ACTIONS OF ANTICHOLINESTERASES ON VISUAL PERFORMANCE IN MAN AND THEIR ANTAGONISM BY ATROPINE

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SUMMARY

This work investigates the effects of the anticholinesterases physostigmine and pyridostigmine and the cholinergic antagonists atropine and homatropine on the human visual system. The antagonism between these classes of drug is also assessed.

Anticholinesterases cause pupillary constriction and an increase in accommodation. As a means of simulating their effects in a controlled situation, a systematic study was performed to determine the effects of artificial pupils and defocusing lenses on visual performance. This was assessed by measuring contrast sensitivity for the detection of sinusoidal grating patterns. Contrast sensitivity was measured in 12 subjects for a range of spatial frequencies (0.5-38 c/deg), for pupil diameters 2 - 8mm and for defocuses of +1 to +4 D, following homatropine eyedrops. Changes in pupil diameter, without any compensation for the change in retinal illumination, had no significant effect on contrast sensitivity, except at 0.5 and 1 c/deg when a significant reduction occurred with the 2mm pupil. This suggests that the expected improvement in optical quality associated with smaller pupil diameters had been annulled by the accompanying reduction in retinal illumination. On the other hand, defocus caused an appreciable reduction in contrast sensitivity at spatial frequencies higher than the peak of the contrast sensitivity function (3 c/deg) and a smaller reduction below the peak. With increasing defocus a downwards parallel shift of the contrast sensitivity function above the peak was observed. Each dioptre of defocus reduced contrast sensitivity by about 50% at spatial frequencies higher than peak and 19% at spatial frequencies lower than peak in the homatropinised eye. The decrements were slightly less in the natural eye.

An oral dose of 60mg pyridostigmine bromide which causes at least a 20% inhibition of blood cholinesterase, caused a small but significant increase of 7% in contrast sensitivity to stationary oscilloscope-generated grating patterns over 3-38 c/deg, for a group of 13 subjects. This was attributed to an increase in ocular quality due to the small reduction in pupil diameter. Contrast sensitivity to laser interference fringes observed in the Maxwellian view, were unchanged after pyridostigmine. It is concluded that pyridostigmine may be used as a pre-treatment against organophosphorus anticholinesterases without adverse effects on stationary visual function.

Instillation of 0.25% physostigmine sulphate eyedrops in 12 subjects caused a sustained miosis, a transient increase of near point accommodation and amplitude of involuntary accommodation. This last effect was maximal at 30 min and subsided by 90 min, though its amplitude varied greatly between subjects from +0.5D to +10D. Comparisons between two families of three siblings suggested involvement of a genetic trait in the amplitude of response of the ciliary body to physostigmine. Contrast sensitivity to externally-viewed oscilloscope grating patterns was transiently reduced after physostigmine and correlated with the increase in amplitude of accommodation. Physostigmine had a transient deleterious effect on contrast sensitivity to laser interference fringes, particularly at higher spatial frequencies, which was not affected by defocus of the image. Physostigmine also caused a prolonged reduction in contrast sensitivity to low spatial frequency grating patterns. Since the control eye showed no miosis, systemic absorption of physostigmine seems improbable. This suggests that there is a direct effect from trans-corneal absorption of physostigmine on the retinal neurones.

The effects of a single intramuscular injection of 2mg atropine sulphate on visual performance were studied in 13 subjects. The well known actions of atropine on heart rate, secretion of saliva, dilatation of pupils and reduction in the amplitude of accommodative range, were observed. However, visual acuity, stereoacuity, red-green colour balance and reaction time to a visual stimulus were unaffected by atropine, although extra-ocular muscle balance was transiently changed. There was no significant change in contrast sensitivity to stationary sinusoidal gratings of spatial frequencies 1-30 c/deg for oscilloscope-generated patterns and laser interference fringes. However, contrast sensitivity to low spatial frequency (1-5 c/deg) grating patterns phase-reversed at 5.5 Hz showed a sustained reduction over six hours post-injection. Thus, it is concluded that atropine has an adverse effect on movement detection but not on stationary visual function.

An intramuscular injection of 2mg atropine sulphate was given at either 8 min or 124 min prior to 0.25% physostigmine eyedrops. The maximum increase in accommodation expected with physostigmine would thus coincide with the peak plasma concentration of atropine or with the fully developed mydriasis and reduction in near point accommodation caused by atropine. Atropine applied at either time had no affect on the miosis, the increase in near point accommodation, the increase in involuntary accommodation or the resulting reduction in contrast sensitivity caused by physostigmine. The reduction in contrast sensitivity to a phase-reversed grating pattern of 3 c/deg caused by physostigmine, was actually exacerbated by atropine although this was not statistically significant. Thus, the standard intramuscular dose of atropine is ineffective against an anticholinesterase. This was due primarily to insufficient delivery of atropine to ocular tissues following intramuscular injection as shown by the effectiveness of topically applied homatropine against physostigmine.

Homatropine hydrobromide eyedrops (2%) were applied 100 min prior to physostigmine eyedrops, so that antagonism of the muscarinic receptors would be optimal prior to exposure to physostigmine. Pupil diameter was still reduced by physostigmine, but to a much smaller extent than that seen with physostigmine alone. Although physostigmine transiently reversed the homatropine-induced paralysis of near point accommodation, no large increase in involuntary accommodation was induced and the marked reduction in contrast sensitivity was effectively antagonised by homatropine at all spatial frequencies.

Thus, pre-treatment with homatropine eyedrops prior to exposure to anticholinesterases would be far more effective, as far as the visual system is concerned, than intramuscular injection of atropine, especially since atropine itself may have adverse effects on perception of moving objects.

INTRODUCTION

The work presented in this thesis is a study of the effects of the anticholinesterases pyridostigmine and physostigmine and the cholinergic antagonists homatropine and atropine on the human visual system. The possible antagonism between these two classes of drug was also investigated. Throughout this work, the principal test of visual performance was to measure contrast sensitivity to sinusoidal grating patterns, which will be described first in this introductory section.

Measurement of visual function; contrast sensitivity

Contrast sensitivity was measured as the ability to detect a grating pattern of a specified spatial frequency. In a sinusoidal grating pattern, the luminance varies sinusoidally across the horizontal axis producing a repeated sequence of light and dark cycles as shown in Fig. 1. The two important variables to consider are spatial frequency and contrast. The number of cycles per degree of visual angle is the spatial frequency and the difference in luminance between light and dark half cycles is the contrast, being conventionally defined as

$$C = L_{max} - L_{min} / L_{max} + L_{min}$$

where L_{max} and L_{min} are the maximum and minimum luminance of the cycles of the grating pattern.

If the contrast of the grating pattern is increased from below visibility to where the grating is just seen, then the grating is said to have reached threshold contrast. Contrast sensitivity is the reciprocal of this threshold contrast. Gratings of different spatial frequencies require different contrasts to reach threshold and collectively give the contrast sensitivity function (CSF) which relates contrast sensitivity to spatial frequency. The CSF thus describes a person's ability to detect an object across the range of object sizes.

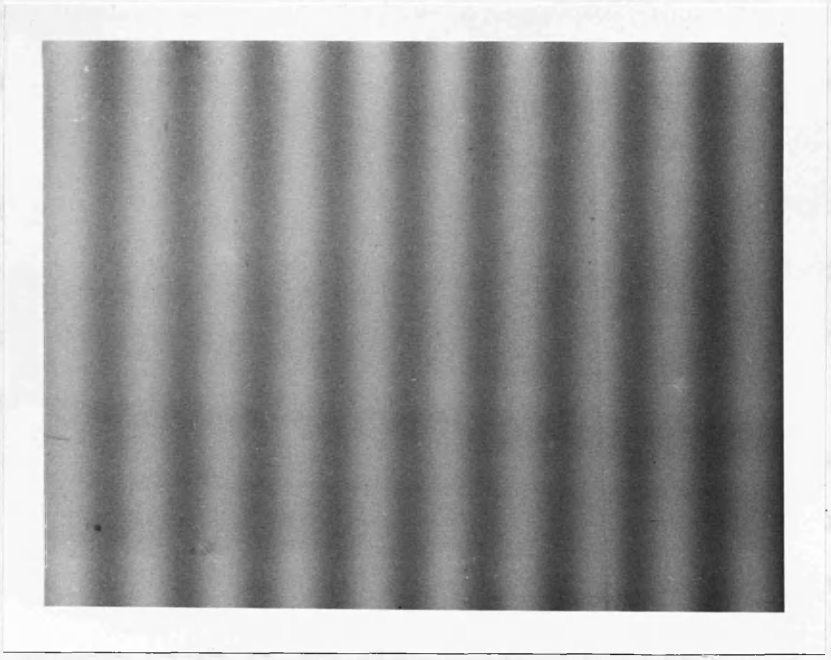


Fig. 1. A sinusoidal grating pattern generated on a Tektronix 606B oscilloscope as used in this study.

The CSF of the human visual system was first measured by Schade (1956). He found that contrast sensitivity is highest for spatial frequencies of 3-5 c/deg with a decline in performance at both higher and lower spatial frequencies. Schade's technique was subsequently used by F.W. Campbell and colleagues. Campbell & Green (1965) determined the modulation transfer function (MTF) of the human eye by comparing contrast sensitivity measured to oscilloscope generated grating patterns which are focused by the eye and represent an assessment of the overall visual system, with that for the neural component alone. The latter assessment consisted of measurement of contrast sensitivity to a pattern formed directly on the retina by the interference of two laser beams. Interference fringes have been used as stimuli for psychophysical measurements since the work of LeGrand (1935). As the interference fringes were formed by coherent light and were observed in the Maxwellian view, they were unrefracted by the optical media and gave a measure of retinal/central sensitivity. Campbell & Green demonstrated that the resolution limit of the visual system was set by the retina and not by the optical media of the eye. Campbell, Kulikowski & Levinson, (1966) showed that the visual system is sensitive to the orientation of a grating pattern, as contrast sensitivity to vertical or horizontal patterns was greater than for oblique orientations. This was not due to any optical factors as it was also valid for interference fringe patterns. The CSF is believed to reflect the contributions of a series of independent channels each detecting over a limited range of spatial frequencies. The presence of these channels was first suggested by Campbell & Robson (1964; 1968) because they found the contrast sensitivity for the fundamental frequency of a square waveform (of low spatial frequency) was lower than predicted from a model based on the CSF of the whole visual system measured with sine waves. Thus, the

mechanism detecting low fundamental frequencies had a lower sensitivity to high frequency harmonics than would be supposed from the CSF. Complex waveforms could only be recognised as distinct from sine waves when the higher harmonic components of the waveform reached their own thresholds, indicating that the channels operate independently. Blakemore & Campbell (1969) showed that there was an indeterminate number of individual spatial channels with peak sensitivities closely spaced through the range of frequencies 3-20 c/deg. Each channel had a band-width of about an octave. Recent studies suggest the presence of six or seven discrete spatial frequency channels in central vision (Wilson, McFarlane & Phillips, 1983; Watson & Robson, 1981 respectively). These frequency-selective channels are not only involved in the detection of contrast, but also in the suprathreshold perception of spatial frequency (Blakemore & Sutton, 1969). Campbell & Kulikowski (1966) also demonstrated the presence of narrow orientationally selective channels, thus, it seems that each of the channels which transmit visual spatial information filters a narrow band of both orientations and frequencies: these channels are believed to be cortically located (Braddick, Campbell & Atkinson, 1978; Maffei, 1978).

