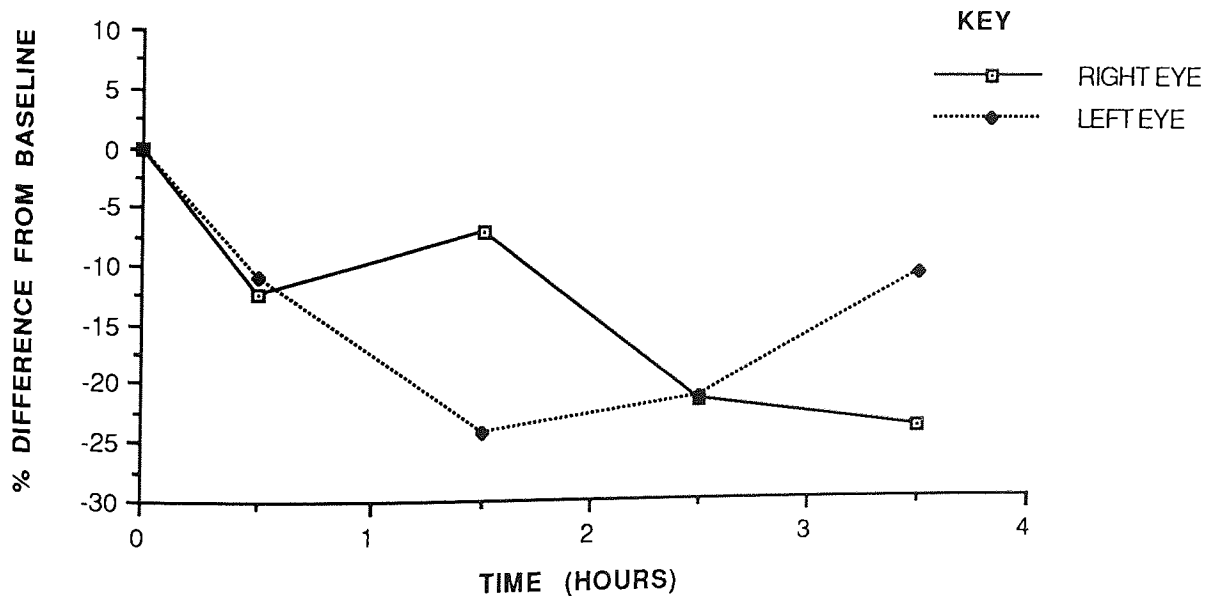


Figure 9.10 Effect of topical administration of scopolamine on the flash P2-N3 amplitude, in the control eye (solid line) and test eye (broken line). Data from channel O2-C4.

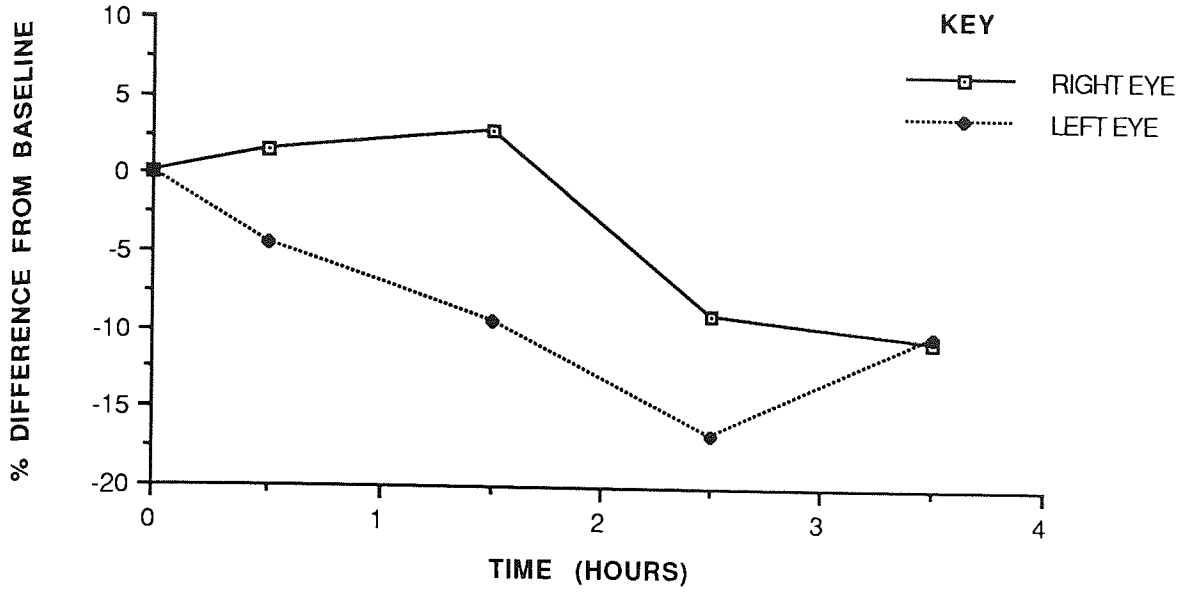
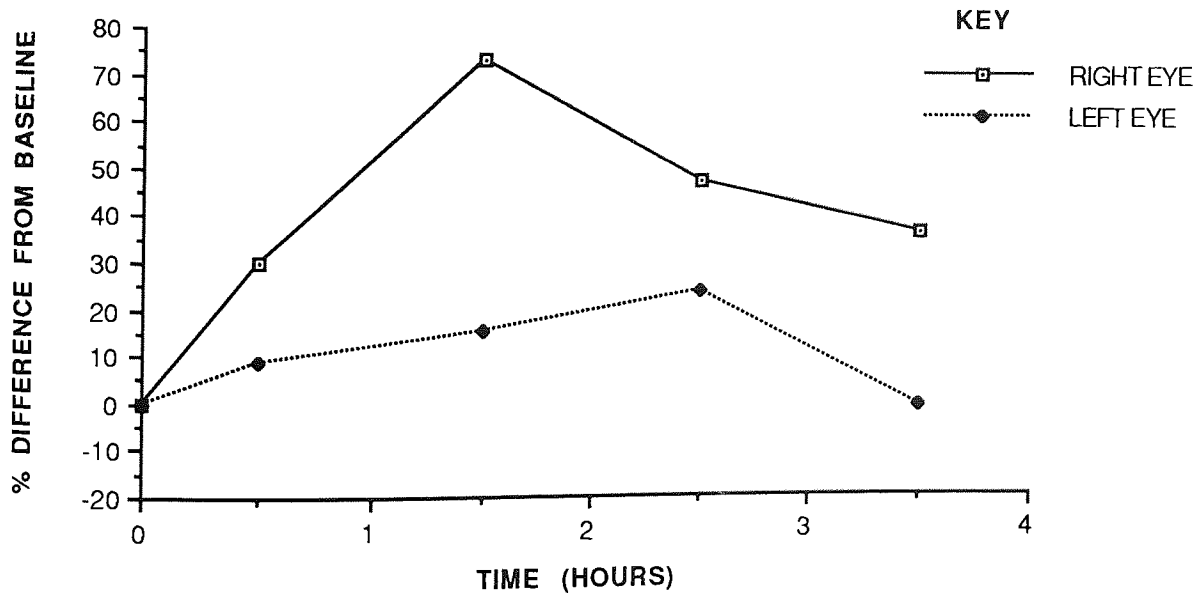


Figure 9.11 Effect of topical administration of scopolamine on the flash N3-P3 amplitude, in the control eye (solid line) and test eye (broken line). Data from channel O2-C4.



9.3.6 Pattern reversal VEP

An example of the response to a pattern reversal stimulus is shown in fig. 9.12. There was a slight intraocular difference in the P100 component but this was not significant and remained consistent throughout the day. The results of the group mean values of the pattern reversal VEP have been presented in table 9.2. Again in order to compare and overcome any intraocular differences, the effects of scopolamine eye-drops over the day, the percentage change from the baseline values were calculated.

The effects of scopolamine eye drops have been illustrated for the pattern reversal N75 (fig. 9.13), P100 (fig. 9.14), N140 (fig. 9.15) and P160 (fig. 9.16). The N75 and P100 latencies displayed similar trends (figs. 9.13 and 9.14 respectively). There was little change in the N75 and P100 latency in the test eye compared to the control eye, the latter showing a slight decrease (<5%) in latency over the day. The N140 and P160 also displayed similar trends (figs. 9.15 and 9.16 respectively). The test and control eye displayed opposing trends, i.e. the test eye showed an increase in latency followed by a decrease in latency towards baseline, whereas the control eye showed a decrease in latency followed by an increase towards baseline. Overall, the control eye displayed similar trends (general decrease) in latencies of the pattern reversal VEP components. But the test eye displayed little change for the early N75 and P100 components and an increase for the later N140 and P160 components. Statistical analysis also revealed no significant difference between the control and test eyes for the latency of the pattern reversal VEP components. There was significant inter-individual variation ($P<0.05$) as with the flash VEP components, due to the small number of subjects included in the study.

The effects of scopolamine eye drops for the pattern reversal N75-P100, P100-N140 and N140-P160 inter-amplitudes have been shown in figs. 9.17, 9.18 and 9.19 respectively. There was a slight increase in the N75-P100 amplitude in the control eye at 0.5 hours followed by a decrease up to 2.5 hours and a return towards baseline by 3.5 hours. But in the test eye, the N75-P100 amplitude displayed a gradual decrease in amplitude throughout the day. The P100-N140 amplitude for the control eye also displayed an increase at 0.5 hours followed by a slight decrease compared to baseline levels for the rest of the day. In contrast, the N140-P160 amplitude displayed similar effects in both the control and test eye. There was an initial increase in the N140-P160 amplitude at 0.5 hours, followed by a decrease up to 2.5 hours and a return towards baseline by 3.5 hours. However there was no statistical difference in the amplitude measurements when comparing the control and test eyes.

Figure 9.12 An example of a pattern reversal VEP as recorded from subject AG. The solid and broken lines represent the right (control) and left (test) eyes respectively. There was little difference in waveform between the eye or runs. Waveform was recorded from channel O2-Fz.

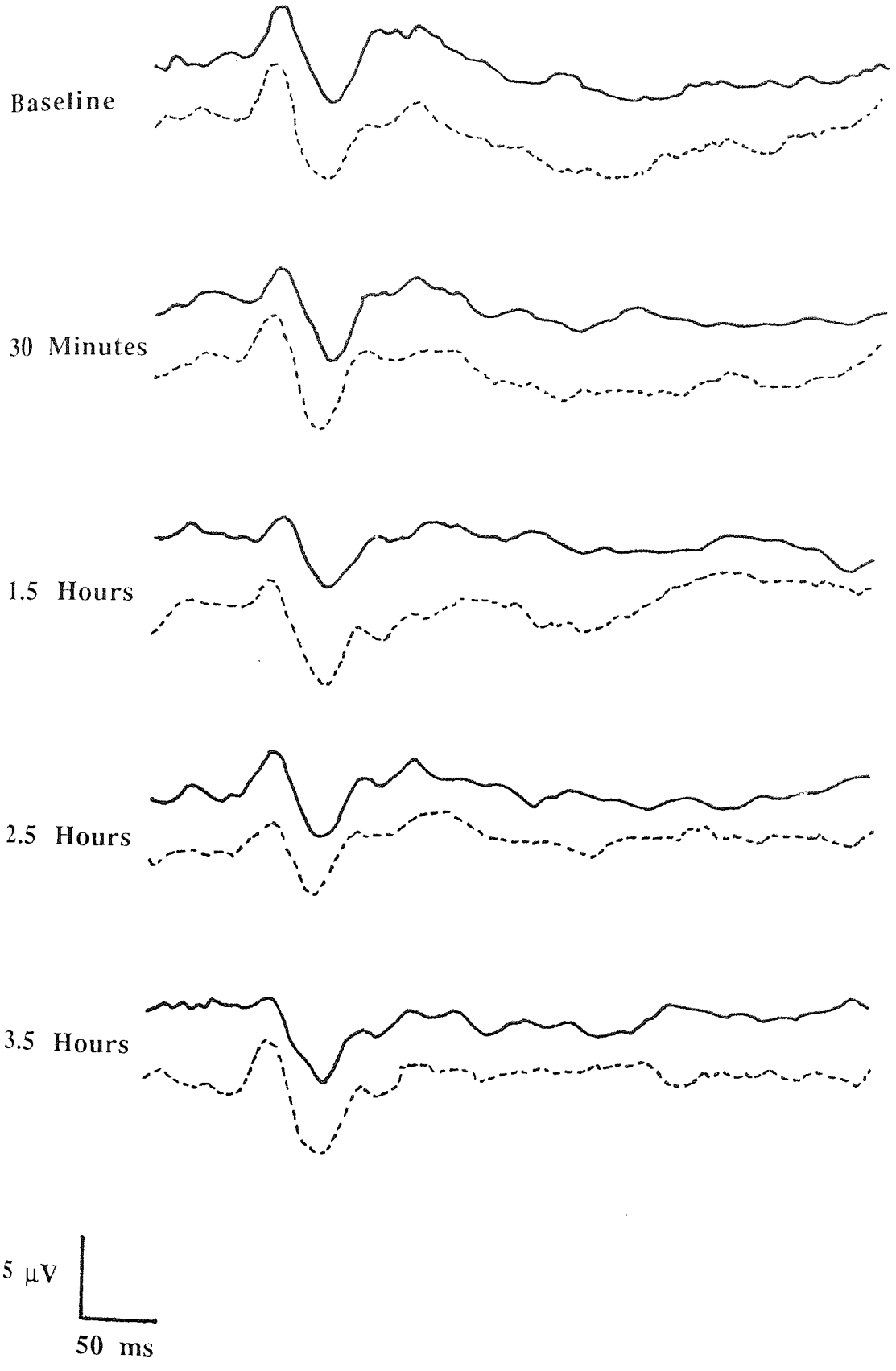


Table 9.2 The group mean values of the pattern reversal VEP latencies in the right (control) and left (test) eye. Data from channel O2-C4.

	BASELINE	30mins	1.5h	2.5h	3.5h
N75 LATENCY (ms)					
RE MEAN	80.33	77.50	77.17	80.00	77.00
SD	3.20	1.97	3.06	4.60	1.79
LE Mean	77.00	77.17	78.00	77.17	76.83
SD	3.74	4.40	2.10	3.71	1.94
P100 LATENCY (ms)					
RE Mean	120.67	116.17	122.83	118.67	122.17
SD	7.06	3.76	11.32	6.28	5.53
LE Mean	115.67	117.17	117.33	117.33	117.33
SD	6.44	8.35	7.87	9.24	6.86
N140 LATENCY (ms)					
RE Mean	178.33	171.83	176.67	180.17	182.67
SD	20.10	18.67	23.70	17.22	16.93
LE Mean	165.83	173.67	174.83	171.17	171.67
SD	14.86	19.71	21.34	15.82	13.49
P160 LATENCY (ms)					
RE Mean	228.50	220.17	230.83	221.33	226.33
SD	31.94	29.44	36.35	23.47	27.46
LE Mean	214.83	235.60	220.80	223.80	219.50
SD	38.12	27.74	25.92	29.42	25.52
N75-P100 AMPLITUDE (μV)					
RE Mean	6.82	8.02	5.86	5.85	6.70
SD	2.41	5.67	2.27	1.80	2.32
LE Mean	8.04	6.98	6.20	5.83	5.98
SD	2.83	2.90	2.88	1.49	2.98
P100-N140 AMPLITUDE (μV)					
RE Mean	6.71	7.46	6.84	6.59	6.38
SD	2.80	3.41	3.08	2.41	3.20
LE Mean	7.35	7.45	7.39	6.28	6.21
SD	3.17	3.01	2.61	1.98	3.35
N140-P160 AMPLITUDE (μV)					
RE Mean	2.36	3.49	2.10	1.92	2.34
SD	1.49	2.32	1.01	1.16	1.01
LE Mean	1.94	2.72	2.04	2.63	1.86
SD	1.44	2.27	1.04	1.02	1.12

KEY: RE -Right eye; LE -Left eye; SD -standard deviation.

Figure 9.13 Effect of topical administration of scopolamine on the pattern reversal N75 latency, in the control eye (solid line) and test eye (broken line). Data from channel O2-Fz.

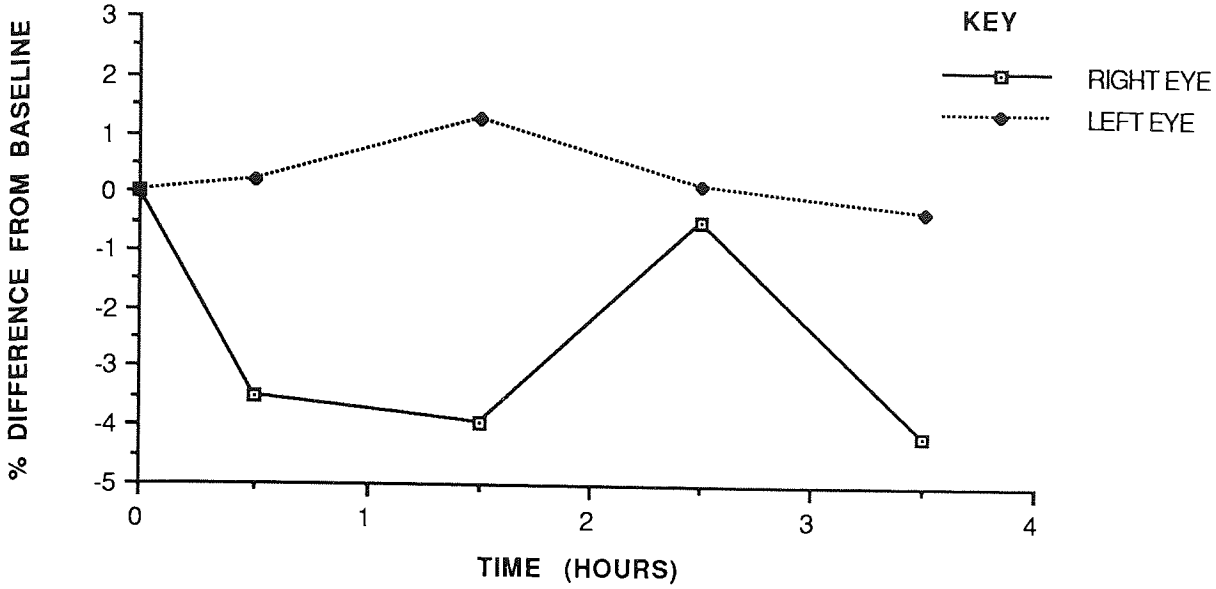


Figure 9.14 Effect of topical administration of scopolamine on the pattern reversal P100 latency, in the control eye (solid line) and test eye (broken line). Data from channel O2-Fz.

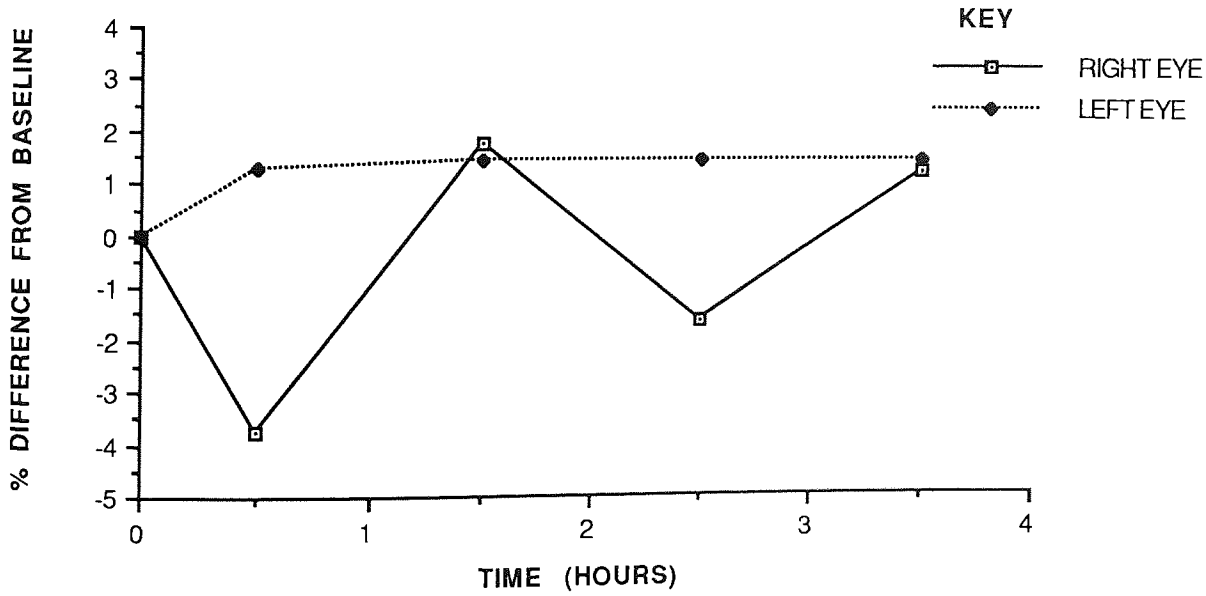


Figure 9.15 Effect of topical administration of scopolamine on the pattern reversal N140 latency, in the control eye (solid line) and test eye (broken line). Data from channel O2-Fz.

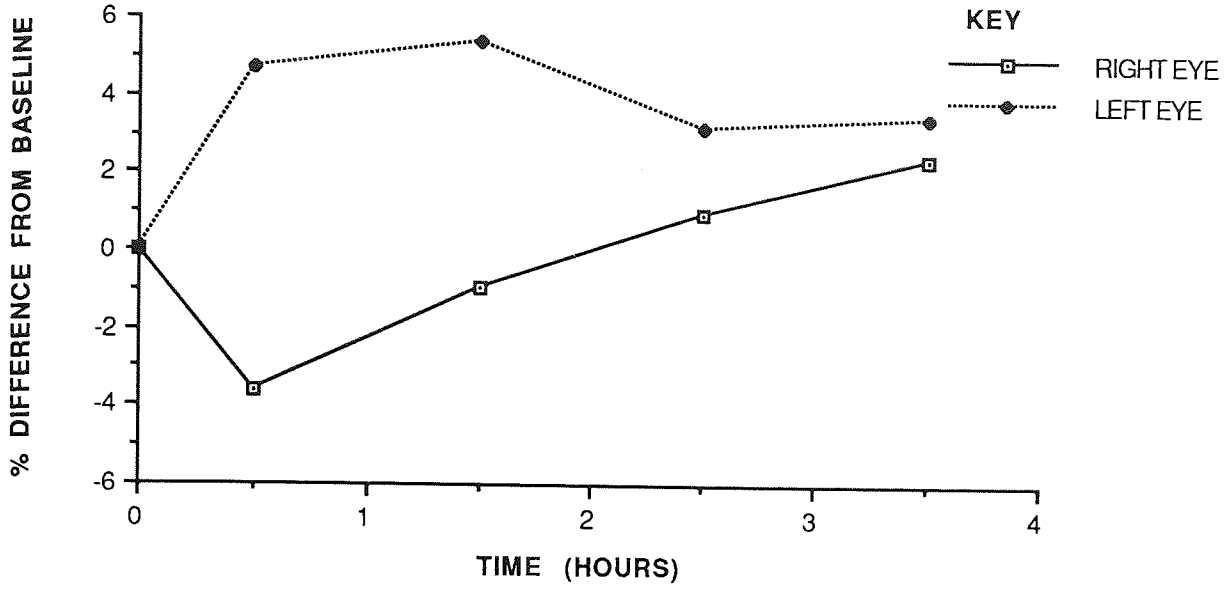


Figure 9.16 Effect of topical administration of scopolamine on the pattern reversal P160 latency, in the control eye (solid line) and test eye (broken line). Data from channel O2-Fz.

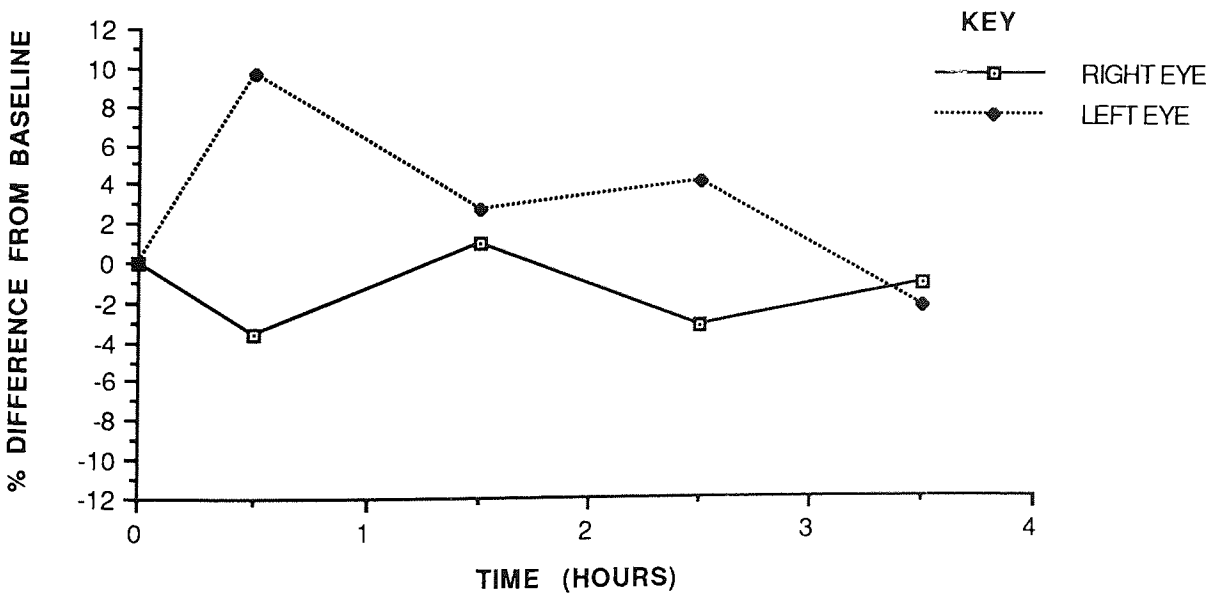


Figure 9.17 Effect of topical administration of scopolamine on the pattern reversal N75-P100 amplitude, in the control eye (solid line) and test eye (broken line). Data from channel O2-Fz.