Contrast sensitivity is influenced by many factors. Decreasing the mean retinal luminance reduces the peak sensitivity and shifts the high frequency cut-off of the CSF to lower frequencies. Also, the low spatial frequency decline in contrast sensitivity is absent for scotopic luminances (Patel, 1966; van Meeteren & Vos, 1972). Contrast sensitivity to a grating may also depend on the number of cycles visible (McCann et al., 1974; Hoekstra et al., 1974) and the presence of a dark or an illuminated surround (Estevez & Cavonius, 1976). Due to the anatomical and functional specialisation of central retina for spatial resolution, the part of the retina which is tested is also important

(Daitch & Green, 1969; Hilz & Cavonius, 1974; Skrandies, 1985). Focus and pupil size also affect the CSF but they will be discussed in detail later. Finally, the temporal modulation of the grating can also affect the shape of the CSF. At very low spatial frequencies there is an increased sensitivity to moving and flickering gratings (Robson, 1966; Tolhurst, 1973). It seems that two distinct CSFs can be determined for the detection of pattern and movement corresponding to two different detection mechanisms. Tolhurst has suggested that the movement-independent mechanism may be analagous to the "X" or "sustained" class, and the movement-dependent mechanism to the "Y" or "transient" class of cat retinal ganglion cells (Enroth-Cugell & Robson, 1966).

The fundamental advantage of measuring contrast sensitivity for a range of spatial frequencies is that it gives a more complete description of the visual system's functional range than, for example, the Snellen test which measures only the resolution limit. The importance of this has been demonstrated by Ginsburg et al., (1982), who found a correlation between the ability to detect a target and the peak of the CSF, but not with Snellen acuity. Also, contrast sensitivity measurements are often more sensitive to visual disturbances than other means of visual assessment (Arden, 1978; Comerford, 1983). Contrast sensitivity has been useful in assessing visual abnormalities in cases of amblyopia (Gstalder & Green, 1971; Hess & Howell, 1977), myopia (Fiorentini & Maffei, 1976), cataract (Hess & Woo, 1978), macular degeneration (Hyvarinen, Laurinen & Rovamo, 1983), glaucoma (Arden & Jacobson, 1978) and in multiple sclerosis (Regan, Silver & Murray, 1977; Ginsburg, 1981) and Parkinson's disease (Skrandies & Gottlob, 1986). Contrast sensitivity measurements have also been used to study changes in the visual system associated with normal ageing (McGrath & Morrison, 1981; Morrison & McGrath, 1985; Owsley, Sekuler & Siemsen, 1983) and to

study the effects of dopaminergic drugs on normal vision (Domenici et al., 1985).

Factors affecting the retinal image

The size of the pupil is an important factor in determining the final image quality as not only does it control the amount of light which reaches the retina, it also limits which rays from the object form the image. Pupil diameter determines the amount of geometrical aberration, diffraction and depth-of-focus available. Even in a perfect optical system, with a round pupil, the image of light from a point object is spread into an Airy disc on the retina, known as the point-spread function. Theoretically, for two incoherent point objects to be resolvable, the maximum of the Airy disc of one object should fall on the first minimum of the second. The minimum angular separation (θ, in radians) is given by the Rayleigh criterion of

$\theta = 1.22 \lambda / a$, where λ, is wavelength and a, pupil diameter

Relating this to the Fourier theory of optical images, for a diffraction-limited optical system, in monochromatic light, image contrast declines approximately linearly with spatial frequency (this relationship is the MTF), until the cut-off frequency determined by a / λ (cycles/rad) is reached (Westheimer, 1964).

In the human eye, the performance of the ocular media is poorer than that of a diffraction limited system. The eye may be approximated as an optical system made up of spherical surfaces and is thus subject to the five Seidel monochromatic aberrations; spherical aberration, coma, oblique astigmatism, field curvature and distortion (details in Fincham & Freeman, 1980). The latter two are compensated for by the curvature of the retina and in the higher centres of the visual system respectively, and are not thought to significantly degrade the final

image (Charman, 1983). Of the three remaining Seidel aberrations, only spherical aberration will produce blurring of the image on the optical axis. This results from the formation of a blur circle due to marginal and paraxial rays coming to slightly different foci. However, in the human eye, on average only ~ 0.5 D of positive spherical aberration exists (Millodot, 1978), as the eye is made aspheric by the flattening of the peripheral cornea (Westheimer, 1963). The lens also corrects its own spherical aberration by means of the gradients of refractive indices from its centre to the periphery (Nakao & Ono, 1969), and also by flattening of its surfaces towards the periphery (Howcroft & Parker, 1977). The optical axis and visual axis are misaligned in the living eye by ~ 5-10 deg. In the past, off-axis aberrations were thought to have little effect as the peripheral retina is not capable of good resolution (Bennett & Francis, 1962). Recent measurements of the overall monochromatic wavefront aberration of the eye, however, suggest that off-axis aberrations dominate (Howland & Howland, 1976, 1977; Walsh & Charman, 1985). In Howland & Howland's original study, the subject monocularly viewed a point source of light through a crossed-cylinder lens and grid assembly, and drew a picture of the retinal shadow of the grid. Since each individual aberration produces a characteristic deformation, the contribution of each to the overall wavefront aberration could be calculated. Walsh & Charman repeated this approach but objectively measured the aberrations by photography of the retinal image and measured the phase relationship as well as the amplitude change. The general conclusions from the two groups were essentially the same; spherical aberrations are often limited to one meridian (i.e. a "cylindrical" aberration) and that coma (an off-axis aberration which causes point objects to appear as a core of light with a spreading tail) was the dominant aberration at all pupil sizes. They also concluded

that aberrations vary from subject to subject and are usually asymmetrical.

The final Seidel aberration to consider is oblique ray astigmatism which causes blurring in a plane either through or perpendicular to the image, this will then either blur horizontal lines and sharpen vertical lines or vice versa. However, no difference in peripheral visual acuity was found in a study made both with and without correction for this peripheral refractive error (Millodot et al., 1975). Other functions of the peripheral retina such as motion threshold or absolute threshold were reduced by off-axis aberrations (Millodot, 1978).

In white light, aberrations due to chromatic differences in focus are important (van Meeteren, 1974), since the eye can be in focus for only one wavelength, the retinal image formed by the other wavelengths must be blurred. The longitudinal chromatic aberration of the human eye (distance along optical axis of foci for the different wavelengths) is approximately 2D over the whole visible spectrum (Charman, 1983). Transverse chromatic aberration or chromatic difference in magnification may also be present, due to the misalignment of the optical and visual axes (van Meeteren, 1974). However, the overall effect of chromatic aberration on the retinal image is not generally considered to be large, although contrast sensitivity to medium spatial frequencies was reduced, contrast sensitivity to high spatial frequencies (and hence cut-off frequency) was little changed (Campbell & Gubisch, 1967). Chromatic aberration also increases the threshold for out-of-focus blurring, which leads to a greater depth-of-focus (Campbell, 1957). Bour (1980) found that monochromatic MTFs were generally no better than those for white light, suggesting that the monochromatic aberrations dominate over chromatic aberrations.

Further degradation of the retinal image results from intraocular scatter of light, due to small heterogeneities in the ocular media, particularly in the cornea and lens. The combined effects of all these aberrations become progressively more important as the pupil size increases. However, under photopic conditions the visual system partially compensates for this due to the narrow acceptance angle at the cone photoreceptors (Stiles-Crawford effect). Light entering the periphery of the pupil falls obliquely on the cones and is not reflected or "trapped" by the cone inner segments which then funnel the rays to the outer segments which contain the light sensitive pigment (Miller & Snyder, 1977). This reduces the "effective" pupil diameter for large pupils and results in an increased depth-of-focus.

Experimental measurements of the ocular quality of the human eye for a range of pupil sizes fall into two categories. The first is objective assessments of the MTF of the ocular media by measuring the line spread function of the faintly reflected fundal image of a thin line of light (Campbell & Gubisch, 1966; Charman & Jennings, 1976). Second, subjective assessments have been made of the effect of pupil size on visual acuity, as measured by discrimination of parallel vertical lines (Campbell & Gregory, 1960), the Landolt C (Jenkins, 1963) and Snellen letters (Tucker & Charman, 1975) and on contrast sensitivity (Campbell & Green, 1965; Charman, 1979). Generally, ocular quality is found to increase as pupil diameter decreases, with maximal quality at around 3mm; below this it again declines due to increased diffraction. Experimental measurements of the effects of pupil diameter (with constant retinal illumination) on the MTF of the eye agree closely with theoretical predictions based on the combined effects of aberrations and diffraction (van Meeteren, 1974). However, in studies where no compensation has been made for the changes in retinal

illumination with pupil size, less variation in visual acuity with pupil diameter was seen (Jenkins, 1963). Previously, no significant change in contrast sensitivity was reported over a range of spatial frequencies between the natural pupil (8mm) or a 3mm artificial pupil (Morrison & McGrath, 1985).

So far, we have been considering optical quality for an eye in optimal focus. Defocus, however, will cause further degradation of the image due to the formation of a blur circle around the true focus of a point source. The size of the blur circle is directly related to the pupil diameter. Out-of-focus imaging improves at smaller pupil diameters when the depth-of-focus increases (Campbell, 1957; Tucker & Charman, 1975). Errors of refraction are commonplace especially since the refractions required to bring different spatial frequencies into focus differ viz. high spatial frequencies require an additional +0.8D compared with low spatial frequencies for large pupil diameters, due to the effects of spherical aberration (Green & Campbell, 1965). Also errors of accommodation are common even in the natural eye, for the resting state of accommodation is not at 0 D but at 1.0 -1.5 D (Morgan, 1944; Hennessy et al., 1976) and visual resolution is closely related to accommodation accuracy over a wide range of luminances and stimulus distances (Johnson, 1976). Viewing a brightly illuminated field like a featureless blue sky leads to an involuntary accommodative effort of some 1.7D which is referred to as empty-field or space myopia (Whiteside, 1952). Accommodation also increases in twilight when the eye becomes myopic by 1.5-2.0D (Campbell & Primrose, 1953), though there is an element of 0.5-1.5D in this case due to increased spherical aberration resulting from dilation of the pupil (Koomen, Scolnik & Tousey, 1951). Another condition related to the resting state of accommodation is instrument myopia, where subjects overaccommodate by

~2D when viewing through an optical instrument like a microscope (Hennessy, 1975).

The visual system is able to tolerate a degree of defocus blur, this has been measured psychophysically to be 0.20-0.25D (Whiteside, 1957; Campbell & Westheimer, 1958). Theoretically however, appreciable degradation should occur when the Rayleigh criterion of λ / 4 (i.e. greater than only 0.03D) is exceeded (Hopkins, 1962). Hopkins has also calculated the MTFs for a diffraction limited optical system corresponding to different levels of defocus. In theory, the changes in retinal image quality associated with defocus lead to an accelerated fall in image contrast with increasing spatial frequency as shown in Fig. 2. which is taken from Hopkins (1955). When defocus reaches a certain level (n = 2 in Fig. 2.), the contrast ratio becomes negative for certain spatial frequencies. This is known as spurious resolution and results in an image of a grating with reversed contrast and occurs at a lower spatial frequency for greater amounts of defocus.