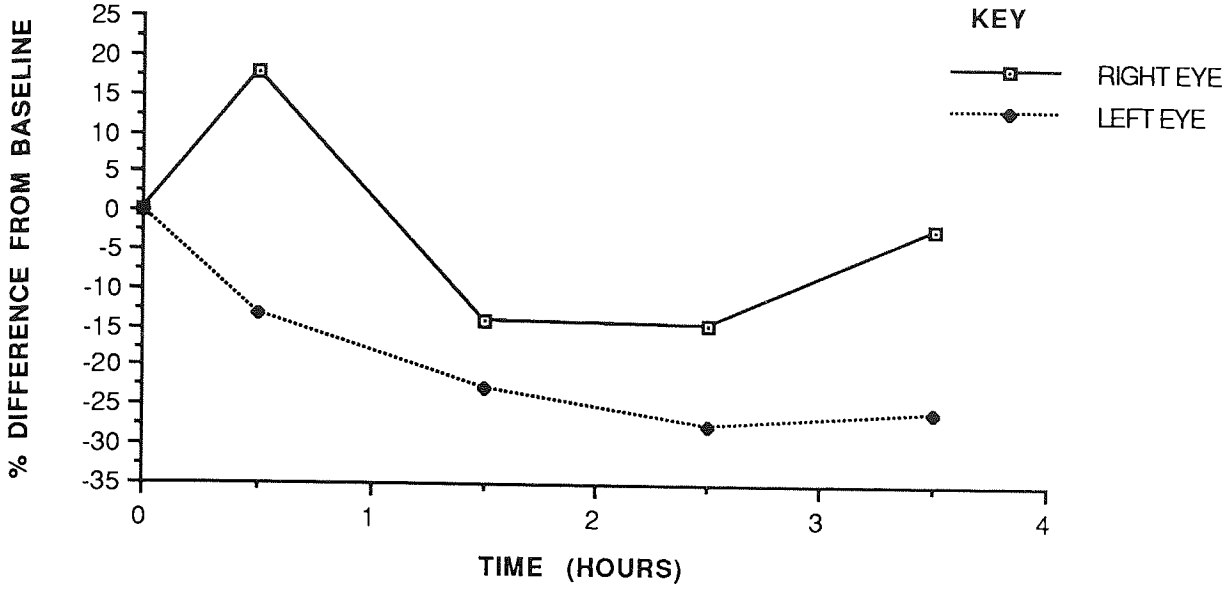


Figure 9.18 Effect of topical administration of scopolamine on the pattern reversal P100-N140 amplitude, in the control eye (solid line) and test eye (broken line). Data from channel O2-Fz.

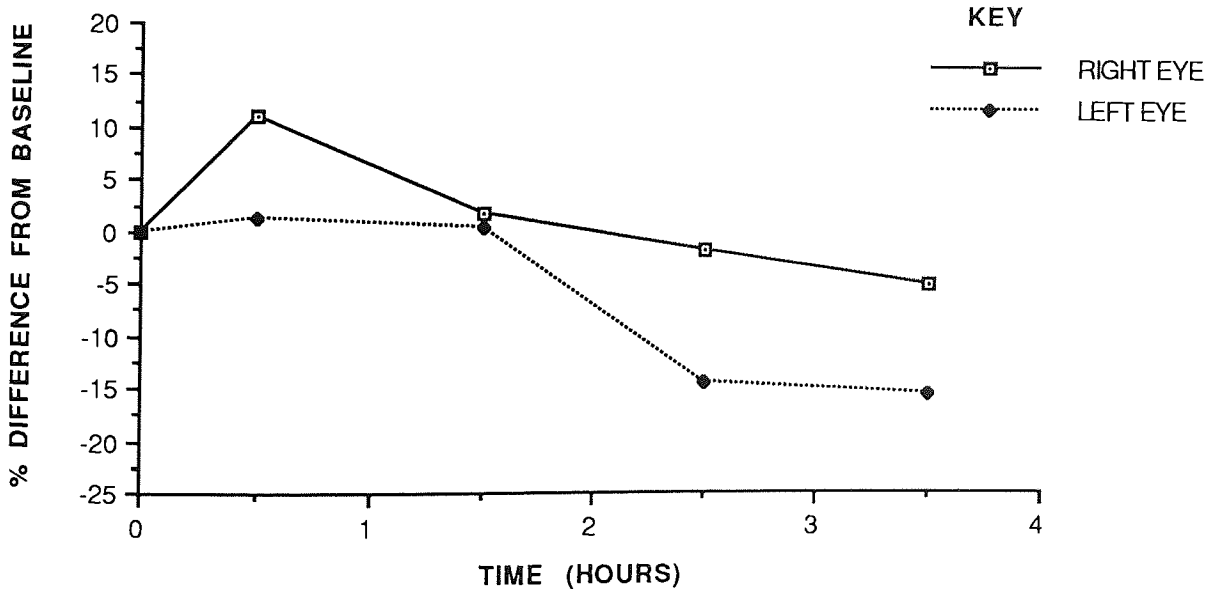
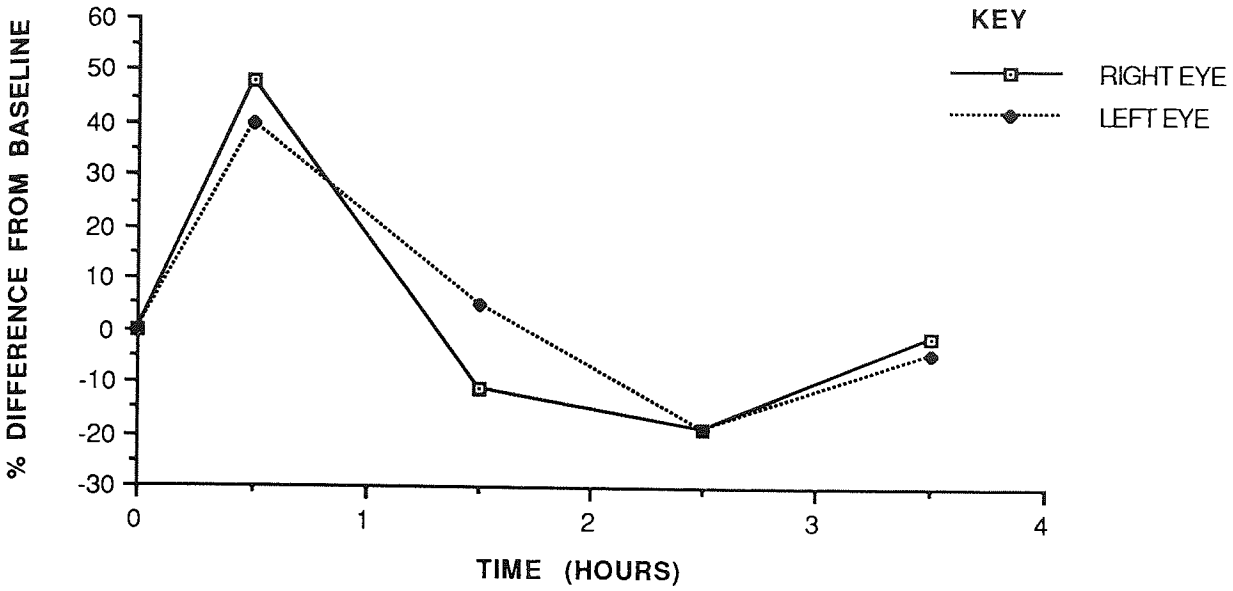


Figure 9.19 Effect of topical administration of scopolamine on the pattern reversal N140-P160 amplitude, in the control eye (solid line) and test eye (broken line). Data from channel O2-Fz.



Overall the magnitude of the percentage change in the amplitude values were greater than for the latency. This is because the change in the measurements are expressed in percentage terms. Since the amplitude values were smaller than the latency values, any difference in the amplitude were exaggerated. For example, an increase in the N140-P160 amplitude in the left eye of only 0.1 μV at 1.5 hours equated to a 40.2% increase due to the small amplitude values, hence illustrating the exaggeration due to conversion of the data.

The amplitudes of the flash VEP and pattern reversal VEP displayed similar changes. The amplitudes of the flash N2-P2, P2-N3, pattern reversal N75-P100 and P100-N140 all displayed a reduction in amplitude in the test eye post-dosing. These amplitude values in the control eye also displayed a reduction but was of lesser magnitude when compared to the test eye. The flash N3-P3 displayed a small increase in amplitude post-dosing which was of greater magnitude in the control eye compared to the test eye. The pattern reversal N140-P160 displayed very similar effects in both the control and test eye of an initial increase followed by a decrease in amplitude over the day. Hence the P2 and P100 amplitudes were generally reduced in the test eye to a greater extent than in the control eye, but the differences between the eyes were not statistically significant. Therefore, there were no significant effects of scopolamine eye-drops on the flash and pattern reversal VEPs.

9.4 Discussion

The results showed that there was no significant effect of topically administered scopolamine on the VEPs examined, although mydriasis and cyclopegia was achieved. The lack of an effect on the flash VEP was unexpected, since it was thought that the flash VEP would probably be transmitted by retinal M-ganglion cells. A possible explanation could be that the dose of 0.125% scopolamine used in the present study was insufficient. However, Morrison and Reilly (1989) found that a dose of 0.025% was enough to cause a transient deleterious effect on the contrast sensitivity function to a grating pattern of low but not high spatial frequencies. However, the dose of scopolamine used in the present study was low, it was much higher than that of Morrison and Reilly (1989). In addition, the use of scopolamine eye drops can lead to toxic symptoms in some patients and thus low doses (below 0.5%) have been recommended (Freund and Merin 1970).

A second explanation for the lack of an effect on the VEPs may be that scopolamine failed to reach the retina and thus could not affect the response of the ganglion cells to the VEP stimuli. But Morrison and Reilly (1989) concluded their findings were due to the a direct action of scopolamine upon retinal ganglion cells. In agreement, Alpern and Jampel (1959) also suggested that the deleterious effect of physostigmine eye drops on critical fusion frequency was due to a direct action on the retina. However, these suggestions are speculative and there is no concrete evidence that the actions of scopolamine are due to a direct action on the retina. But, since the actions of scopolamine (Morrison and Reilly 1989) and physostigmine (Alpern and Jampel 1959) were unrelated to their effects on the iris and ciliary muscles, the most probable explanation has to be that these drugs acted on the retina through transcorneal absorption. Systemic absorption of scopolamine from the nasal mucosa after drainage through the nasolacrimal canal (Shell 1982) can be ruled out in the present study as there was no effect on scopolamine on the iris and ciliary muscles of the control eye.

Finally, scopolamine may have reached the retina, but failed to have any deleterious effect on the response of the ganglion cells. The levels of scopolamine at the retina may not have been sufficient to cause an effect on the VEPs. To test this, a study would need to be carried out examining the effects of scopolamine on both the VEPs (as in the present study) and on the contrast sensitivity on a range of low and high spatial frequency gratings similarly to Morrison and Reilly (1989).

Assuming that scopolamine reached the retina, the lack of any deleterious effects on the flash and pattern reversal VEPs suggests that the action of scopolamine occurred beyond the level of the retina. In addition, the retinal pathology reported in patients

with AD (Hinton et al. 1986; Sadun and Bassi 1990) do not account for the delay in flash P2 observed in this disease. Therefore in conclusion, delay in the flash P2 component occurring in AD and that due to systemic administration of scopolamine is likely to be due to cholinergic deficits occurring beyond the retinal level, i.e. either at the level of the dLGN, superior colliculus, or visual cortices.

CHAPTER 10

Discussion

10.1 Summary of results

The following is a summary of the major findings of the study :-

1. Recording VEPs to the flash and pattern reversal stimuli hourly over daylight hours, produced no statistically significant change in the latency or amplitudes of the major VEP components. The study found fluctuations of 5.0%, 2.2%, 4.6% and 6.5% in the flash N2, P2 and N3 latencies respectively and 36.5%, 23.8% and 48.6% in the N2-P2, P2-N3 and N3-P3 peak to peak amplitudes respectively. Similarly, the pattern reversal (to 56' check size), there was a fluctuation of 4.5%, 2.7%, 4.7% and 13.3% in the N75, P100, N140 and P160 latencies respectively and 27.2%, 28.4% and 33.1% in the N75-P100, P100-N140 and N140-P160 peak to peak amplitudes respectively. The pattern reversal VEP to a smaller check size (27') resulted in fluctuations of 3.5%, 2.1%, 7.0% and 13.5% in the N75, P100, N140 and P160 latencies respectively and 31.9%, 38.1% and 43.9% in the N75-P100, P100-N140 and N140-P160 amplitudes respectively. These values represent the natural variation that could be expected when recording VEPs during daylight hours.

2. The muscarinic agonist SDZ 210-086 had no effect on the latencies of the flash and pattern reversal VEPs. However, the amplitudes of these VEPs were reduced by SDZ 210-086. The flash N2-P2 amplitude was reduced at both doses of SDZ 210-086 (0.5 and 1.0 mg/day), whereas the pattern reversal N75-P100 amplitude was reduced only at the higher dose (1.0 mg/day). Further examination of SDZ 210-086 at higher doses, which were nearer therapeutic levels was abandoned due to an indication of possible hepatotoxicity.

3. Administration of the muscarinic antagonist scopolamine resulted in a significant delay of the flash P2 latency ($P<0.05$), but no effect on the pattern reversal P100 latency, confirming the findings of Bajalan et al. (1986). The flash N2-P2 amplitude displayed an increase, an effect opposite to that observed with SDZ 210-086.

The pattern reversal VEP displayed no significant change in latency post scopolamine dosing. However, the pattern reversal N75-P100 amplitude was significantly increased post dosing ($P<0.05$), an effect opposite to that observed with SDZ 210-086.

4. The combination of scopolamine with the acetylcholinesterase inhibitor SDZ ENA 713, revealed that the flash P2 was slightly delayed but failed to reach statistical significance as observed with administration of scopolamine alone. The flash N2-P2 amplitude showed little change, although the later P2-N3 and N3-P3 amplitudes were reduced. There was no significant effect of the combination treatment on the pattern reversal VEP. Therefore, SDZ ENA 713 probably reversed the scopolamine induced changes to a limited extent.

5. Topical application of scopolamine did not produce any statistically significant changes in the flash or pattern reversal VEPs. Therefore, the cholinergic influence on the VEP is likely to be at post retinal levels, i.e. at the cortical rather than peripheral level.

10.2 Discussion of results

The studies described in this thesis were carried out with two main objectives in mind. Based on the findings reported above, these can now be answered.

Objective 1: To investigate the effectiveness of the flash and pattern reversal VEPs in clinical studies investigating new cholinergic agents developed for the treatment of AD.

Clinical trials of cholinergic agents have reported variable degrees of success (chapter 4). The only sign of success in treating AD by increasing cholinergic transmission has been with the use of AChEi. However, there are still a number of problems associated with these agents, such as diagnostic inaccuracy (Katzman et al. 1988), a narrow and variable therapeutic window (Bartus et al. 1982; Rosenberg et al. 1983) and of non-invasive indicators of central cholinergic agonist efficacy (O'Mahony et al. 1991). Since the flash P2 component is sensitive to the central cholinergic manipulation (Bajalan et al. 1986; Gilles et al. 1989), it may be of value in reducing some of these problems. In addition, the findings of the present study also suggest there is clear evidence for the use of the flash and pattern reversal VEPs as a monitor in clinical trials.

A useful property of using the flash in conjunction with the pattern reversal VEP, is the selective and reversible delay of the flash P2, but not of the pattern reversal P100 component when using anticholinergic agents such as scopolamine in young healthy subjects (Bajalan et al. 1986; Gilles et al. 1989; present study). Scopolamine has already been used as a model in humans for some of the memory and cognitive deficits associated in AD (Drachman 1977; Sitaram et al. 1978; Bartus et al. 1982). Hence

most clinical trials examine the ability of the drug under investigation to reverse these memory and cognitive deficits observed with scopolamine (Drachman 1977; Bartus et al. 1982; Coyle et al. 1983). If a potential cholinergic agent developed for AD could reverse the deficits in the flash VEP together with deficits in memory and cognition caused by anticholinergic agents such as scopolamine, this would provide both a physiological as well as a subjective evidence for the potential therapeutic advantages for that agent in the treatment of AD.

The present study has provided a basis for investigating the use of VEPs in clinical trials. For example, the study described in chapter 6, was carried out in Edinburgh, all the equipment necessary for studying the VEPs were provided and transported by Aston University in Birmingham. Hence the instrumentation required for recording a VEP is highly mobile and fairly easy to set up in most environments e.g. hospitals. In addition, the time taken to record the flash and pattern reversal VEPs is around 5-10 minutes, hence they could be included in clinical trials, even those incorporating a battery of memory and cognitive tests. Overall, it the opinion of the author that the flash and pattern reversal VEPs would be a complementary addition in testing the therapeutic efficacy of cholinergic agents undergoing clinical trials.

Objective 2: To examine further the nature of the generators of the flash P2 and pattern reversal P100 components in relation to cholinergic function.

The results of the present study confirm the presence of a cholinergic influence on the VEP. Administration of the anticholinergic agent scopolamine to young healthy volunteers, resulted in the selective delay of the flash P2 component without affecting the pattern reversal P100 latency (Bajalan et al. 1986; Gilles et al. 1989; present study). These effects mimic those observed in patients suffering from AD who show a delay in the flash P2 latency co-existing with a normal pattern reversal P100 latency (Doggett et al. 1981; Harding et al. 1981, 1984, 1985; Wright et al. 1984a, 1986; Danesi et al. 1985; Engedal et al. 1987; Philpot et al. 1989; Coburn et al. 1991; Sloan and Fenton 1992).

The question thus arises, why is there a selective delay of the flash P2 but not of the pattern reversal P100 component observed in patients with AD, which is also mimicked by the systemic administration of scopolamine in healthy volunteers? In order to answer this, the possible generators of the flash P2 and pattern reversal P100 components need to be considered. The primary response to the flash stimulus (occurring prior to 100 ms after the onset of the stimulus), is thought to be generated

in the striate cortex (Brodman's area 17), whereas the secondary response (occurring after 100 ms and includes the P2 component) is thought to be generated in extrastriate cortices (areas 18 and 19) (Ciganék 1961; Vaughan 1966; Spekreijse et al. 1977; Regan 1989). The pattern reversal P100 component is thought to be generated in the striate cortex (Barrett et al. 1976; Leuders et al. 1980; Halliday 1982; Edwards and Drasdo 1987).

In AD, distribution of pathology (plaques and tangles) is greater in association cortices with relative sparing of the primary sensory areas (Brun and Gustafson 1976; Sim 1979; Lewis et al. 1987). Lewis et al. (1987) found low quantities of NFTs in Brodman's area 17 which increased 20 fold in the adjacent association area 18 and doubled further in association area 20. In addition, the visual association areas show a reduction in CAT activity in patients with AD (Procter et al. 1988) and reduction in ACh binding (Whitehouse et al. 1986). Therefore, pathology in the visual association areas (possible generator site of the flash P2) seems consistent with the selective delay in the flash P2 component. However, the flash P2 delay can be present in the absence of cortical atrophy, as measured by CT scans (Cosi et al. 1982; Harding et al 1985). Cosi et al. (1982) therefore suggested other metabolic, biochemical and circulatory factors must also be involved. There are also reports of patients exhibiting a clinical picture of AD in the absence of the pathological changes (Earnest et al. 1979; Hagnell et al. 1983), whereas others have presented pathological changes in the absence of dementia (Newton 1948; Jacoby and Levy 1980). The reduction in ACh markers is not confined to the extrastriate areas, as CAT activity has also been shown to be reduced in Brodman's area 17 (possible generator of the pattern reversal P100 component) as well as area 19 (Davies 1979; Rossor et al. 1982). Hence pathology of extrastriate areas alone cannot account for the selective flash P2 delay.

The visual signal can be transmitted to the visual cortex via either the geniculate pathway (section 2.1.3) or the non-geniculate pathway (section 2.1.4). Approximately 80% of the signal from retinal ganglion cells is transmitted via the geniculate pathway (Davson 1990). Hence it is reasonable to assume that information about both the flash and pattern reversal VEPs are processed via the geniculate pathway. The tectal pathway represents an alternative route whereby retinal signals reach the visual cortex (Davson 1990). In monkey, up to 10% of retinal ganglion cells are thought to project to the superior colliculus which in turn projects to the visual association areas (Perry and Cowey 1984). The superior colliculus has been reported to respond to unstructured spots of light and not to stimuli of any spatial detail (Humphrey 1968; Cynader and Berman 1972; Schiller et al. 1979; Wurtz and Albano 1980). Therefore, the tectal association pathway could also transmit information about

the flash VEP, but not the pattern reversal VEP. Based upon these and VEP findings in patients with optic neuritis and AD, Harding and Wright (1986) and Wright et al. (1987) proposed a model whereby the flash P2 is transmitted along the tectal association pathway which passes through the superior colliculus and pulvinar to areas 18 and 19 of the visual cortex and the pattern reversal P100 component is transmitted along the geniculostriate pathway which passes through the LGN to area 17 of the visual cortex. Evidence suggests that the neurotransmission in the geniculostriate pathway is non-cholinergic (Spehlmann 1971; Spehlmann et al. 1971) whereas the tectal association pathway is cholinergic (Shute and Lewis 1967). Hence reduction of ACh levels in AD and scopolamine administration is likely to affect the tectal association pathway which is also likely to be responsible for the flash P2, whereas there would be no affect on the geniculostriate pathway which is likely to be responsible for the pattern reversal P100 component.

However the question arises, does the cholinergic influence on the flash P2 arise in the cortex itself or in other areas of the visual pathway afferent to the cortex, or both? In chapter 9, topical administration of scopolamine did not affect the flash or pattern reversal VEPs. Therefore, assuming that scopolamine had reached the retinal ganglion cells in sufficient concentration, the cholinergic influence on the flash P2 occurs beyond the level of the retina. Hence the cholinergic influence on the flash P2 occurs either at the level of the dLGN, superior colliculus or the visual cortex itself.

There is evidence that both the LGN and striate cortex may be under a cholinergic influence. In the cat, laminae A and A1 have shown strong AChE activity (Dean et al. 1982). These layers coincide with the site of neurophysiologically recorded X-cells. In the visual cortex, presence of AChE activity was reported in layer IV of areas 17 and 18. Dean et al (1982) thus suggested that the corticothalamic pathway may provide the source of the geniculate AChE activity. In the monkey, AChE activity was observed in both the magnocellular and parvocellular layers, with greatest activity in the magnocellular layers. But the cat X-cells, correlate with the P-cell pathway in humans which in turn has been proposed to be responsible for transmission of the pattern reversal VEP. Hence based on studies in the cat, this would suggest that the pattern reversal VEP was under cholinergic influence. However, the findings from the monkey suggest that the P-cell and hence the pattern reversal VEP was under less influence of ACh, whereas the M-cell and hence the flash VEP, would be influenced by ACh.

Fitzpatrick and Diamond (1979) demonstrated that the parvocellular but not the magnocellular layers in the monkey dLGN displayed AChE activity. In addition,

these workers found that the origin of the cholinergic activity was not from the retinal ganglion cells. Hence this cholinergic influence was proposed to arise either from the striate cortex where these geniculate fibres terminated and which also displayed AChE positive cells, or alternatively, the cholinergic fibres formed part of the dorsal tegmental pathway which have shown to innervate the tectum, pretectum and portions of the thalamus including the intralaminar nucleus and lateral and medial geniculate bodies (Shute and Lewis 1963 and 1967). This suggests that the geniculostriate as well as the tectal association pathway can influenced by ACh. In addition, Graybiel and Ragsdale (1982) reported staining of the parvocellular layers with the pseudocholinesterase butyrylcholinesterase (BuChE) which hydrolyses higher esters but can hydrolyse ACh. The striate cortex revealed detailed patterning of BuChE most densely in layer 4Calpha and a thin dark lamina at the border of layer V. In contrast, AChE activity showed activity confined to the magnocellular layers of the LGN. The staining in the striate cortex was most pronounced in layers 4A and both 4Calpha and 4Cbeta, and strong in layers I and VI. The staining of BuChE in magno-recipient layers of the striate cortex suggested that intrinsic systems of area 17, extrastriate systems or the pulvinar may be the source of its activity. Hess and Rockland (1983) also reported BuChE activity in the parvocellular layers of the monkey dLGN, but found the activity was weak in comparison to the AChE activity in the magnocellular layers.