Measurements for the human eye show that defocus broadens the line-spread function and reduces its central amplitude. For a given amount of defocus, image quality deteriorates more rapidly with a larger pupil (Westheimer & Campbell, 1962). Optical performance measured in this way is comparable to that which would be predicted from geometrical optics provided the errors of focus are greater than 0.5D (Charman & Jennings, 1976). However, contrast sensitivity and ocular quality measured for various amounts of defocus are generally higher than predicted from theory (Campbell & Green, 1965; Charman, 1979), due to geometrical aberrations which reduce the MTF at optimal focus relatively more than at out-of-focus positions. In practice, defocus has a severe effect on contrast sensitivity at medium and high spatial frequencies, but a much higher tolerance of defocus exists at low spatial frequencies

(Campbell & Green, 1965; Green & Campbell, 1965). Defocus has less effect on contrast sensitivity at small pupil diameters (Charman, 1979).

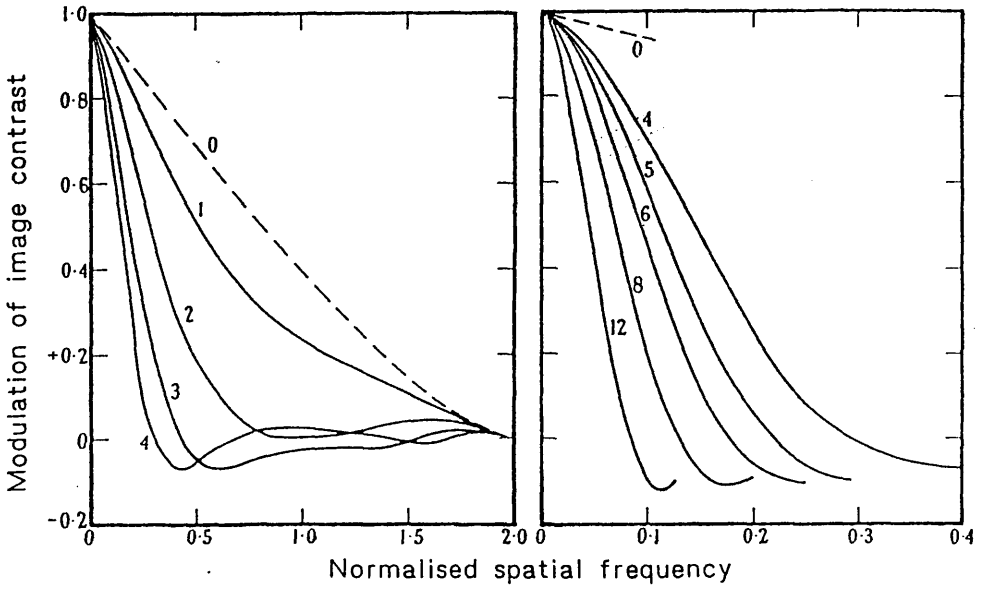


Fig. 2. Computations by Hopkins (1955) of the effects of defocus on the MTF for a diffraction-limited optical system. Spatial frequency is normalised so that the value of 2.0 corresponds to the cut-off value for the system [this cut-off is given by $(1.746 \times 10^4 a)\lambda$ c/deg for the eye, where a is pupil diameter in mm and λ is wavelength in nm]. The figures on the curves give the defocus terms of the parameter n, where the corresponding wavefront aberration is $w_{20} = (n/\pi)\lambda$. A value of n = 1 corresponds to a defocus of approximately 0.125 D when the eye has a pupil diameter of 3.3 mm and the wavelength is 555 nm. Note the expanded abscissa on the right hand graph. Reproduced with permission from Hopkins (1955).

Influence of cholinergic drugs on visual function

Cholinergic drugs influence visual function largely because of their effects on the parasympathetically innervated ciliary muscles and sphincter pupillae of the iris leading to changes in accommodation (focus) and pupil diameter, respectively. There may also be an action via the sympathetic nervous system through an effect on the sympathetic ganglia. Topical and systemic administration of cholinergic drugs may also affect central visual function (Alpern & Jampel, 1959; Trussov, 1962). Before considering the pharmacology of the cholinergic drugs investigated in this work, the role of cholinergic transmission in central visual processing will be discussed.

Cholinergic mechanisms in the retina

It is now well established that acetylcholine is a neurotransmitter in the vertebrate retina: the evidence for this has been recently reviewed by Neal (1983) and Hutchins (1987). Histochemical studies have shown that the retina contains significant quantities of the enzymes cholineacetyltransferase (ChAT) which catalyses the synthesis of acetylcholine from choline and acetyl-coenzyme A, and acetylcholinesterase (AChE) which hydrolyses acetylcholine not bound to receptors and thus terminates its physiological action (Graham, 1974). Both enzymes are mainly confined to the inner retinal layers with the highest activity in the inner plexiform layer (IPL) (Neal, 1983). ChAT activity has also been found in human retinal homogenates (Hutchins & Hollyfield, 1986) and AChE activity has been reported in the human IPL (Hutchins & Hollyfield, 1987a).

The identity of the cholinergic cells was first established in the rabbit retina using autoradiography (Masland & Mills, 1979). Two

populations of amacrine cells were found to synthesise acetylcholine; one in the inner nuclear layer and one in the ganglion cell layer, which were later identified as displaced amacrine cells (Hayden, Mills & Masland, 1980). ChAT-like immunoreactivity has also been demonstrated in the retinae of rat (Voight, 1986), cat (Schmidt, Wassle & Humphrey, 1985; Pourcho & Osman, 1986), monkey (Mariani & Hersch, 1988) and human (Hutchins & Hollyfield, 1987b). In each case the reactivity was located in specific types of amacrine cells. Morphologically, these cholinergic amacrine cells have "starburst" dendritic fields in rabbit (Famiglietti, 1983; Masland & Tauchi, 1986), rat (Voight, 1986) and cat retinae (Schmidt, Humphrey & Wassle, 1987). Starburst amacrine cells have also been found in human retinae (Golgi stain; Rodieck, 1986) but they have not yet been shown to be cholinergic.

Both muscarinic and nicotinic acetylcholine receptors have been reported in the retina of many species. The localisation of nicotinic receptors has been studied using alpha bungarotoxin (BTX) although the binding of this molecule may not be totally selective for this receptor (Neal, 1983). For muscarinic receptors, however, the binding of [^{3}H]quinuclidinylbenzylate and [^{3}H] propylbenzilylcholine is generally accepted to be specific. In human retina, binding sites for both muscarinic markers and BTX have been found in the IPL (Hutchins & Hollyfield, 1985; Zarbin et al., 1986). The cellular localisation of receptors has been determined in chick retina; BTX binding sites occur on ganglion, bipolar and amacrine cells, whereas muscarinic receptors occur only on amacrine and/or bipolar cells (Morgan & Mundy, 1982). In rat retina, Redburn et al., (1984) proposed that muscarinic receptors occur on amacrine cells but that BTX binding sites occur only on ganglion cells and Lipton, Aizenman & Loring (1987) have shown pharmacologically that cholinergic receptors on isolated rat retinal ganglion cells are nicotinic.

Physiological evidence that acetylcholine is a retinal neurotransmitter has come from several sources. Masland & Livingstone (1976) first demonstrated that rabbit retina, in vitro, synthesises acetylcholine from labelled precursors, which has also been reported in human retina (Hutchins & Hollyfield, 1986). More importantly, Masland & Livingstone showed that rabbit retina released acetylcholine in response to stimulation by light and that rapid changes of illumination were most effective in producing this release. Masland and his co-workers have shown that there is a burst of acetylcholine released at light onset and a second burst of acetylcholine after the off stimulus. It has been suggested that displaced cholinergic amacrine cells are responsible for the "ON" response and the conventionally placed amacrines for the "OFF" release of acetylcholine (Masland & Tauchi, 1986). Other neurotransmitters can affect the release of acetylcholine from the retina (Neal, 1983). Glycine reduces the light-evoked release of acetylcholine as does the cholinergic agonist muscarine, whereas the muscarinic antagonist, atropine increases the release (Cunningham, Dawson & Neal, 1983). Strychnine, a glycinergic antagonist blocked the effects of both atropine and muscarine, suggesting that they may have been acting via an inhibitory glycinergic amacrine cell (ibid).

The functional postsynaptic effects of cholinergic amacrine cells have been studied principally by the effects of cholinergic agonists and antagonists on the activity of retinal ganglion cells.

In the rabbit, Masland & Ames (1976) showed that most ON-centre or directionally selective and many OFF-centre ganglion cells were excited by acetylcholine and their spontaneous and light evoked activity was enhanced by anticholinesterases and depressed by cholinergic antagonists: nicotinic antagonists having greater efficacy than muscarinic antagonists. Ariel & Daw, (1982a) found that all ganglion

cells with complex receptive fields received a light-driven cholinergic input. Furthermore, the light evoked response of directionally selective cells were, of all ganglion cell types studied, the most affected by cholinergic drugs (Ariel & Daw, 1982b) which may reflect a stronger cholinergic excitatory input to these cells (from cholinergic amacrine cells) compared with other ganglion cells.

In the cat retina, Ikeda & Sheardown (1982) showed that ionophoresis of acetylcholine enhanced both the spontaneous and visually-driven activity of "transient" ganglion cells (with the periphery effect). This response was blocked by dihydro-β-erythroidine (dHβE), a nicotinic antagonist but not by atropine. However, Schmidt et al., (1987) showed that acetylcholine excited both "transient" and "sustained" ganglion cells and that hyoscine, a muscarinic antagonist, blocked the effects of ionophoretically applied acetylcholine in both cases, whereas dHβE only blocked the effect of acetylcholine on OFF-centre cells. Despite the apparent difference in results between these two groups of workers, there appears to be unequivocal evidence of the involvement of cholinergic inputs to at least one class of retinal ganglion cell.

Cholinergic influences in other parts of the visual pathway

The optic nerve is notable for its absence of ChAT (Hebb & Silver, 1956) and neither atropine nor dHβE greatly affect the response of lateral geniculate nucleus (LGN) relay neurones to stimulation of the optic tract, indicating that acetylcholine is not the neurotransmitter released by the axon terminals of retinal ganglion cells (Phillis, Tebecis & York, 1967). Nevertheless, there is a large amount of AChE present in the LGN which seems to be associated with terminals originating from the pontine and mesencephalic reticular formation and

may be involved in the arousal response (Singer, 1979). Indeed Phillis et al., (1967) found that many LGN neurones were excited by acetylcholine and stimulation of the reticular formation facilitated the response of these cells. Sillito, Kemp & Berardi (1983) have shown ionophoresis of acetylcholine has a strong excitatory action on neurons of the dorsal lateral geniculate nucleus (dLGN) and suggest that the function of the cholinergic input may be to enhance stimulus-specific inhibitory interactions within the dLGN. More recently, McCormick & Prince (1987) showed that application of acetylcholine can result in both excitation and inhibition of dLGN and medial geniculate cells in the cat and guinea pig. Three separate responses could be demonstrated: a rapid depolarisation due to activation of nicotinic receptors which in some cells was followed by a hyperpolarisation; this was then followed by a slow depolarisation above the resting potential in some cells. The latter two responses were due to activation of muscarinic receptors. Such mechanisms may not exist in all species, however, for in the rat dLGN only a very few cells respond with a hyperpolarisation (Crunelli et al., 1988). In cat, the only difference reported between "X" and "Y" dLGN cells' responses following ionophoresis of acetylcholine has been a specific enhancement of the late part of the "Y" cell "shift" response (elicited by stimulation outside the conventional receptive field): this was presumed to be a disinhibition effect (Eysel, Pape & van Schayck, 1987).

There is also some evidence for cholinergic neurotransmission in the superior colliculus due to the presence of AChE and a high affinity choline uptake mechanism in rabbit (Wichmann, Illing & Stark, 1987).

The visual cortex also receives a cholinergic input and the reticular formation again appears to be the source (Singer, 1979). However, the visual cortex has the lowest concentration of ChAT in the

entire cerebral cortex (Hebb & Silver, 1956). Anticholinesterases are known to reduce the cortical visual evoked response (VER) in cat (Harding, Wiley & Kirby, 1983; Harding, Kirby & Wiley, 1985; DeBruyn, Gajewski & Bonds, 1986) and this effect can be reversed by atropine, suggesting that muscarinic receptors are involved.

Ionophoretically applied anticholinesterases also affect the responses of single cells in the primary visual cortex of cat (DeBruyn & Bonds, 1987). Physostigmine, for example, caused an increased firing to visual stimulation in 61% of cells and a decreased firing in 30% of cells; 9% of cells were unaffected. Similarly, Sillito & Kemp (1983) found 92% of striate cortex cells exhibited a modification in their visual response during ionophoresis of acetylcholine; 61% of responses being facilitated and 31% depressed. The effect was antagonised by atropine. Sato et al., (1987) suggested that the responses of striate cortical cells may be tonically modulated by acetylcholine, to maximise the signal to noise ratio of visual signals.

Cholinergic Pharmacology

Nature and function of cholinesterase

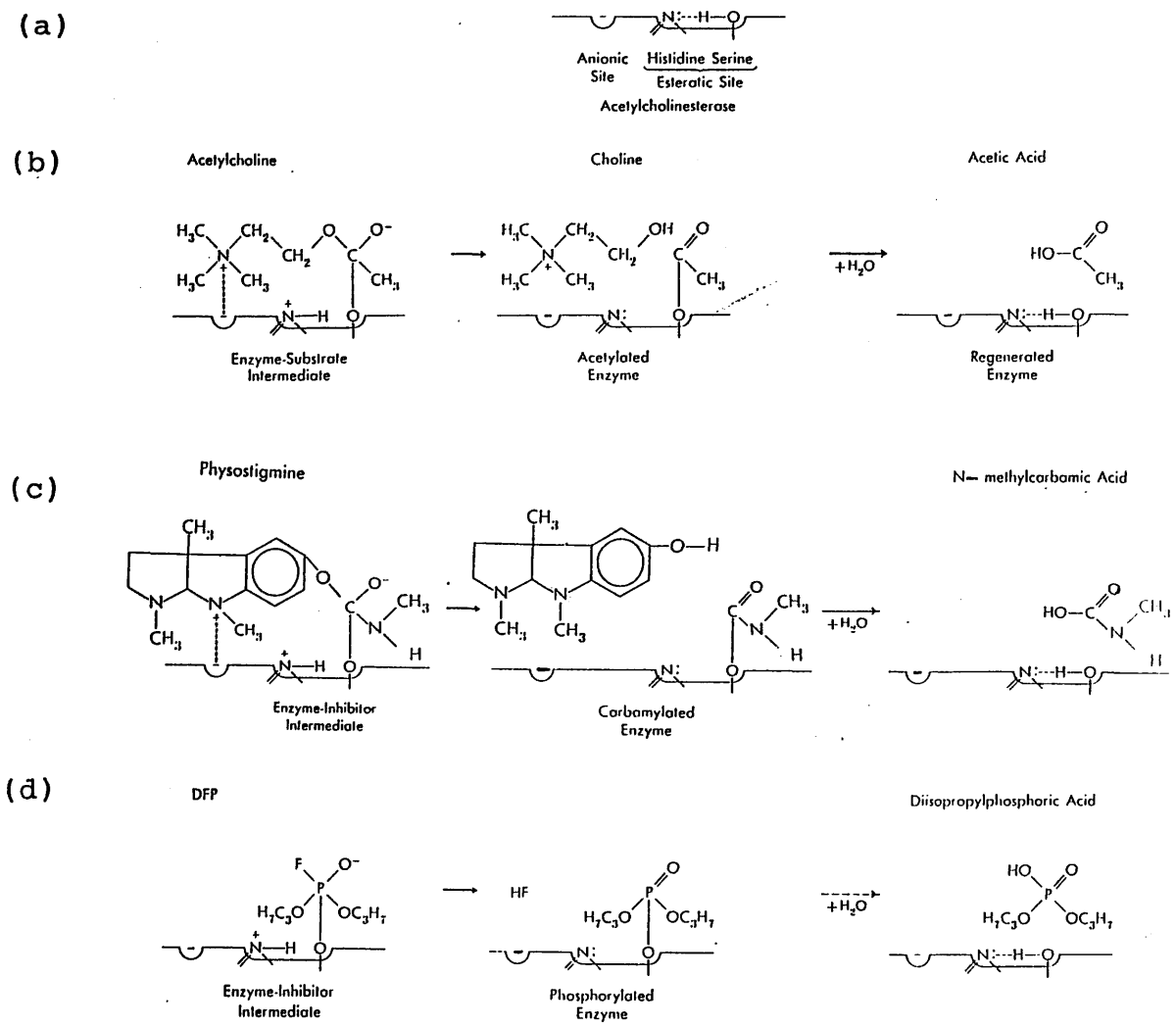
The duration and magnitude of a response to acetylcholine, as with other neurotransmitters, depends not only on release but also on the removal of the neurotransmitter from the region of the receptor. For example, at the skeletal neuromuscular junction, if acetylcholine is not removed rapidly from the synaptic cleft then the sub-synaptic membrane remains depolarised and hence unresponsive to further stimuli. Removal of acetylcholine from the neuromuscular synaptic cleft is accomplished by the enzyme acetylcholinesterase and also by diffusion of the transmitter away from the receptor area. In fact, two kinds of cholinesterase enzyme are found in mammals, acetylcholinesterase

(AChE, true cholinesterase) and butyryl-cholinesterase (BuChE, pseudocholinesterase). AChE is found largely in the post-synaptic membranes at cholinergic synapses, and in red blood cells (where its function is not known; Day, 1979). BuChE is found in mammalian plasma, the intestine and in many glial cells in the central nervous system. The function of BuChE is not clear but may include a role in active sodium transport (Hobbiger, 1968) and in regulating the proposed action of acetylcholine on intestinal tone (Day, 1979). BuChE is little involved with the hydrolysis of endogenous acetylcholine (Cullumbine, 1963).

The AChE molecule has two active sites for combination with acetylcholine; the anionic site which bears a negative charge and the esteratic site which bears a positive charge (Fig. 3a). AChE combines with acetylcholine and forms a reversible enzyme-substrate complex as the positively charged head of acetylcholine attaches to the anionic site of AChE. This hydrolyses the ester linkage of the acetylcholine molecule and free choline is released. The acetyl group is then thought to covalently bind with the esteratic site of AChE. Acetylated AChE is very unstable, with a half-life of less than 1ms, and is rapidly hydrolysed to form acetic acid and unbound enzyme which is then ready to combine with another acetylcholine molecule (Fig. 3b).

Mechanism of action of anticholinesterases

Anticholinesterases preserve acetylcholine at sites of cholinergic transmission by preventing its hydrolysis by cholinesterases (diffusion away from the receptor still takes place). The actions of anticholinesterases are almost entirely attributable to the inhibition of AChE since inhibition of BuChE at most sites produces no apparent functional change (Hobbiger, 1968). Anticholinesterases are classified into two groups, reversible and irreversible.



Heavy, light, and dashed arrows represent extremely rapid, intermediate, and extremely slow or insignificant reaction velocities, respectively.

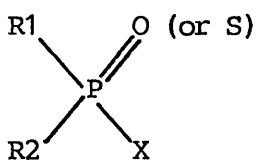
Fig. 3. Representation of the AChE molecule and its active sites (a). The steps involved in the hydrolysis of acetylcholine by AChE (b) and in the inhibition of AChE by physostigmine (c) and by the organophosphorus anticholinesterase DFP (d). Adapted from Taylor, 1980.

A. Reversible anticholinesterases

Examples are physostigmine, neostigmine and pyridostigmine which contain a carbamyl ester group (a condensation product of a carboxylic acid and an amine) and form a reversible complex with AChE in essentially the same way as acetylcholine (Fig. 3c). The carbamylated enzyme is more stable than the acetylated enzyme (Fig. 3b) and is hydrolysed at a much slower rate. Thus, the anticholinesterase competes with acetylcholine for binding sites on the AChE enzyme thereby protecting acetylcholine from hydrolysis. The time course of the inhibition of AChE depends on the structure of the anticholinesterase. For example, neostigmine is longer lasting than physostigmine as its ammonium group provides greater binding stability at the anionic site of AChE, whereas edrophonium, a similar compound to neostigmine but without a carbamyl group is less potent and much shorter acting (Taylor, 1980). A full account of the structure and activity of reversible anticholinesterases is given by Long (1963).

B. Irreversible anticholinesterases

These substances are mainly organic compounds containing pentavalent phosphorus and are extremely long-lasting and potent anticholinesterases. They can be described by the general formula which was first given in 1937 by Schrader (Holmstedt, 1963).



R1 and R2 are almost infinitely variable, e.g. alcohols, phenols, amides or alkyl groups (Holmstedt, 1963). Common X radicals include

fluorine (e.g. in diisopropyl fluorophosphate, DFP) and phosphates (e.g. tetraethyl pyrophosphate, TEPP). Detailed tables with the structure, activity and toxicity of numerous organophosphate anticholinesterases are given by Holmstedt (1959; 1963).

Many organophosphorus compounds do not have a quarternised nitrogen to react with the anionic site of AChE (as is the case for physostigmine) and are thought to react only with the esteratic site of the enzyme (Fig.3d). The stages of the interaction of these compounds with AChE are generally similar to those of the reversible inhibitors. The enzyme and inhibitor again form a complex which is still capable of dissociation. The group X is then removed by hydrolysis leaving the esteratic site of AChE phosphorylated. The phosphorylated enzyme is extremely stable and secondary (as in DFP) or tertiary alkyl groups further enhance this stability. Removal of the phosphoryl group from the enzyme may take weeks or months and thus for most practical purposes the complex is irreversible. The return of cholinesterase activity depends on the synthesis of new enzyme.

Actions of anticholinesterases in vivo

Anticholinesterases allow acetylcholine to accumulate at cholinergic receptor sites and thus enhance and prolong the effects produced by stimulation of pre- and postganglionic autonomic cholinergic nerves. The symptoms of acute poisoning by anticholinesterases in man are quite well documented for both local and systemic absorption and consist of so-called muscarinic and nicotinic components (Table 1; reviewed by Grob, 1963).

At the neuromuscular junction, the persistence of acetylcholine leads to fasciculation of the muscle due to prolongation of the endplate currents and even antidromic firing of the motor nerve fibres due to

binding of acetylcholine to cholinergic receptors on the motor nerve terminal. (Brown, Dale & Feldberg, 1936; Morrison, 1977). With sufficient inhibition of AChE at the endplate a depolarisation block may develop, which, if in the respiratory muscles, will result in death. The spontaneous, quantal release of acetylcholine from nerve terminals which cause miniature endplate potentials (Fatt & Katz, 1952) can in the presence of an anticholinesterase, lead to stimulation of post-synaptic cells as they can lead to the build up of an effective concentration of acetylcholine in the synaptic cleft under these conditions (Katz, 1966). If the anticholinesterase is lipid soluble it can cross the blood-brain barrier and affect the central nervous system. Central effects are characterised by stimulation at low concentrations, followed by depression at higher concentrations, which can lead to changes in the EEG and respiration (Grob & Harvey, 1953; Dille & Smith, 1964).