Overall, there is conflicting evidence of AChE activity in the monkey LGN, with the New World Owl monkey (*Aotus trivirgatus*) and the prosimian bushbaby (*Galago senegalenesis*) showing dense staining for AChE in the parvocellular layers (Fitzpatrick and Diamond 1980), whereas, in Old World macaque monkey (*Macaca mulatta, Macaca fascicularis*) the magnocellular layers are more densely stained (Dean et al. 1982; Graybiel and Ragsdale 1982). Hess and Rockland (1983) suggested that the owl monkey and bushbaby are nocturnal while the squirrel monkey and macaque are diurnal primates, therefore the distribution of the AChE may reflect the adaptation to the different visual habitats. However, since these animals are closely related, and display great inter-species variation, the results are difficult to correlate with the human visual system.

Aguglia et al. (1991) found a flash P2 delay in patients with Creutzfeldt-Jakob's disease (CJD) similar to that in patients with AD. Post-mortem examination of the pathology occurring in the visual pathway of these CJD patients revealed selective degeneration of the superior colliculus, pulvinar, layers II-III, V and VI of area 17 and scattered irregular spongiosis in areas 18 and 19, whereas the dLGN, layer IV of area 17 and Gennari's strip (which constitutes horizontal axon collaterals of spiny stellate

neurones lying in layer IV) were relatively spared. Therefore, the preservation of the geniculostriate pathway would be in agreement with preservation of the early flash (prior to P2) (Harding et al. 1981; Wright et al. 1984; Danesi et al. 1985) and pattern reversal (up to P100) VEP components. The lack of subcortical input (from the superior colliculus and pulvinar) and cortical (from layers II-III of area 17) to the visual association cortex and the degeneration of these areas could account for the flash P2 delay.

Therefore, it seems that there are three possibilities to explain the flash P2 delay seen in patients with AD and healthy subjects injected with scopolamine: Firstly, the deficit in the cholinergic input from the tectal pathway (superior colliculus and pulvinar) may results in a delay of the afferent input to the visual cortex. Secondly the deficit in the magnocellular region of the geniculostriate pathway may also result in a delay of the afferent signal to the visual cortex. Thirdly, intrinsic cortico-cortical connections which may be under cholinergic influences may cause a delay in the flash P2. A regional and laminar distribution of tangles implied deterioration of cortico-cortical projections (Lewis et al. 1987). There is evidence of small intrinsic bipolar cholinergic interneurones that exist in the cortex, providing the connections between these areas (Bradford 1986).

In addition, the proposed model of the flash VEP being processed by the tectal pathway and the pattern reversal VEP by the geniculostriate pathway (Harding and Wright 1986) may be modified. The optic nerve signal from the retina is divided such that approximately 80% of fibres go to the dLGN (section 2.1.3) and 10-20% to extrageniculate pathways (section 2.1.4) (Davson 1990). Approximately 10% of the retinal ganglion population have been estimated to project to the magnocellular layers of the dLGN, whereas about 80% project to the parvocellular layers (Perry et al. 1984). The remaining 10% (known as P gamma and P epsilon cells) project to the superior colliculus and pretectum (Perry and Cowey 1984; Leventhal et al. 1981). Therefore, the flash VEP may be processed by both the tectal pathway to the extrastriate cortex and the geniculo-magnocellular pathway to the striate cortex. This would then take into account the presence of a cholinergic influence at the level of the dLGN and layer IV of the striate cortex. The pattern reversal VEP would be processed via the geniculo-parvocellular pathway to the striate cortex. Since there is less evidence of a cholinergic influence on this latter pathway, this would be in agreement with the normal P100 latency occurring patients with AD and healthy volunteers injected with scopolamine.

An important consideration of this model is that 80% of retinal ganglion cells seem to be responsible for generation of the pattern reversal P100 component, but only 20% seem to be responsible for the flash P2. However, the the flash VEP is reported to be of foveal origin (Rietveld et al. 1965; Copenhaver and Perry 1964). In addition, the foveal projection of retinal M-cells to the dLGN is weaker than that of the P-cells, with almost 35 times more parvocellular neurones (Connolly and Van Essen 1984). Therefore there may be an overlap of both the M- and P-cell transmission of the flash VEP. This would be consistent with the findings of AChE activity in the parvocellular layers of the dLGN of the cat (Dean et al. 1982) and the monkey (Fitzpatrick and Diamond 1979). Alternatively, although there are more P-cells in the foveal region of the retina, the M-cells are larger than P-cells (DeMonasterio 1978), hence based on the area covered by an individual cell, the M-cells would transmit more than just 20% of signal from the retina.

But what about the selective delay of the flash P2 in AD and with scopolamine? Studies show that ACh is not involved in either the retinogeniculate or the corticogeniculate transmission (Miller et al. 1969; Bigl and Schober 1977). However there is considerable cholinergic input to the dLGN from the brainstem (Woolf and Butcher 1986; De Lima and Singer 1987; Hallanger et al. 1987; section 2.4.2). In addition, there is a neuromodulatory influence of ACh in the visual cortex (Sillito and Kamp 1983). The basal forebrain (septum, vertical and horizontal limb of the diagonal band of Broca, nucleus basalis of Meynert and substantia innominata) provide a cholinergic input to the striate cortex (Mesulam 1990; Singer 1990), which in turn is reduced in AD (Whitehouse et al. 1981). Therefore the pathology of the basal forebrain in AD and the anticholinergic action of scopolamine in healthy volunteers is the most likely to affect the M-cells of the dLGN and the magnorecipient layers of the striate visual cortex, in addition to superior colliculus and its extrastriate projection.

10.3 Future studies

The present study has shown that the flash and pattern reversal VEP are useful tools in clinical trials investigating the potential effects of cholinergic drugs developed for AD. However, the clinical trials reported in this thesis were prematurely discontinued due to potentially dangerous side-effects. Therefore a full investigation of the effects of the drugs on the VEPs in comparison to psychometric testing could not be evaluated. Hence a suggestion for a possible future study would be to compare the efficacy of psychometric tests and the flash and pattern reversal VEPs in such a clinical trial.

The effects of scopolamine on the flash and pattern reversal VEPs have been observed with doses ranging from 0.25-0.6 mg (Bajalan et al. 1986; Gilles et al. 1989; Ray et al. 1991; Sannita et al. 1992; Sloan et al. 1992; study presented in chapter 8). Therefore a study investigating the effects of scopolamine over a range of doses, would provide further information about the anticholinergic effects on the flash and pattern reversal VEPs. This type of study would evaluate the least dose of scopolamine required to achieve a significant flash P2 delay with no effect on the pattern reversal P100, as well evaluating the optimum dose for achieving such an effect.

Observations reported by Aguglia et al. (1991) on the post-mortem examination of the pathology occurring in the visual pathway of patients with Creutzfeldt-Jakob's disease, led some way to explain the selective affect on the flash VEP. However, the observations were limited to 4 CJD patients. Therefore, similar studies are required in patients with AD who display a delay in the flash P2 latency whilst sparing the pattern reversal P100 latency. Such a study would provide an anatomical basis for the selective VEP effects observed. Such studies would also need to take into account the ethical issues involved. In addition, post-mortem examinations would involve patients who have reached extremes of the disease and hence may present a complicated picture.

The arguments discussed above (section 10.2) proposed that the flash VEP is transmitted via the M-cell pathway and the pattern reversal VEP via the P-cell pathway. This is a very simplified model of the parallel processing of visual signals, crudely based on the spatial properties of the VEP stimuli. The M- and P-cell pathways are dependent upon other factors of a visual stimuli such as contrast sensitivity, chromatic sensitivity and temporal sensitivity (section 2.3). In addition, these pathways are thought to overlap, as well as be subdivided further into at least the Mx, My, Pi and Pii pathways (table 2.2). Hence another future study would be to develop stimuli to the VEP which preferentially discriminated between these pathways. A possible stimulus for the My system could be an achromatic pattern reversal type stimulus of low spatial frequency, low contrast and modulated at a high temporal frequency. The Mx could be stimulated by a pattern similar to that of the My but which was modulated at low temporal rates. The Pi and Pii could be stimulated by a slow pattern onset type stimuli of high spatial frequency, high contrast and slow modulation, e.g. a pattern taking 300 ms to reach peak onset and offset. The Pi and Pii would be differentiated by using chromatic and achromatic stimuli respectively, e.g. equiluminant red-green checkerboard. Hence the effect of various disease processes such as AD could then be evaluated using such stimuli. In addition, the

neurotransmitter systems involved in the processing of these stimuli could also be investigated further.

However the pedictability of any such discrete relatively weak stimuli in a patient population such as AD, has to be considered further. The ability to to focus attention for a long time in patients with AD is reduced (Huisman et al. 1987) and thus find it difficult to cooperate. One of the advantages of flash and pattern reversal VEPs is that they require relatively little cooperation. Such a study of stimuli subdividing the M- and P-cell pathways, therefore would have to carried out on controls receiving scopolamine in order to identify the cholinergic pathways in humans. In addition, such studies could highlight even better electrophysiological markers to be used to identify the benefits of cholinergic drug treatment to patients with AD.

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APPENDIX 1

1.1 Pharmacology of SDZ 210-086

The muscarinic activity of SDZ 210-086 has been demonstrated in several *in vitro* and *in vivo* functional test systems including the guinea-pig ileum, the superior cervical ganglion of the rat, rat hippocampal slices and cultured rat hippocampus (Palacios 1987). In addition, intraperitoneal and oral administration of SDZ 210-086 to mice resulted in effects characteristic of muscarinic receptor activation such as hypothermia, tremor, analgesia, salivation, lacrimation, diarrhoea and miosis-mydriasis. Other tests demonstrating central muscarinic activity of SDZ 210-086 in rats included: modification of brain regional glucose uptake; reduction in ACh turnover in rat striatum, cortex and hippocampus; increase in dopamine and noradrenaline but not serotonin turnovers; production of a discriminative cue in rats trained to discriminate pilocarpine or oxetremorine from saline; and modification of the sleep-wake cycle in a way similar to other muscarinic agonists by increasing the "wake" part of this cycle. However, SDZ 210-086 did not reverse scopolamine-induced amnesia in mice. The cardiovascular effects in conscious rats included a slight centrally mediated increase in blood pressure and in the anaesthetised cat, SDZ 210-086 produced a hypotensive effect.

1.2 Toxicology of SDZ 210-086

A 26-week toxicity study (Van Ryzin 1987) was carried out in both rats and dogs. In dogs, SDZ 210-086 was well tolerated at doses of 0.06 and 0.36 mg/kg/day. Pharmacological effects observed included lacrimation, salivation, loose stool and injected sclera. In addition, the higher 0.36 mg/kg/day dose, resulted in emesis and diarrhoea. Doses of 1.2 mg/kg/day resulted in ataxia, tremor, decreased and increased locomotion, blood in the saliva and vomitus, and 2 out of 6 animals died. The target organs for these toxic effects were the liver and gastrointestinal tract. Therefore, the no toxic-effect level of SDZ 210-086 in dogs was set at 0.36 mg/kg/day.

SDZ 210-086 was better tolerated in rats, where up to a maximum dose of 6 mg/kg/day resulted in no organ toxicity and no death rate was observed. Doses of 2.4 and 6 mg/kg/day resulted in lacrimation, salivation, diarrhoea and loose stool. One animal in the maximum dose group showed repeated tonic convulsions. Other observations included dose-dependent inhibition of weight gain and loss of weight at the highest doses. The no toxic-effect level in the rat was set at 0.3 mg/kg/day.

1.3 Clinical observations with SDZ 210-086

Single-dose tolerability trials have been carried out in healthy volunteers (Tinney 1988). SDZ 210-086 was given to a total of 70 healthy male volunteers such that at each dose level, 7 subjects received active medication and 3 received matching placebo. A total of seven dose levels were tested: 0.1; 0.25; 0.5; 1.0; 2.0 and 2.5 mg, administered 30 minutes after a light breakfast. No adverse events were noted up to a dose of 1.5 mg. At 2.0 mg, the following effects were observed: sweating (5/7), nausea and vomiting (1/7) and blurred vision (1/7). One subject from the placebo group also reported dizziness, vomiting, headache and blurred vision. At 2.5 mg, salivation was reported (3/7), sweating (5/7), nausea (1/7) and blurred vision (3/7). There were no disturbances in the laboratory values except an increase in salivary amylase at 2.0 and 2.5 mg doses. Thus it was concluded that doses up to 1.5 mg of SDZ 210-086 were well tolerated. Beyond this dose level, results at 2.0 and 2.5 mg confirmed peripheral cholinergic activation.

APPENDIX 2

Criteria for subjects participating in the study of the effects of SDZ 210-086 on the flash and pattern reversal evoked potential.

Inclusion criteria

1. Male 18 to 50 years of age.
2. No clinically significant abnormal physical findings at the screening examination which would interfere with the objectives of the study.
3. No clinically relevant abnormalities in the results of their laboratory screening evaluation.
4. Normal ECG.
5. Normal blood pressure (BP) and heart rate (HR) at the screening visit, i.e. BP 100-140 mmHg systolic, 60-90 mmHg diastolic and HR 50-100 bpm in sitting position.
6. Body weight between 50-95 kg and within ±10% of their ideal body weight.
7. Capable of communicating well with the investigators and complying with the requirements of the entire study.
8. Written consent to participate signed by the subject.
9. Completion of hydroxylator phenotype determination.

Exclusion criteria

1. clinically relevant abnormal findings at the screening physical examination which would interfere with the objectives of the study.
2. Clinically relevant abnormalities in the screening laboratory profile or abnormal ECG at the screening evaluation.
3. symptoms of a significant clinical illness in the two-week period preceding drug administration and during the study.
4. Presence or history of hypertension or other cardiovascular abnormalities.
5. Resting heart rate less than 50 bpm.
6. Administration of any investigational drug twelve weeks prior to entering the study.
7. A need for any concomitant medication during the five days prior to and during the entire study.
8. Existence of any surgical or medical condition which might interfere with the absorption, distribution, metabolism, or excretion of the drug.
9. History of drug or alcohol abuse.

10. Loss of greater than 400 ml of blood within the twelve week period before the study,either as a blood donor or as a subject in an investigational trial.
11. Known serious adverse reactions or hypersensitivity to any drug.
12. Presence or history of allergy requiring acute or chronic treatment.
13. Inability to communicate well with the investigator, (i.e. language problem, poor mental development or impaired cerebral function).
14. Positive results from the serology examination (evidence of HIV or hepatitis infection not due to immunisation).
15. Past history of peptic ulcer disease or gastrointestinal bleeding.
16. History of mental disease.
17. history of asthma or lung disease.

APPENDIX 3

Time of VEP recording for subjects involved in the study of SDZ 210 086 on the flash and pattern reversal VEPs.

NAME	TIME OF VEP RECORDING (hours:minutes)		
	Day 0	**Day 2/3**	**Day 10/11**
PLACEBO			
GRey	15:06	10:48 (-4:18)	10:21 (-4:45)
KA	21:55	11:47 (-10:08)	13:18 (-8:37)
SCr	17:48	10:49 (-6:59)	11:03 (-6:45)
DC	11:18	11:07 (-0:11)	11:24 (+0:06)
DR	15:45	09:55 (-5:50)	10:04 (-5:41)
GM	16:13	10:20 (-5:53)	12:01 (-4:12)
GROUP 1			
AD	14:00	07:58 (-6:02)	09:51 (-4:09)
MT	17:38	12:05 (-5.33)	10:47 (-6:56)
GRei	18:20	12:41 (-5:39)	11:13 (-7:07)
KM	19:22	10:04 (-9:18)	11:46 (-7:36)
KB	20:09	10:41 (-9:28)	10:48 (-9:21)
IM	21:18	11:14 (-10:04)	12:55 (-8:23)
GROUP 2			
SCo	10:02	10:07 (+0:05)	10:07 (+0:05)
JA	10:47	10:36 (-0:11)	10:53 (+0:06)
BB	12:52	11:32 (-1:20)	11:53 (-0:59)
BM	13:36	12:01 (-1:35)	12:15 (-1:21)
BW	15:20	13:22 (-1:58)	10:36 (-4:44)
HA	17:07	11:13 (-5:54)	10:34 (-6:33)

KEY: ( ) Difference in time between baseline and test days (hours: minutes).

APPENDIX 4

4.1 Pharmacology of SDZ ENA 713

The pharmacological investigation of SDZ ENA 713 included both *in vivo* and *in vitro* studies from the rat brain (Enz 1988). SDZ ENA 713 produced a general inhibition of the enzyme AChE with corresponding increases in ACh levels in all the brain regions examined, but especially marked in the cortex, followed by the hippocampus and corpus striatum. There was a relatively weak effect of SDZ ENA 713 on the brainstem and heart, which theoretically reduces the risk of brainstem and cardiovascular depression. In parallel with the increase in ACh levels, there was a decrease in ACh turnover, but this was thought to be secondary to the rise in ACh levels, due to the negative feedback loop (fig. 4.2), i.e AChEi prevented the breakdown of ACh; ACh levels rose in the synapse and feedback on to presynaptic "autoreceptors" on cholinergic nerve terminals which prevented release of ACh. The effects of SDZ ENA 713 on AChE activity in the rat brain commenced rapidly, reaching a peak within 15-30 minutes and persisting for at least 6 hours. There was no residual activity of SDZ ENA 713 on AChE 24 hours after a single oral administration.

Changes in the functional activity of the CNS are associated with altered deoxyglucose utilisation in the brain which can be visualised using autoradiographic techniques. An oral application of SDZ ENA 713 (1.88 mg/kg) in the rat brain induced significant changes in deoxyglucose utilisation. The most marked changes were in the visual regions, the anteroventral thalamus and in the lateral habenula nucleus. There was an increase in deoxyglucose uptake of 17% in the dLGN, 71% in the superior colliculus (superficial gray layer) and 42% in the medial terminal nuclear optic tract, compared to control animals. The preadministration of mecamylamine, which blocks nicotinic receptors, reduced the influence of SDZ ENA 713 in the visual regions such as superior colliculus and simultaneously potentiated the effects in other regions. The study therefore provided clear evidence of in vivo activation of central nicotinic and muscarinic receptors by SDZ ENA 713.

A characteristic effect of muscarinic receptor stimulation is synchronisation of hippocampal EEG, with induction of theta waves (0.2-4.2 Hz). These effects were observed in rats after administration of SDZ ENA 713. These EEG effects were further blocked by scopolamine but not by the peripheral muscarinic antagonist N-methylscopolamine, providing evidence of the central cholinergic action of SDZ ENA

713. The effect of SDZ ENA 713 on the hippocampal EEG lasted over 6 hours after a single dose of 0.75 mg/kg.

Centrally acting cholinergic agents also induce stimulation of "alert non-mobile behaviour", which is a decrease in locomotor activity in the absence of sedation. SDZ ENA 713 induced this behaviour at doses well below those causing any peripheral effects such as salivation. SDZ ENA 713 also partially reversed scopolamine induced impairment of learning in the water maze exploration memory test in rats.

There was no change in blood pressure reported in either the anaesthetised cat or conscious squirrel monkey with SDZ ENA 713, even at doses inducing marked tremor (1 mg/kg), indicative of excessive central cholinergic stimulation. In the rat, there was a slight decrease in heart rate, -a peripherally mediated effect as it was blocked by N-methylscopolamine. There was also a rise in blood pressure (by 29%) in the rat, after a high dose of SDZ ENA 713 (5.6 mg /kg), -a centrally mediated effect as it was blocked by scopolamine but not N-methylscopolamine.

ACh induces bronchospasm in the anaesthetised, ventilated guinea-pig, manifested by a dose-dependent increase in airway resistance. SDZ ENA 713 (dose range 0.01-0.1 mg/kg) had no effect on airway resistance but potentiated the bronchospasm induced by ACh in the ventilated guinea-pig, similarly to other AChEis. The drug was therefore contraindicated in patients with asthma or chronic obstructive pulmonary disease.

4.2 Toxicology of SDZ ENA 713

A number of the toxicology studies of SDZ ENA 713 were carried out in rats, dogs and monkeys (Van Ryzin 1989).

In the rat, drug-related clinical signs, characteristic of excessive cholinergic stimulation were observed at the highest dose administered (6 mg/kg/day SDZ ENA 713). These signs included decreased locomotor activity, flattened body posture, loss of the righting reflex, ataxia, tremor, exophthalmos, red tears, diarrhoea or loose stool. In addition, laboured breathing, soiled fur and vasoconstriction were observed in many of these high dose animals. The food consumption was also reduced by about 17-23% at the highest dose. This resulted in a marked significant decrease in the body weight gain by about 9-18% when compared to control animals. These clinical signs were reduced as the dose of SDZ ENA 713 was reduced, while the placebo group appeared normal throughout the study. There were no significant changes in the haematology or biochemical parameters, with the exception of a small rise in serum

urea at SDZ ENA 713 doses of 2.4 and 6 mg/kg/day. There were also no drug-related microscopic or macroscopic changes observed at post-mortem. In conclusion SDZ ENA 713 was reported to be well tolerated in rats up to a dose of 2.4 mg/kg/day, with no target organ toxicity observed histopathologically.

In the dog, administration of SDZ ENA 713 (3.6 mg/kg/day, reduced subsequently to 3.0 mg/kg/day) induced severe signs of excessive cholinergic stimulation, including ataxia, convulsions, muscle fasciculations, decrease in locomotor activity, bloody diarrhoea, emesis, salivation, and lacrimation. Of the 6 dogs in the group, 1 died and 3 were killed in moribund condition. Intussusception in the ileum (in 2 animals) and gastrointestinal congestion and/or haemorrhage were observed in all 4 animals that died or were sacrificed moribund. Other microscopic findings attributed to SDZ ENA 713 in the 4 animals that died, included lymph node congestion, liver congestion, congestion and necrosis in the adrenal cortex, thymus atrophy/involution and hypoplasia of splenic follicles. However, no microscopic lesions related to SDZ ENA 713 were found in the dogs sacrificed at the end of the study. There were no changes observed in the electrocardiographs during the study. The haematology and serum biochemistry parameters were also not significantly altered, with the exception that in the two surviving animals there was a rise in sorbitol dehydrogenase level, possibly indicating liver dysfunction. However, other liver function tests were unaltered and there was no evidence of liver damage histologically. In general, doses up to 0.42 mg/kg/day were well tolerated in the dogs.

In a special study to determine whether excessive pharmacodynamic effects of a high dose of SDZ ENA 713 could be reversed by atropine, 6 Beagle dogs received a single oral dose of 1.5 mg/kg SDZ ENA 713 followed by 3 mg/kg SDZ ENA 713 one week later. Within one hour of dosing (1.5 mg/kg), the animals exhibited tremor, ataxia, vomiting, diarrhoea and pale mucous membranes. These effects were reversed within 5-10 minutes after subcutaneous injection of atropine (0.1 mg/kg). At the dose of 3 mg/kg SDZ ENA 713, the animals exhibited moderate to severe hypersalivation, tremor, ataxia, vomiting and diarrhoea, tachypnoea and cyanotic oral mucosa. These signs disappeared completely after intravenous injection of atropine (0.1 mg/kg). Thereafter only slight muscle fasciculation persisted for 45 minutes.

SDZ ENA 713 was administered orally for 2 weeks to one male and one female Rhesus monkey. An increasing dosage was used: 3 mg/kg/day for days 1-7; 4 mg/kg/day for days 8-10; 6 mg/kg/day for days 11-13 and 10 mg/kg/day on day 14. Clinical signs observed at all dose levels included decreased locomotor activity and reduction in amount of and paleness of faeces. Ataxia and tremor were observed

occasionally at dose levels of 6-10 mg/kg/day. The female showed erythema and puffiness around the eyes at all dose levels and mild dehydration from days 10-14. A decrease in food consumption and loss in body weight (by about 10%) occurred throughout the study. The monkeys tolerated SDZ ENA 713 fairly well even at high doses (3 mg/kg/day increased gradually to 10 mg/kg/day). However, the tolerability of SDZ ENA 713 may be species specific, since in pharmacological experiments, Squirrel monkeys tolerated SDZ ENA 713 poorly even at a dose of 0.1 mg/kg (section 7.1.1).

In conclusion, the findings of the toxicology studies in the rats, dogs and monkeys were typical for an AChEi with excessive pharmacodynamic effects of cholinergic stimulation occurring at higher doses in a dose-dependent manner. These effects were rapidly reversed by administration of atropine in dogs. The non-toxic effect level was estimated at 2.4-10 mg/kg/day for rats and Rhesus monkeys, and at 0.42 mg/kg/day for dogs.