The most frequent causes of systemic anticholinesterase poisoning are inhalation of sprays or dust of insecticides or contamination of the skin in the case of agricultural workers (Wolfe, Durham & Armstrong, 1967), contamination of crops or food (Hayes, 1967) and direct ingestion (Toivonen, Ohela & Kaipaieu, 1965). However, the development of organophosphorus nerve agents has produced another potential risk of anticholinesterase poisoning (see p. 45)

Table 1. Signs and symptoms of anticholinesterase poisoning in man. Reproduced, with permission, from Grob (1963).

Site of action	Signs and symptoms
	Following Local Exposure
Muscarinic	
Pupils	Miosis, marked, usually maximal (pin-point), sometimes unequal
Ciliary body	Frontal headache; eye pain on focusing; slight dimness of vision; occasional nausea and vomiting
Conjunctivae	Hyperemia
Nasal mucous membranes	Rhinorrhea; hyperemia
Bronchial tree	Tightness in chest, sometimes with prolonged, wheezing expiration suggestive of bronchoconstriction or increased secretion; cough
Sweat glands	Sweating at site of exposure to liquid
Nicotinic	
Striated muscle	Fasciculation at site of exposure to liquid
	Following Systemic Absorption
Muscarinic	
Bronchial tree	Tightness in chest, with prolonged, wheezing expiration suggestive of bronchoconstriction or increased secretion; dyspnea; slight pain in chest; increased bronchial secretion; cough; pulmonary edema; cyanosis
Gastrointestinal	Anorexia; nausea; vomiting; abdominal cramps; epigastric and substernal tightness (? cardiospasm) with "heartburn" and eructation; diarrhea; tenesmus; involuntary defecation
Sweat glands	Increased sweating
Salivary glands	Increased salivation
Lacrimal glands	Increased lacrimation
Heart	Slight bradycardia
Pupils	Slight miosis, occasionally unequal; later, more marked miosis
Ciliary body	Blurring of vision
Bladder	Frequency; involuntary micturition
Nicotinic	
Striated muscle	Easy fatigue; mild weakness; muscular twitching; fasciculation; cramps; generalized weakness, including muscles of respiration, with dyspnea and cyanosis
Sympathetic ganglia	Pallor; occasional elevation of blood pressure
Central nervous system	Giddiness; tension; anxiety; jitteriness; restlessness; emotional lability; excessive dreaming; insomnia; nightmares; headache; tremor; apathy; withdrawal and depression; bursts of slow waves of elevated voltage in EEG, especially on overventilation; drowsiness; difficulty in concentrating; slowness of recall; confusion; slurred speech; ataxia; generalized weakness; coma, with absence of reflexes; Cheyne-Stokes respiration; convulsions; depression of respiratory and circulatory centers, with dyspnea, cyanosis, and fall in blood pressure

Historical review of physostigmine

Daniell, a British Army medical officer, stationed in West Africa first reported in 1840 the use of the "ordeal bean of Calabar", in native judicial procedure. Those on trial were made to swallow a solution containing the crushed bean and then to walk continuously until it took effect. If, however, after the lapse of a predetermined period the accused vomited the poison, his innocence was considered vindicated and he was set free. Following Daniell's report of this to the Edinburgh Ethnological Society, a botanist at Edinburgh University, Balfour, became interested in the tall woody vine which produced the Calabar beans, identified it as a member of the pea family, and named it Physostigma venenosum.

Christison, a toxicologist also of Edinburgh University, was first to experiment with the use of the Calabar bean on animals and also on man. Indeed, Christison ate a small amount of the bean himself and found it caused a great feeling of weakness with some giddiness and nausea and a weak, irregular pulse. Thomas Fraser, an assistant of Christison, continued the study of the Calabar bean and isolated the active principle, an alkaloid, which became known as eserine, from the native name for the ordeal poison. Fraser (1863) was first to observe the constriction of the pupil associated with the use of physostigmine in man, and noted that the pupil constricted within a few minutes if physostigmine was applied either to the eyeball or to the skin around the eye. He also noted that the immediate reaction to placing a drop of the physostigmine solution onto the eyeball was a copious discharge of tears. Ten minutes after application, the pupil had constricted to 1/16 inch diameter (1.6 mm) and vision was described as being imperfect. By 30 minutes, however, vision was almost lost and there was redness of the eye and tenderness on exposure to light. These uncomfortable symptoms

lasted for 1-2 hours, the dimness of vision lasted for 4 hours and the pupil remained fully constricted for 24 hours after which it slowly returned to normal over a period of 5 days.

Douglas Argyll Robertson, a practising ophthalmologist, also experimented with physostigmine on his own eye. He wished to find a drug which would produce an effect on the pupil exactly opposite to that of belladonna (atropine). He confirmed that physostigmine constricted the pupil and increased accommodation to cause indistinct distance vision which was relieved by concave glasses. He then repeated the experiment with one drop of physostigmine followed by a drop of atropine 30 minutes later. Following physostigmine, the pupil had constricted and the near point had moved closer to a distance of 5 inches (~13 cm) from the eye. A concave lens of 8 inches negative focus (- 5.0 D) was required for distant objects to be clearly seen. The atropine solution then caused the pupil to dilate and all objects beyond 3 feet could then be distinctly viewed unaided. These experiments led Robertson to propose that physostigmine and atropine were antagonistic in their action on the eye (Argyll Robertson, 1863). Shortly after this, physostigmine was first used as a treatment for systemic atropine poisoning by another ophthalmologist, Kleinwachter, in Prague in 1864 (translated by Nickalls & Nickalls, 1988). Nowadays, physostigmine is used as an antidote for poisoning by over 600 varieties of cholinergic antagonists (Daunderer, 1980). Conversely, Fraser (1870) in an animal study, demonstrated that the lethal effect of physostigmine could be prevented by atropine. Atropine is still used today to treat anticholinesterase poisoning (see p. 55).

Argyll Robertson did not realise the value of the Calabar bean extract as a miotic in glaucoma, but suggested that it be applied after atropine eyedrops given as a mydriatic prior to ophthalmological

examination of the eyes. He also proposed its use for the relief of photophobia and paralysis of the ciliary muscles. Ludwig Laqueur (1876) was the first ophthalmologist to use physostigmine therapeutically in glaucoma and Weber first measured the reduction in intraocular pressure caused by physostigmine in 1876. The development of physostigmine as a miotic agent has been reviewed by several authors (Rodin, 1947; Lebensohn, 1963; Kronf..eld, 1970; Holmstedt, 1972).

Finally, in this historical review of physostigmine, the work of Anderson (1905), which advanced the understanding of its mechanism of action, should be mentioned. He demonstrated that physostigmine can constrict the pupil in normal cats but not in cats in which the ciliary ganglion has been removed and time allowed for degeneration of the post-ganglionic fibres. After post-ganglionic nerve fibre degeneration there are no nerve terminals present to release acetylcholine and hence anticholinesterases are without effect.

Effects of topically instilled physostigmine

Physostigmine, instilled into the conjunctival sac is absorbed through the cornea and reaches the intraocular smooth muscle of the sphincter pupillae and the ciliary body. These muscles are innervated by parasympathetic cholinergic fibres arising from the ciliary ganglion and physostigmine acts by preserving the tonic and reflex release of acetylcholine from the parasympathetic nerve endings. This leads to a sustained contraction of the sphincter pupillae (pupil constriction or miosis) and of the ciliary muscles (which may lead to a spasm of accommodation). As the ciliary muscle fibres contract, the diameter of the ciliary ring reduces and the ciliary body is pulled forwards. This reduces the tension on the suspensory ligaments and the elasticity of the lens capsule causes it to become more spherical to diminish its

focal length resulting in optimal focus for near vision.

The minimum concentration of physostigmine which will produce pupillary constriction in humans is of the order of 0.01% (Leopold, 1961). The extent and duration of the miosis depends on the dose administered and the viewing conditions under which measurements are made. In rabbits, after instillation into the conjunctival sac, physostigmine can be demonstrated in the iris for 7-8 hours which corresponds to the duration of miosis (Schumacher, 1956). At low concentrations, physostigmine enhances the pupil's contraction to light, because the extra acetylcholine at cholinergic terminals allows each light flash to elicit a contraction which is faster, stronger, and longer lasting than normal. High frequency repeated light flashes lead to a tetanic contraction of the pupil. Under the influence of physostigmine, the iris sphincter becomes tetanically contracted at a lower than normal stimulation rate. Higher doses of physostigmine reduce the light reflex, as only little further contraction (although longer lasting than normal) can be elicited from the already constricted pupil (Loewenfeld, 1963).

The increase in accommodation starts at about the same time as the miosis but is of a much shorter duration. Any intense accommodative spasm tends to disappear in about 2 hours, but may remain irritable so that the slightest effort of accommodation for looking at a near object results in a spasm of accommodation (O'Connor Davies, 1981). Physostigmine also increases the amplitude of voluntary near point accommodation (Fincham, 1955). Again, in rabbits, physostigmine can be demonstrated in the ciliary body for 4 hours, which corresponds to the duration of the effects on accommodation (Schumacher, 1956).

Although the blurring of far vision associated with the accommodative spasm was measured by Argyll Robertson in 1863, very

little quantitative work has been reported since that time. Mattila, Takki & Idanpaan-Heikkila, (1967) studied the effects of one drop of 0.5% physostigmine salicylate and found that two thirds of the subjects reported impaired vision, a decrease in Snellen visual acuity from 6/4 to 6/36 was not uncommon. Rengstorff (1970) investigated the effects of one or two drops of 0.5% physostigmine salicylate, which was instilled into one or both eyes. Two drops were more effective than one. He found that accommodation could increase by up to 7.75D, 30 min after instillation, although there was large variation between subjects as to the extent of the increase in accommodation. Visual acuity decreased from 6/6 to as little as 6/120. No consensual effect was present; physostigmine applied to one eye had no effect on the pupil size or refractive state of the contralateral eye.

The electrical and motor activity of extraocular muscles are also greatly enhanced by physostigmine and may show spontaneous contraction (Brown & Harvey, 1941).

As mentioned previously, topical application of physostigmine decreases intraocular pressure. Although in most cases, there is first a slight rise in intraocular pressure due to dilatation of the blood vessels and engorgement of the vascular supply of the iris and ciliary body. This is then followed by a decrease in intraocular pressure once the pupil has constricted allowing increased aqueous outflow at the sinus venosus sclerae (canal of Schlemm) (Leopold, 1961).

Flicker sensitivity of human eyes is reduced by about 1-2 Hz following 1% physostigmine and this is independent of pupil size (Alpern & Jampel, 1959). The authors believed that the effect was retinal and probably on nicotinic receptors because pilocarpine (muscarinic agonist) did not change the flicker fusion frequency. However, prior administration of homatropine (muscarinic antagonist)

effectively blocked the response, whereas homatropine itself had no measurable effect on the critical flicker frequency, suggesting that muscarinic receptors must in fact be involved. Also, Alpern & Jampel found a reduction in visual sensitivity, independent of pupil size, following topical physostigmine, although small intramuscular (i.m.) doses of physostigmine improved light sensitivity and accelerated dark adaptation (Trussov, 1962).