4.3 Clinical observations with SDZ ENA 713

A single dose tolerability study in young healthy male volunteers was carried out (Sandoz 1989). A total of eight groups each containing 10 subjects were studied. 7 out of 10 subjects were given the active medication while the remaining 3 were given placebo in a double-blind manner. A total of 80 subjects were studied and the doses of SDZ ENA 713 included 0.16, 0.3, 0.6, 1.0, 1.5, 2.3, 3.0 and 4.6 mg. Subjects discontinuing the study for non-medical reasons were replaced. To maximise safety, the starting dose was very low (0.16 mg), being 150 times lower than the non-toxic effect level in the dog. The different doses were given in an ascending manner, such that the tolerability and safety of SDZ ENA 713 was reviewed by the investigator and sponsor at each dose level before proceeding to the next dose level. Adverse events recorded on the day of administration are summarised in table A4.1.

In general, the tolerability up to 3 mg was considered good. With the exception of one subject in group VII who had a headache of moderate intensity, all adverse events up to 3 mg dose were rated as mild by the investigator. However, at the 4.6 mg dose, 5 out of 7 subjects on SDZ ENA 713 reported adverse events, including nausea and vomiting, headache and a "dazed" feeling in the head. A short-lived increase in the supine blood pressure occurred in one subject on 4.6 mg SDZ ENA 713, rising from 122/62 mmHg systolic/diastolic at baseline to 152/104 mmHg at one hour, 142/94 mm Hg at two hours, before returning to normal (118/70 mmHg) by four hours. A slight fall in the mean standing systolic blood pressure also occurred at this dose level, although no episodes of supine or orthostatic hypotension occurred in any case.

Occasional episodes of sinus bradycardis (pulse <50/min.) were observed after medication, but this phenomenon was not dose-related, and was seen after both active and placebo treatment. Furthermore, in all cases the heart rate (as measured by ECG) was relatively low prior to medication and the episodes were considered consistent with the effects of bed-rest in fit young male subjects.

There were no significant changes in the ECG, respiratory function tests or laboratory (haematology and biochemistry) values observed after medication. Salivation was increased in some subjects after 1.5, 2.3 and 3.0 mg SDZ ENA 713, but no increase was observed after the 4.6 mg dose. No consistent changes in pupil size or sweating occurred.

Measurement of plasma levels of SDZ ENA 713 after the 3 mg dose indicated a short half-life of 1-2 hours of the parent compound. Nevertheless, the duration of the biological activity of SDZ ENA 713 as measured by inhibition of plasma cholinesterase activity, was at least eight hours. Plasma butyrylcholinesterase was inhibited in a dose-dependent manner, with a maximum inhibition of 15-20 % after the 3 mg dose, and 40% after the 4.6 mg dose.

Table A4.1 Adverse events recorded after administration of SDZ ENA 713 in healthy male volunteers. Brackets indicate number of subjects.

GROUP	DOSE	PLACEBO	ACTIVE
I	0.16 mg	Headache (n=1)	Headache (n=1)
II	0.3 mg	No adverse events	No adverse events
III	0.6 mg	No adverse events	No adverse events
IV	1.0 mg	Headache (n=2)	Headache (n=2) Dizziness and sweating on standing (n=1)
V	1.5 mg	No adverse events	Headache (n=1)
VI	2.3 mg	No adverse events	Dizziness (n=1)
VII	3.0 mg	No adverse events	Dizziness (n=1) Dizziness on standing (n=1) Headache (n=1) Increased salivation (n=1)
VIII	4.6 mg	Headache (n=1)	Dazed feeling (n=4) Headache (n=3) Nausea and vomiting (n=3) Diarrhoea (n=1) Tiredness (n=1)

APPENDIX 5

Table A5.1. The effect of scopolamine eye drops on pupil diameter (mm) of the right eye (RE) and left eye (LE).

SUBJECT	CONTROL		5 mins		1 hour		2 hours		3 hours	
	RE	LE	RE	LE	RE	LE	RE	LE	RE	LE
AD	7.5	7.5	7.5	7.5	7.5	9.0	7.5	9.0	7.5	9.0
AG	6.5	6.5	6.5	6.5	5.0	8.5	5.0	8.5	6.5	8.5
JB	8.0	8.0	8.0	8.0	7.0	9.0	7.0	9.0	7.0	9.0
JG	6.5	6.5	6.5	6.5	5.0	8.5	5.0	8.5	5.0	8.5
PF	5.5	5.5	5.5	6.0	4.0	7.0	4.0	7.0	4.0	7.0
RD	5.0	5.0	5.0	5.0	5.5	9.0	5.0	8.0	5.0	8.0
Mean	6.5	6.5	6.5	6.6	5.7	8.5	5.6	8.3	5.8	8.3
SD	1.1	1.1	1.1	1.1	1.3	0.8	1.4	0.8	1.4	0.8

Table A5.2. The effect of scopolamine eye drops on the amplitude of accommodation (dioptres) in the right eye (RE) and left eye (LE).

SUBJECT	CONTROL		5 mins		1 hour		2 hours		3 hours	
	RE	LE	RE	LE	RE	LE	RE	LE	RE	LE
AD	8.25	8.75	9.0	9.0	9.0	<2	9.0	<2	9.0	<2
AG	8.0	8.0	8.0	7.5	8.0	<2	8.0	<2	8.0	<2
JB	8.0	8.5	7.75	7.75	8.0	2	7.0	<2	7.75	<2
JG	8.5	10.0	10.0	10.25	8.25	<2	10.0	<2	9.0	<2
PF	6.75	6.75	7.0	6.5	7.0	<2	7.0	<2	7.0	<2
RD	9.0	8.5	8.0	8.5	8.0	<2	8.0	<2	8.0	<2
Mean	8.1	8.4	8.3	8.3	8.0	<2	8.2	<2	8.1	<2
SD	0.8	1.1	1.1	1.3	0.6	0	1.2	0	0.8	0

Table A5.3. The effect of scopolamine eye drops on the intraocular pressure (mm Hg) in the right eye (RE) and left eye (LE).

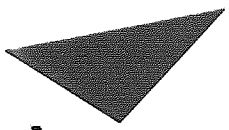
SUBJECT	CONTROL		5 mins		1 hour		2 hours		3 hours	
	RE	LE	RE	LE	RE	LE	RE	LE	RE	LE
AD	20	20	20	12	18	17	18	18	18	20
AG	14	15	15	13	17	14	17	16	16	16
JB	13	13	13	14	12	14	12	12	14	12
JG	14	12	11	11	13	11	12	10	13	14
PF	18	19	17	17	14	17	14	20	14	16
RD	15	13	13	11	9	10	9	11	13	9
Mean	15.7	15.3	14.8	13.0	13.8	13.8	13.7	14.5	14.7	
SD	14.5 2.7	3.4	3.3	2.3	3.3	2.9	3.4	4.1	2.0	3.8

APPENDIX 6

Publications

6.1 S. Padhiar and G.F.A. Harding. The effect of time of day on the flash and pattern reversal visual evoked potentials. *Electroencephalography and Clinical Neurophysiology. May (1991) vol 78 (5): 87P.*

Presented as a paper for the EEG Society conference held on 6th October 1990 at The National Hospital for Neurology & Neurosurgery, Queen's Square, London.



Aston University

6.2 S. Padhiar and G.F.A. Harding. The effect of scopolamine on the flash and pattern reversal visual evoked potentials. *Electroencephalography and Clinical Neurophysiology. (1992) vol 82 (1): 12P.*

Presented as a paper for the EEG Society conference on 12th June 1991 at The Royal Orthopaedic Hospital in Birmingham.

6.3 **G.F.A. Harding, R. Daniels, S. Padhiar, N. Drasdo and S.J. Anderson.** Visual evoked potentials to flash and pattern reversal stimulation following administration of systemic or topical scopolamine. *Documenta Ophthalmologica In Press*

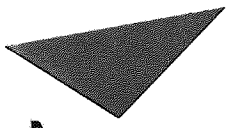


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Figure captions.

Figure 1. Shows the flash and pattern VEP of a control subject (F.T.) and two patients with Alzheimer's disease (J.K. and L.L.). It can be seen by the position of the cursor, that the P2 component of the flash VEP is markedly delayed for both Alzheimer's patients compared to the control. The P100 component of the pattern reversal VEP, however remains unaffected.

Figure 2. Shows the latencies of the P2 (open squares) and N2 (closed squares) components of the flash VEP and the latencies of the P100 (open squares) and N75 (closed squares) of the pattern reversal VEP at baseline level and at 1,2,4 and 6 hours following systemic hyoscine hydrobromide administration. The asterisks show the statistically significant delay in the P2 latency of the flash VEP 1 and 2 hours after administration of the drug - this delay was around 6ms. The error bars indicate one standard deviation from the mean (student's t test).

Figure 3. Shows the interocular difference in latency of the flash P2 latency and pattern reversal P100 latency for each of the 6 subjects at baseline levels and up to 200 min following topical instillation of hyoscine hydrobromide to the left eye. It can be seen that no consistent change in latency occurred between the two eyes.

Figure 4. Shows the group mean intraocular difference in latency of the flash P2 and pattern reversal P100 components of the VEP at baseline levels and up to 200 min following topical administration of hyoscine hydrobromide to the left eye. The error bars represent one standard deviation from the mean (student's t test). No significant difference in latency occurred between the two eyes.

Table 1 gives the mean latencies of the N2 and P2 component of the flash visual evoked potential prior to, and 1,2,4 and 6 hours following an intramuscular injection of hyoscine hydrobromide. The asterisk indicates a statistically significant increase in latency.

Figure 1

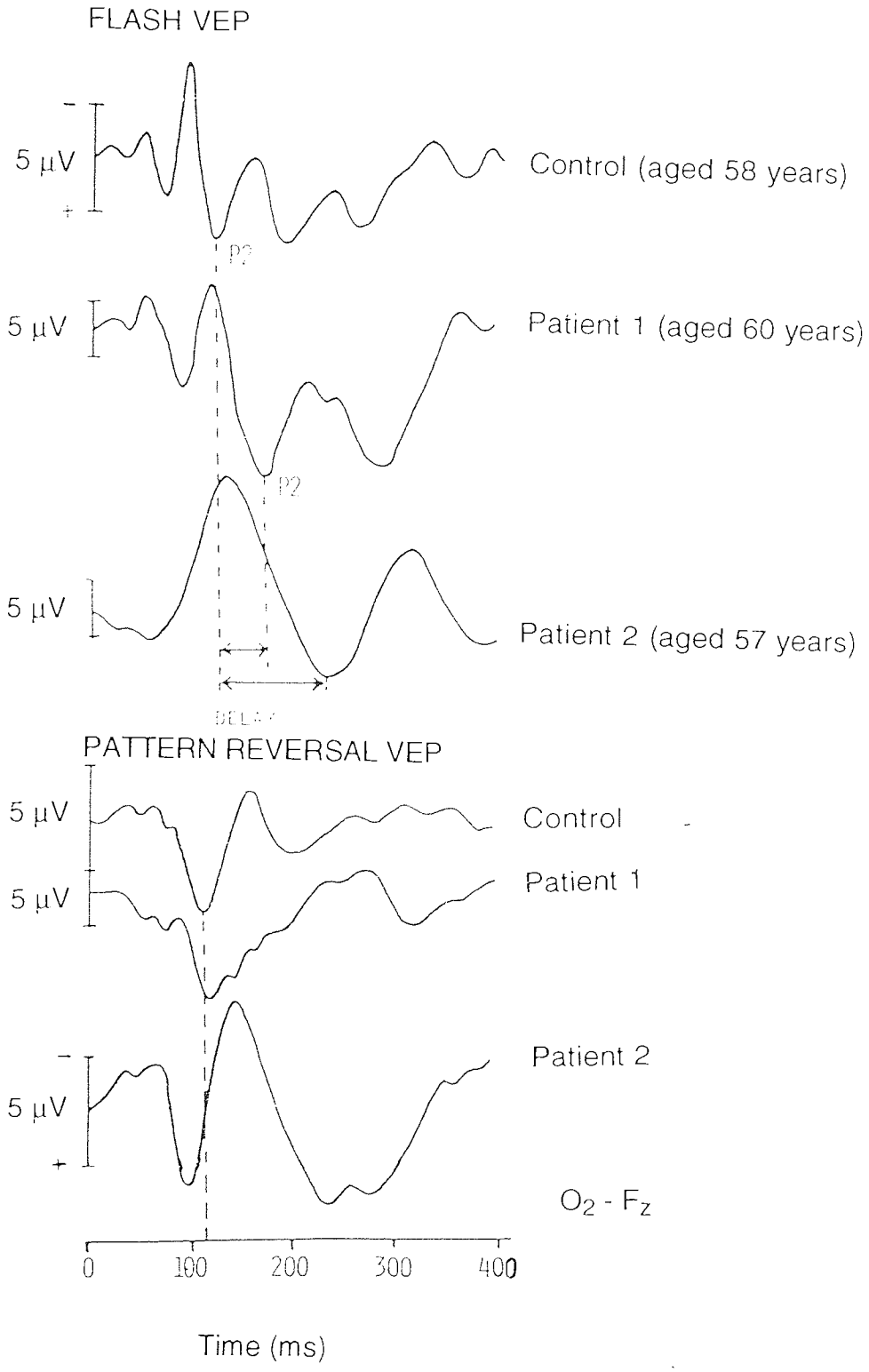

FLASH VEP
5 μV
Control (aged 58 years)
P2
5 μV
Patient 1 (aged 60 years)
P2
5 μV
Patient 2 (aged 57 years)
PATTERN REVERSAL VEP
5 μV
Control
5 μV
Patient 1
Patient 2
5 μV
O2 - Fz
0
100
200
300
400
Time (ms)