Development of organophosphorus nerve agents

Organic phosphorus compounds such as TEPP have been known since the middle of the last century. In fact, as Holmstedt (1963) points out, it was remarkable that De Clermont, the chemist who first synthesised TEPP, was not poisoned in 1854, for he reported tasting the compound he had made. It was not until 1932 that a vigorous interest in their chemistry occurred when Lange and Kruegar gave the first indication of the extreme toxicity of these compounds. During the synthesis of dimethyl and diethyl phosphorofluoridate, the workers found that inhalation of the vapours caused a persistent choking sensation and blurred vision (Holmstedt, 1963). This discovery led directly to the long sequence of synthetic organophosphates with insecticidal and toxic properties. It also led to the synthesis in Germany of chemical warfare agents, chiefly by Schrader at Farbenfabriken Bayer, and also prompted the first synthetic and toxicological work with these compounds in Britain (these studies were carried out during the Second World War but only reported afterwards - Kilby & Kilby, 1947; Aldridge et al., 1947). During the war Schrader is said to have synthesised around 2000 organophosphorus compounds, though most were kept secret by the German Government. Among these compounds were the fluorine-containing compounds including DFP and the pyrophosphorus derivatives including TEPP, and

also the nerve agents Tabun or GA (ethyl N-dimethyl-phosphoramidocyanidate), Sarin or GB (isopropyl methylphosphono-fluoridate) and Soman or GD (pinacolyl methylphosphonofluoridate). The nerve agents are among the most toxic of the chemical warfare agents and are particularly dangerous as they act very rapidly and may be absorbed through any body surface. The so-called "V" agents, e.g. VX (O-ethyl-S-(2-diisopropylaminoethyl) methylphonothiolate) are most persistent as they do not disperse but remain over the area of release.

Effects of organophosphorus compounds on visual function

The first reported systematic study of the effects of organophosphorus compounds involved the exposure of volunteers to low concentrations of DFP (Kilby & Kilby, 1947). A long lasting pupillary constriction and a small increase in near point accommodation was observed. Blurring of far vision was also reported and later quantified by Scholz & Wallen (1946) at 1D to 6D which began 3 hours after exposure and lasted for several days. Aldridge et al. (1947) then went on to investigate the effects of DFP vapour (but at a lower concentration than that tested by Scholz & Wallen) on visual function when just the eyes were exposed (while the volunteer breathed uncontaminated air). They again observed a marked (though sometimes unequal) pupillary constriction and an increase in near point accommodation; however, Snellen visual acuity was unchanged. The scotopic visual threshold increased by a factor of, at most, ten which is slightly less than would have been expected from the changes in pupil diameter which occurred.

The visual effects accompanying the miosis induced by TEPP, which is used as an insecticide, have also been studied in some detail (Upholt et al., 1956). Doses of TEPP sufficient to give a maximal miosis also resulted in an increase in accommodation and a decrease in sensitivity

to light. When miosis was induced in only one eye, subjects complained of an inability to perceive distance properly and experienced some stumbling or other sensorimotor accidents. The authors point out that these effects are the same as the Pulfrich effect, which causes the subject with unequal light intensities in the two eyes to perceive moving objects falsely; thus a pendulum appears to rotate in an ellipse instead of moving in one plane. Any change in accommodation may also cause a change in vergence of the eyes since accommodation and convergence are linked by a common neural mechanism which activates the two together and to which pupil diameter is also related (Davson, 1980), although some people can sever this link (Morrison & Whiteside, 1984). Thus, any change in vergence may add to the difficulties in depth perception.

Pilots engaged in aerial application of organophosphorus insecticides have an accident rate which is very much higher than that of other commercial flyers (Smith, Stavinoha & Ryan, 1968) and visual disturbances may be an important factor leading to pilot-error (Wood et al., 1971). Chronic exposure to organophosphorus insecticides can also produce various central effects and psychiatric symptoms such as depression, confusion, dizziness and an inability to perform familiar tasks (Dille & Smith, 1964). A case of accidental exposure to two organophosphorus insecticides in 19 farm workers reported that all experienced blurred vision on the day of exposure. Also, that 12 of the workers still reported problems of blurred vision, discomfort on reading and photophobia 4 months later (Whorton & Orbinsky, 1983).

The organophosphorus substance which has received the most attention as far as the visual system is concerned is the nerve agent Sarin. At the lowest effective dose of Sarin, miosis which lasts for about 24 hours and a general feeling of heaviness in and behind the eyes

is produced. After four times this dose, extreme miosis is seen which diminishes gradually over a period of 3 to 14 days. Considerable aching in and behind the eyes is also felt with difficulty in focusing and frontal headache which may last up to 2 to 5 days (Grob, 1956). In cases of accidental exposure to Sarin, the miosis may last for many weeks; the time course for pupil recovery and plasma cholinesterase are similar but was not complete for several months (Sidell, 1974).

Stewart, Madill & Dyer (1968) found that a Sarin solution instilled into the eye reduced sensitivity to light in proportion to the decrease in area of the pupil. The miosis reduced visual performance (on an eyesight test) in dim light and the reduction was greater as the visual threshold was approached. However, Sarin may also have some central visual effects, for experiments in which retinal illumination was held constant using a 2mm artificial pupil have indicated that low doses of Sarin vapour elevates both the time course of dark adaptation and the absolute scotopic threshold (Rubin & Goldberg, 1957). The recovery of photopic vision was complete after 4 hours, but scotopic vision was impaired for more than 24 hours. Topical instillation of Sarin solution did not elevate the absolute visual threshold despite a marked miosis (Rubin, Krop & Goldberg, 1957) and protecting both eyes, with goggles, from direct contact with Sarin vapour prevented the miosis but not the action on visual threshold. The authors concluded that Sarin was acting on central visual processing. In a further experiment, Rubin & Goldberg (1958) showed that 2mg atropine sulphate (i.m.) significantly reduced the elevation of threshold whereas 2mg atropine methylnitrate (i.m.) which does not cross the blood-brain barrier had no significant effect, thus providing additional support for some central effect of Sarin.

Gazzard & Thomas (1975) compared the effects of Sarin vapour to those of physostigmine eyedrops on visual function. A trend was found for visual thresholds for stimuli presented within the central visual field to be higher after exposure to Sarin vapour compared with those measured after physostigmine eyedrops. However, these differences were very small and may have been due to slight differences in the miosis (Sarin, 1.5mm compared to 1.5-2.0mm for physostigmine) or from defocus of the retinal image. Since inhibition of AChE occurred in the retina of guinea pigs after exposure to Sarin (Harris, Fleisher & Yamamura, 1971), this further suggests an action at the retina.

More recently, Rengstorff (1985) has studied the visual effects following accidental exposure to small doses of Sarin vapour in two men. Despite miotic pupils of 1mm diameter lasting for up to 45 days, and an increase in near point accommodation, Snellen acuity actually improved after exposure for both men who were hypermetropic, which was attributed to increased depth-of-focus. In more severe cases of accidental exposure to Sarin, visual effects (save the miosis) are not detailed, as immediate treatment must be given to save life (Sidell, 1974).

So, it appears that the visual effects of organophosphorus anticholinesterases are largely due to miosis and an increase in accommodation, the severity of which depends on the dose and method of administration. Anticholinesterases like DFP have been used therapeutically to correct hypermetropia and accommodative esotropia (Gellman, 1963), since they enhance the amplitude of accommodation without reflexly inducing convergence.

Protection of cholinesterase against irreversible inactivation

The reversible anticholinesterases, physostigmine and neostigmine, have been used as pre-treatments against the irreversible

organophosphorus anticholinesterases. In experiments on cat, Koster, (1946) showed that a pre-treatment with physostigmine had a marked protective action against the toxic effects of subsequent doses of DFP. The best protection was obtained when small doses of physostigmine were given just prior to exposure to DFP. When physostigmine was given after DFP, the toxic effects were enhanced. This additive action of physostigmine has also been seen after parathion exposure (Salerno & Coon, 1950). The probable mechanism of physostigmine's protection against DFP was revealed in an investigation by Koelle (1946); physostigmine protected serum cholinesterase from inactivation by DFP in an *in vitro* rat brain preparation. The degree of protection varied directly with the concentration of physostigmine. Similarly, *in vivo*, the cholinesterase activity of serum from cats pre-treated with physostigmine showed a smaller reduction after exposure to DFP than serum from control cats. The recovery of cholinesterase activity was more rapid in the physostigmine treated cats (Koster, 1946).

Pyridostigmine is also being considered as a possible pre-treatment against organophosphorus poisoning in man. Dirnhuber *et al.*, (1979) found pretreatment with pyridostigmine resulted in toleration of a dose of Soman 28 times greater than normal in rhesus monkeys and 15 times in marmosets. These levels of protection were higher than any reported in non-primate species and therefore suggested that this treatment might also be effective in man.

The protective action of physostigmine/pyridostigmine against organophosphorus anticholinesterases depends upon the ability of these compounds to inhibit AChE forming a semi-stable enzyme-complex which can spontaneously break down to free the enzyme. This fraction of the enzyme would therefore be protected against phosphorylation in subsequent poisoning by an organophosphate. Rapid excretion of the free

organophosphate along with spontaneous breakdown of the carbamate-AChE complex would provide sufficient free AChE to support vital functions.

Cholinergic antagonists; atropine and homatropine

Atropine (hyoscyamine), the classical antagonist to the action of acetylcholine at muscarinic receptors, is a naturally occurring alkaloid obtained from the deadly nightshade plant Atropa belladonna. Preparations of belladonna were known to the ancient Hindus and have been used by physicians for many centuries. During the time of the Roman Empire and again during the Middle Ages the deadly nightshade plant was frequently used as a poison. It also became popular in Renaissance Italy as both a poison and a cosmetic (to dilate the pupils), after which Linnaeus named the plant in the 18th century.

Atropine was first isolated in pure form by Mein in 1831 and its characteristic effect in blocking the cardiac effects of vagal stimulation was reported by Bezold & Bloebaum in 1867 (quoted by Weiner, 1980). Atropine is an ester of trophic acid and the complex organic base tropine. Homatropine, another muscarinic antagonist similar to atropine but with about one tenth the potency, has a much shorter duration of action. It is used clinically as a topical mydriatic (dilates the pupil) and cycloplegic (paralyses accommodation). Homatropine is a semi-synthetic compound produced by combining the base tropine with mandelic acid and acts in essentially the same manner as atropine. The structural formulae of atropine and homatropine are given in Fig. 4.

Combination of atropine with muscarinic receptors

Atropine and related compounds compete with acetylcholine for identical binding sites on muscarinic receptors. Although there are two

subtypes of muscarinic receptor; M1 and M2, these are equally sensitive to atropine and hyoscine (Hammer et al., 1980). First, the binding of acetylcholine with the muscarinic receptor must be considered. The acetylcholine molecule consists of three parts, each of which is involved in the combination with the muscarinic receptor (Fig. 5). First, the qua ternary nitrogen moiety is the centre of the "cationic head" bearing a strong positive charge and fits into a depression in the receptor surface, the anionic site, which bears a negative charge. Second, the acetyl group of the acetylcholine molecule which bears an overall negative charge binds with the receptor at the positively charged esteratic site. Third, the alkylamine chain of acetylcholine provides a bridge between the cationic head and the acetyl group and allows both ends to "fit" into the receptor. The overall binding is therefore a combination of electrostatic attraction and spatial fit (Day, 1979).

Similarly, the combination of atropine with the muscarinic receptor results from at least three points of attachment. Atropine also contains a nitrogen moiety which may be attracted to the anionic site of the muscarinic receptor. The hydroxyl group and the benzene ring of the trophic acid moiety of atropine are the other proposed active sites.

Atropine binding with the muscarinic receptor prevents access of the acetylcholine molecule. The antagonism is competitive and can be overcome by increasing the concentration of acetylcholine at the receptor sites. The selectivity of antagonism is very high at muscarinic receptors of smooth muscle, cardiac muscle and exocrine glands. However, very large doses of atropine may also partially block the nicotinic actions of acetylcholine at ganglia and in skeletal muscle (Weiner, 1980). Also, very low concentrations of atropine, below those

needed to block muscarinic receptors, have been shown to stimulate muscarinic receptors and cause contraction of isolated gut, showing that atropine can act as a partial agonist (Bowman & Rand, 1980).

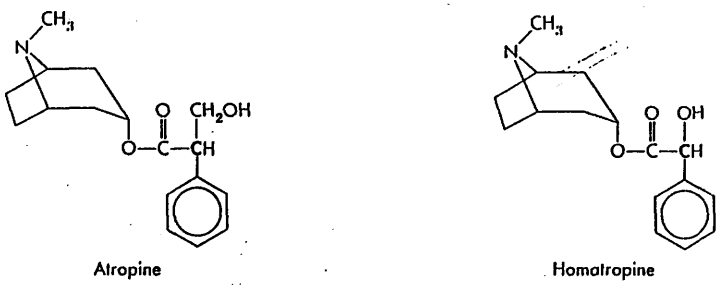


Fig. 4. Structural formulae of atropine and homatropine.

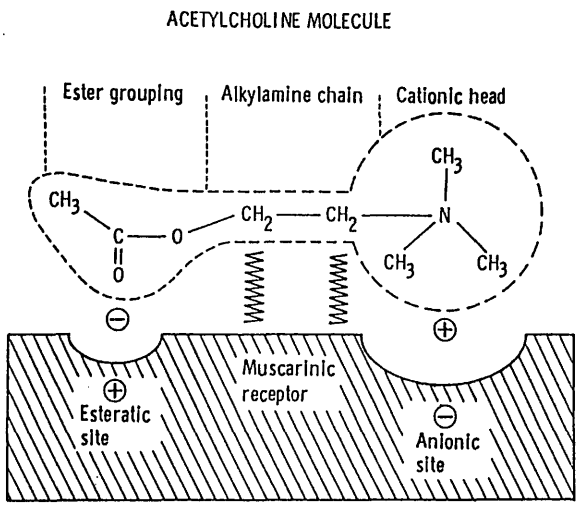


Fig. 5. Hypothetical mechanism involved in the combination of an acetylcholine molecule at three sites with the muscarinic receptor (from Day, 1979).

Pharmacological effects of atropine

As muscarinic receptors occur throughout the body, the effects of systemic atropine are very widespread (Martindale, 1982). Atropine causes a tachycardia due to diminished vagal tone and reduces gastrointestinal motility and salivary, bronchial and gastric secretions. The sweat glands of the skin are also inhibited and, at high doses, an increase in body temperature occurs. Micturition is also inhibited and the parasympathetic tone of the bronchi and bronchioles is reduced which leads to a dilation of the airways. In the sulphate form, atropine crosses the blood-brain barrier to cause central effects such as giddiness and ataxia. Finally, and most importantly as far as this work is concerned, atropine causes dilation of the pupils by antagonism of endogenously released acetylcholine, thus relaxing the circular smooth muscle of the iris, and paralysis of accommodation by blocking the excitation of the ciliary muscles. Photophobia and increased intraocular pressure also occur due to the mydriasis caused by atropine.

Clinical uses of atropine

In adults, one drop of 1% atropine produces mydriasis which is maximal within 30 to 40 min and may last for as long as 12 days (pupil diameter increased from 3.4mm to 8.3mm). Atropine cycloplegia is attained within a few hours and constitutes, on average, an 80% loss of accommodation which may last 2 weeks or longer (Wolfe & Hodge, 1946). In comparison, in the same study one drop of 1% homatropine produced maximal mydriasis in 10 to 30 min with recovery in from 6 hours to 4 days (pupil diameter increased from 3.4mm to 5.9mm). Homatropine caused a 45% loss of accommodation which was maximal at 30 to 90 min after instillation with recovery in 10 to 48 hours. A greater degree of cycloplegia can be obtained by the repeated administration of one drop

of 2% homatropine solution given at 10 minute intervals for one hour (Thorne & Murphy, 1939).

Clinically, atropine eyedrops are used routinely in children to produce mydriasis and cycloplegia in preparation for examination of the retina or for refraction. Cycloplegic refraction is important in children because of their very strong accommodative reserve which can, in the case of hypermetropes, mask a large part of their refractive error (O'Connor Davies, 1981). Atropine eyedrops are used therapeutically for certain diseases characterised by inflammation of the iris (iritis) since the prolonged immobilisation of the ciliary muscle and iris help to prevent the formation of adhesions and post-operatively after cataract extractions (details in Havener, 1983). Systemic atropine is also used as a premedication in surgery to obviate vagal inhibition of the heart and to inhibit salivary, gastro-intestinal and pulmonary secretions.

Atropine as an antidote to organophosphorus poisoning

The antagonism between atropine and physostigmine has been known since the work of Argyll Robertson (1863) and Fraser (1870) and since this time, atropine has become the drug of choice for all cases of anticholinesterase poisoning including that of organophosphorus agents. However, skeletal muscle neuromuscular block will still occur, thus the effective treatment for organophosphorus poisoning combines atropine sulphate with artificial respiration and general supportive therapy (Gordon & Fry , 1955). More recently, a number of specific oximes have been investigated which act by freeing AChE from its combination with the inhibitor (by removing the phosphoryl group from the phosphorylated ACHE) and thus reactivate the AChE (Durham & Hayes, 1962). These agents are now used in conjunction with atropine therapy and are particularly

effective in reversing the neuromuscular block of skeletal muscle (Anon., 1972, Headley, 1982).

Atropine should not be given for preventive purposes prior to an anticipated exposure to nerve agents (Durham & Hayes, 1962; Grob & Harvey, 1953) as it might mask the early occurrence of symptoms of poisoning and allow the patient to expose himself to dangerous levels of the toxicant without realising it. Furthermore, Wills (1959) noted that animals given atropine before respiratory exposure to Sarin vapour were somewhat more susceptible to the anticholinesterase than control animals given no antidote. The bronchoconstriction which otherwise occurs to some degree after inhalation of nerve gas vapour had been prevented by the prior administration of atropine, thus allowing greater inhalation and absorption from the respiratory tract.

The standard first aid measure in cases of organophosphorus poisoning is a 2mg i.m. dose of atropine sulphate Though this may seem large by comparison with the usual therapeutic dose of 0.4 to 0.6mg, it represents no hazard to an adult man (Gordon & Frye, 1955). Also, there is a marked tolerance for atropine in the presence of anticholinesterase poisoning (Grob & Harvey, 1958) and in severe cases doses of 24-48 mg of atropine may be needed in a day (Grob, 1963). U.K. servicemen carry, as part of their standard equipment, 3 self-inject syringes each containing 2mg atropine sulphate in 1ml saline. There arises the question of what would be the effects of premature injection of atropine. The possibility of inappropriate or untimely antidote usage would only occur in a military setting and much of the early work concentrated on the effects of atropine on physical performance and military tasks with less attention paid to visual function.

Effects of atropine on physical performance

Moylan-Jones (1969) showed that 6mg atropine (i.m.) impaired soldiers' physical work rate (digging) but shooting ability was unimpaired. Subjects had little difficulty in completing a light exercise protocol after 2mg atropine but in a more severe test atropinised subjects were less likely to complete the exercise than were control subjects (Miles, 1955). The environmental temperature and humidity can limit the amount of work possible after atropine (Cullumbine & Miles, 1956). This would be especially relevant if any form of protective clothing was being worn.

Symptoms of giddiness, lightheadedness and dizziness are also experienced after atropine (Cullumbine, McKee & Creasey, 1955) and soldiers often require very strong motivation from their superiors in order to overcome the lassitude caused by the drug (Headley, 1982).

Following 1mg atropine sulphate (i.m.), the plasma concentration rises to a peak after 30 minutes and then declines gradually with a measurable amount still present at 240 minutes post-injection (Berghem et al., 1980). Similar results were reported for 2mg (i.m.) monitored over 60 minutes and a close correlation was found between plasma atropine concentration and heart rate (Kalser and McLain, 1970). The initial effect is a bradycardia, beginning at 2 minutes post-injection for a 2mg i.m. dose (Cullumbine et al., 1955). The main effect, however, is a tachycardia which reaches a maximum at 30 minutes (Cullumbine et al., 1955; Kalser & McLain, 1970) or 1 hour (Mirakhur, 1978; Baker et al., 1983).

Effects of intramuscular atropine on visual performance

The ocular effects of atropine viz. paralysis of accommodation and dilatation of the pupil, develop rather slowly and remain pronounced

beyond 6 hours post-injection for a 2mg i.m. dose (Mirakhur, 1978; Rozsival & Ciganek, 1978; Baker et al., 1983), making close tasks requiring accommodation very difficult (Moylan-Jones, 1969; Rozsival & Ciganek, 1978). Atropine sulphate (2mg i.m.) has no effect on the course of dark adaptation nor on the absolute scotopic visual threshold (Rubin, 1956). Distance vision, assessed by the Snellen test, was also unaffected by atropine i.m. (Cullumbine, et al., 1955; Miles, 1955; Rozsival & Ciganek, 1978). Performance of two complex tasks of visual function: a visual search task and a task requiring repeated accommodative changes to identify high and low contrast targets was also unaffected by atropine i.m. Recently, however, 2mg and 4mg atropine sulphate i.m. have been shown to impair visual tracking of a moving stimulus (Penetar & Beatrice, 1986; Haegerstrom-Portnoy et al., 1987).

AIMS OF THIS STUDY

Part 1: A quantitative investigation of the effects of defocus, pupil diameter and spatial frequency on contrast sensitivity.

As a first stage in this work, the well-known effects of anticholinesterases on the pupil and accommodation were reproduced by the use of artificial pupils and defocusing lenses. Contrast sensitivity was measured over a wide range of spatial frequencies of sinusoidally modulated grating patterns. Sinusoidal stimuli are particularly useful in a study of defocus since the fundamental frequency remains constant regardless of the optical blur while the amplitude and phase of the modulation are altered. Thus, the psychophysical judgements of threshold are always made for a sinusoidally modulated image.

The effects of defocus and pupil size on contrast sensitivity have been investigated before on only a small number of subjects viz. two by Campbell & Green (1965) and one by Charman (1979). In the present study, the responses of a relatively large sample of 12 subjects were investigated to allow for individual variations in response. Furthermore, this study differs from previous work in that, as for natural viewing, the changes in retinal illumination which result from changes in pupil diameter were not compensated.

The eye was homatropinised in order to remove the fluctuations in accommodation and pupil diameter which normally occur under steady viewing conditions for a near target (Campbell, Robson & Westheimer, 1959). However, in the event of homatropine itself exerting an effect on contrast sensitivity, the study was also repeated with the natural eye.

The results of this work have been reported in Kay & Morrison (1985; 1987a).

Part 2: The effects of a single oral dose of pyridostigmine bromide on contrast sensitivity.

The effects of an oral dose of pyridostigmine bromide on the visual system were of interest for two reasons. First, because pyridostigmine is a reversible anticholinesterase and second, because this dose and route of administration is being considered as a possible pre-treatment against organophosphorus anticholinesterases. A 60mg oral dose of pyridostigmine bromide causes a 20% inhibition of blood cholinesterase, which gives optimal prophylactic effect at between 1 and 5 hours post-ingestion (Dr. R.I. Gleadle, personal communication). Thus, measurements of visual performance were made during this period.