Figure 2

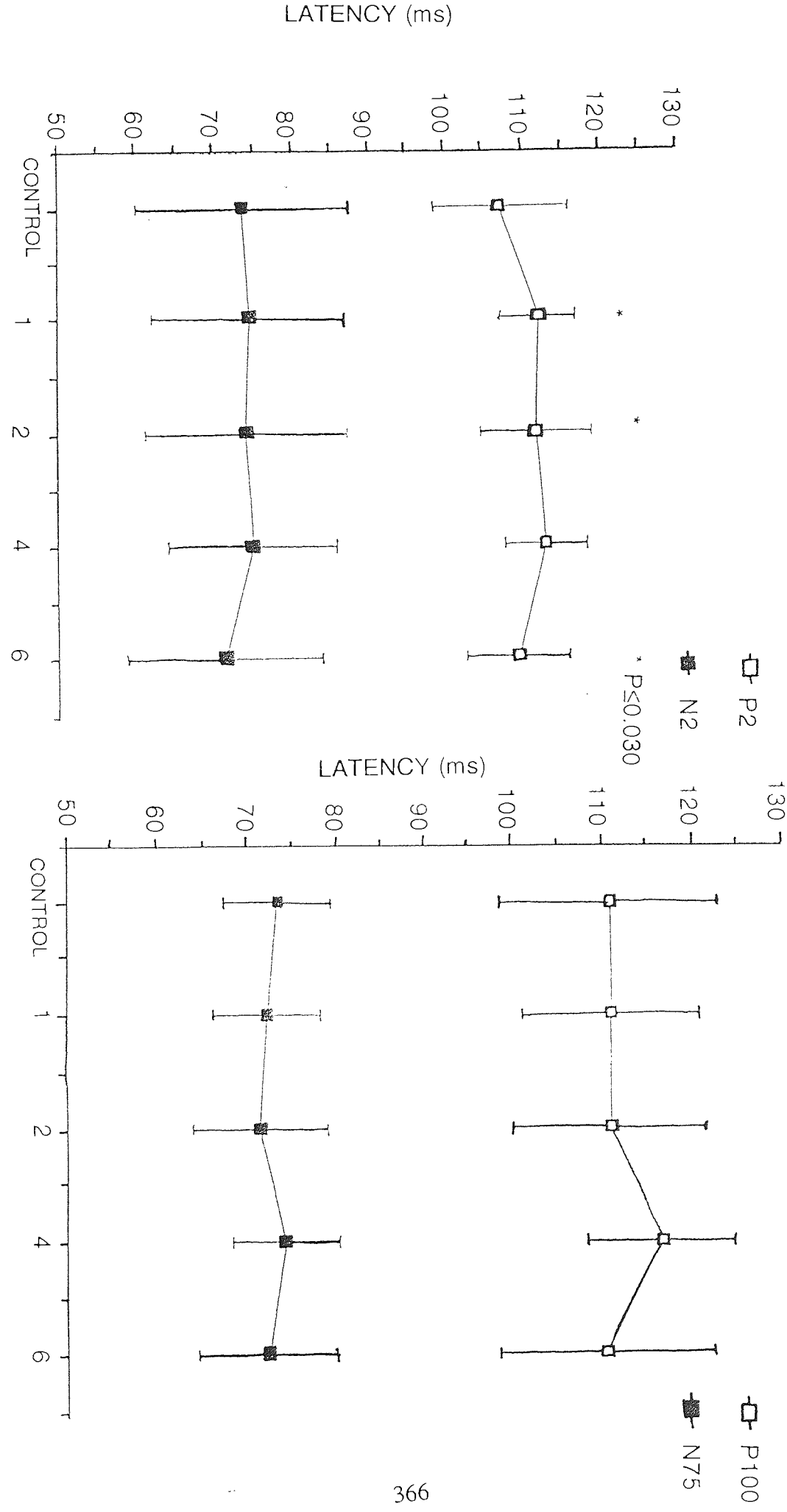
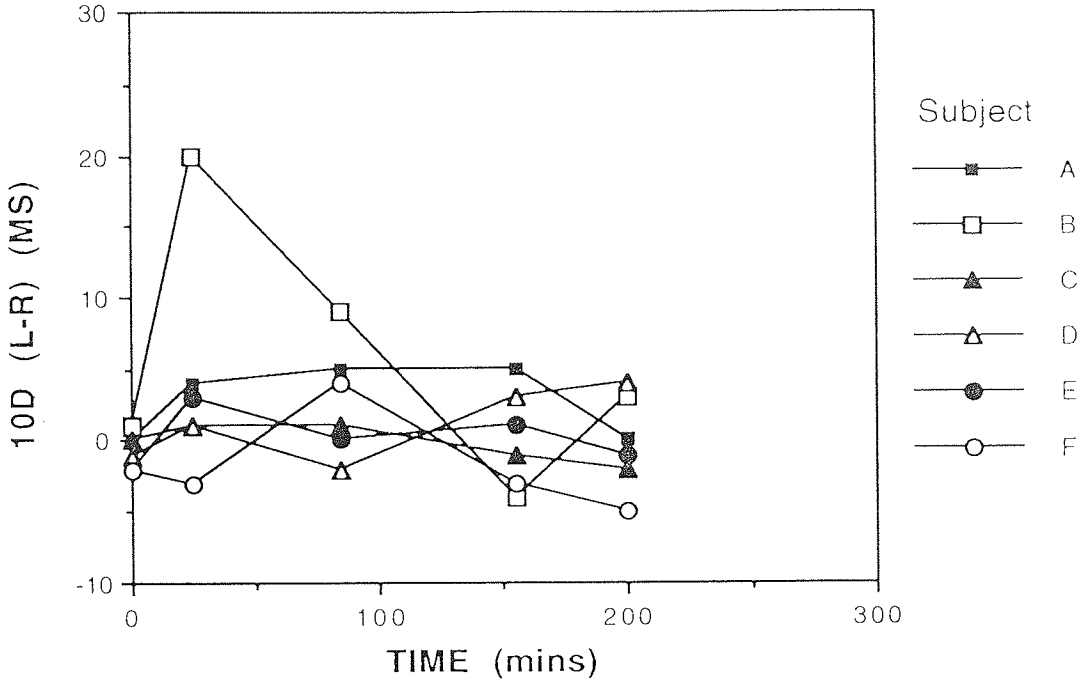

LATENCY (ms)
50
60
70
80
90
100
110
120
130
CONTROL
1
2
4
6
FLASH VEP LATENCIES
P2
N2
* P≤0.030
LATENCY (ms)
PATTERN REVERSAL VEP LATENCIES
P100
N75

Figure 3

PREV P100 LATENCY: INTEROCULAR DIFFERENCES



FLASH P2 LATENCY: INTEROCULAR DIFFERENCES

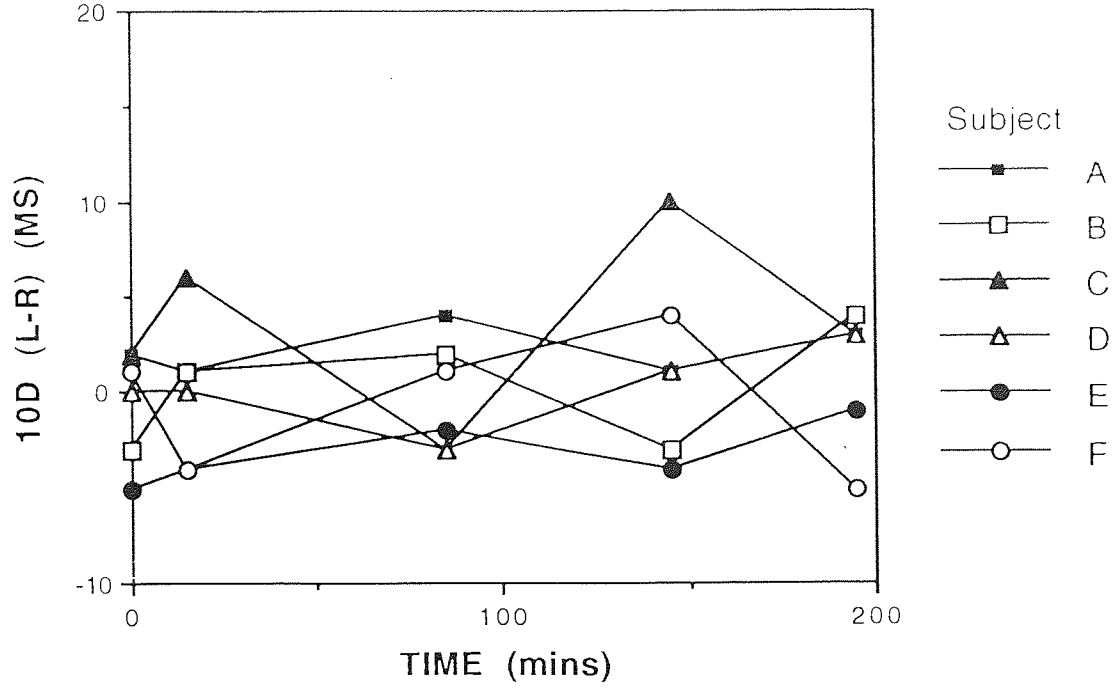
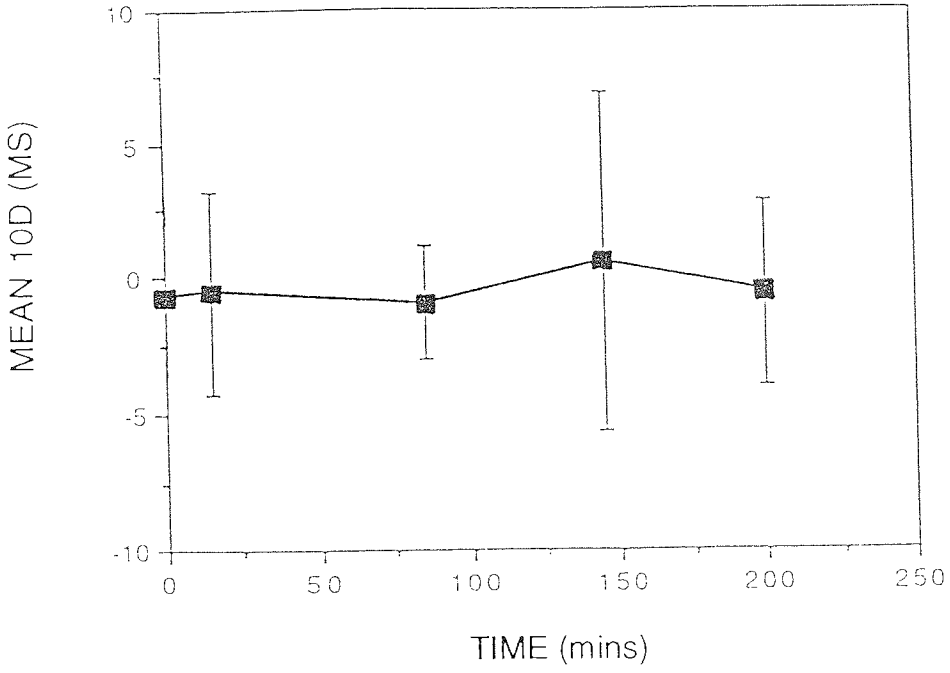


Figure 4

FLASH P2 LATENCY: GROUP MEAN INTEROCULAR DIFFERENCE



PREV P100 LATENCY: GROUP MEAN INTEROCULAR DIFFERENCE