In order to exclude any psychological overlay to the effects of pyridostigmine, the experiments were performed on a double-blind basis, which meant that the control and test trials were performed on different days. To reduce any diurnal variations in the measurements the tests were carried out at the same time of day. Also, several practice runs were performed during the one hour period allowed for drug absorption to cover any learning effects on first acquaintance with the measurements.

Pyridostigmine bromide is a qua ternary compound and crosses the blood-brain barrier with difficulty but although central effects are unlikely they cannot be excluded. Thus, contrast sensitivity to laser interference fringes was measured to assess any change in retinal/central vision.

These results have been reported in Kay & Morrison (1988a).

Part 3: The effects of topical application of 0.25% physostigmine sulphate on visual performance.

The effects of topical application of the reversible anticholinesterase, physostigmine sulphate, were studied to simulate the effects of organophosphorus anticholinesterases. Since many aspects of visual function were investigated, the experiments had to be performed in three separate parts. Also, because of the transient nature of the accommodation response to physostigmine eyedrops (Rengstorff, 1970), the protocol was designed to consist of short series of measurements which could be repeated at intervals to follow the time course of the response.

Pupil diameter, near point accommodation and the increase in accommodation caused by the eyedrops were monitored for 3 hours post-administration, along with other aspects of visual function (different

for each of the three parts). Contrast sensitivity was measured to stationary sinusoidal gratings of spatial frequencies of 3, 10, 20 and 30 c/deg to assess stationary pattern function. To assess movement sensitivity, contrast sensitivity was measured in response to a phase-reversed sinusoidal grating pattern, which provides an adequate stimulus to the "movement detectors", at the temporal and spatial frequencies most sensitive to movement, viz. 5.5 Hz and low spatial frequencies below 5 c/deg (Kulikowski & Tolhurst, 1973). Topical application of physostigmine has previously been shown to affect critical fusion frequency (Alpern & Jampel, 1959). Thus, a more detailed study of retinal/central vision, involving measurement of contrast sensitivity to laser interference fringes along with the critical fusion frequency, was performed in the present study.

These results have been reported in Kay & Morrison (1988b).

Part 4: The effect of an intramuscular dose of 2mg atropine sulphate on visual performance.

Due to the increasing importance of rapid detection of objects, for example, on radar displays or in flying an aircraft, even the smallest decrement in visual function may be significant. As the possibility exists of premature injection of atropine sulphate a comprehensive investigation was made of the effects of a 2mg i.m. injection of atropine sulphate per se, on visual function. In one set of experiments the general changes in heart rate, pupil diameter and accommodation were followed over 6 hours post-injection. The study also included other aspects of visual function, viz.. extraocular muscle balance, colour perception, reaction time and choice reaction time to a visual stimulus, stereoacuity, contrast sensitivity to a moving grating

pattern and visual acuity. Movement sensitivity at low spatial frequencies was of particular interest for in the cat, after intravenous (i.v.) administration of physostigmine, visual evoked responses to low spatial frequencies were often abolished whilst responses to higher spatial frequencies were only minimally affected (Harding et al., 1983). These effects were reversed by atropine sulphate indicating the involvement of cholinergic neurones. In this study, the effect of atropine per se on contrast sensitivity to low spatial frequency phase-reversed grating patterns was investigated.

In the second set of experiments, contrast sensitivity was measured over an extended range of spatial frequencies to oscilloscope generated stationary grating patterns: this was a substantial undertaking which required a separate study in itself. To take account of atropine sulphate crossing the blood-brain barrier to stimulate the central nervous system, the response of the retina/brain was assessed directly by measuring contrast sensitivity in response to laser interference fringes.

Although contrast sensitivity measurements are widely used, little work has been done on the reproducibility of such measurements in an extended recording session on a given day, bar one assessment of the variability of measurements as part of a study comparing three different methods of determining threshold (Ginsburg & Cannon, 1983). To resolve this question of reproducibility and to establish control levels, each individual first underwent a full "dummy" run of the test procedure without atropine.

The results of this part of the study have been reported in Kay & Morrison (1986; 1987b).

Part 5: The effectiveness of prior administration of an intramuscular injection of atropine sulphate or instillation of homatropine eyedrops against physostigmine eyedrops.

It was of interest to find out whether the prior administration of atropine (i.m.) would provide any protection against the topical application of an anticholinesterase. Atropine sulphate (2mg i.m.) was given at two different time intervals:

(a) 8 min prior to physostigmine so that peak plasma concentration of atropine (Berghem et al., 1980) would coincide with the peak defocus.

(b) 120 min prior to physostigmine so that the ocular effects of atropine viz. cycloplegia and mydriasis, could be fully developed before physostigmine was applied.

To investigate if a higher dose of a cholinergic antagonist would afford any protection against topical application of an anticholinesterase, a third test of the effects of physostigmine was performed:

(c) Homatropine hydrobromide eyedrops were instilled 100 min prior to physostigmine eyedrops.

The topical application of a cholinergic antagonist was preferred to a higher dose of atropine (i.m.) due to the possible risk to the volunteer subjects. Homatropine eyedrops were preferred to atropine eyedrops which have a much more persistent action.

Control runs of the following tests were made prior to atropine or homatropine and were repeated every 20-30 min following physostigmine. Contrast sensitivity to stationary grating patterns of 10, 20 and 30 c/deg and to a phase-reversed pattern of 3 c/deg, together with pupil diameter, accommodation and near-point were measured.

These results have been reported in Kay & Morrison (1987c; 1988c).

METHODS

In the course of this work contrast sensitivity was measured in response to vertical sinusoidal grating displays generated by both cathode ray tube (CRT) and laser interferometer.

Apparatus

(1)CRT Display

In parts 1 and 2 of this study the display used was generated by a Telequipment DM53S oscilloscope with a P_{31} phosphor, peak wavelength 520nm, with the time base running at 1ms/div. A triangular wave, peak-to-peak voltage of 12V and frequency 220 kHz was fed into the vertical amplifiers of both beams of the oscilloscope, which had been superimposed, to produce a uniform green raster. The grating pattern was produced by feeding a sine wave of variable voltage and variable frequency into the z-modulation of the oscilloscope. The sine wave was obtained from a Feedback sine square oscillator SS0603, the output of which could be amplified to a maximum of 70V peak-to-peak by a control unit. The amplitude of this sine wave was varied by adjustment of either the oscillator output or the ten-turn potentiometer which attenuated the output of the control unit, and was monitored on a Tektronix 5103 oscilloscope.

The luminance of the grating pattern remains constant as the depth of modulation is varied, and was measured with a Pentax Spotmeter V as 1.4 cd/m^2. Psychophysical determinations showed luminance to be 3.83 log units above foveal threshold i.e. in the mesopic range where both rod and cone pathways may be operational. To exclude the distortions which occurred at the edges of the screen, the display was viewed through a window in a surround of green card. The surrounding card was not illuminated except for the background room lighting of circa 0.5 metre-candela at the level of the subject. The horizontal aspect of the viewing aperture subtended 1.5 deg when viewed from 3m and 4.5 deg when

viewed from 1m. These two viewing distances were used in order to obtain a sufficiently wide range of spatial frequencies without exceeding the limit (of number of cycles of the grating) at which the performance of the CRT declined (Morgan & Watt, 1982) and without going below the limit at which contrast sensitivity was determined by the number of grating cycles rather than by the spatial frequency itself (i.e. ~ 3 cycles for this display luminance; Hoekstra et al., 1974). Thus spatial frequencies of 8 c/deg and above were measured from 3m and spatial frequencies of below 8 c/deg were measured from 1m. The combination of measurements from near and far viewing distances into a common CSF had previously been established by Campbell & Robson (1968).

The psychophysical method described by Campbell & Green (1965) was used to determine the relation between z-modulation voltage and contrast. One cycle of a square wave grating, located in a narrow window, was viewed from 1m. A calibrated neutral density filter was placed in the window covering the brighter half of the cycle and the z-modulation voltage was increased until the two half-cycles were judged to be of equal luminance. At this point, the z-modulation voltage was numerically equivalent to the transmissivity of the neutral density filter. This was repeated at cycle widths corresponding to 5, 10 and 20 c/deg at 3m by two observers. Contrast was linearly related to z-modulation voltage with a slope of 0.022 contrast units/V peak-to-peak up to 25V, attaining a maximum contrast of 0.85 at 70V.

Contrast thresholds were measured for one eye only with the other being covered either by the subject's hand or more usually by an occluder placed in a trial frame over the appropriate eye. For the experiments on the homatropinised eye in part 1, thresholds were measured by an ascending method in which the experimenter steadily increased the contrast of the grating pattern from zero contrast until

the subject was just able to detect the grating in the centre of the screen and no more. This measure, in fact, agrees very closely with the contrast threshold based upon 50% detection (Morrison & Reilly, 1986). Furthermore, Ginsburg & Cannon (1983) demonstrated that an ascending method of measuring contrast threshold produced more conservative values with less variance between subjects, than methods of adjustment which incorporated descending contrast measurements which may be confounded by after-images. The experimenter controlled procedure for measuring contrast was adopted since, in these experiments, the majority of subjects were recruited from outside the academic world and unfortunately did not prove to be completely reliable in their objectivity. In the second experiment in part 1 for the natural eye and, in fact, for all remaining parts of this study, the method used was again ascending but now under the subject's control. This was possible due to the employment of subjects who were better motivated to cooperate to produce objective results. In this case, the experimenter still had independent control of the z-modulation voltage, the sensitivity of the control unit could be varied between determinations so that the subject could not arrive at the same threshold simply by counting the number of turns of the potentiometer. Throughout this study contrast sensitivity was calculated as the mean of five contrast threshold determinations at each spatial frequency measured.

In all experiments from part 3 onwards, the sinusoidal grating pattern was generated on a Tektronix 606B monitor, which also had a P_{31} phosphor, peak wavelength 520 nm. The raster was now produced by feeding a 770 kHz triangular wave into the Y-plates, whilst the time-sweep was driven by a saw-tooth waveform from the X-output of the Tektronix 5103 oscilloscope with time base running at 0.2 ms/div. This oscilloscope also monitored the frequency and amplitude of the z-

modulation which was produced by a sine wave oscillator. The z-modulation signal was connected via a 1:2 or 1:10 attenuator to the control unit with an adjustable ten-turn potentiometer; the attenuation ensured the subject had a reasonable number of turns of the potentiometer in cases where contrast was very low.

The 606B display was viewed through a window 10 x 8 cm in a green card screen. This subtended 2 deg at 2.86m and 4 deg at 1.43m, these being the two distances used to obtain a sufficiently wide range of spatial frequencies without the number of cycles limiting contrast sensitivity as mentioned earlier. Contrast sensitivity was measured from 2.86m for spatial frequencies of 8 c/deg and above and from 1.43m for spatial frequencies below 8 c/deg. The luminance of this display was considerably greater than that of the previous oscilloscope and was measured as 10.2 cd/m^2, equivalent to 4.89 log units above foveal threshold in the upper mesopic range. The surrounding card was not illuminated other than by a background illumination provided by two spotlamps of combined intensity 0.5 metre-candela measured at the location of the subject with a Lightmaster Photometer (Diffusion Systems Ltd.).

The relationship between the z-modulation voltage and contrast was determined as described for the other CRT. The calibration was repeated several times for a range of contrasts with three observers. Contrast was linearly related to z-modulation voltage with a slope of 0.253 contrast units/V peak-to-peak up to 3V, levelling off to a maximum of 0.85 at 5V.

In some experiments, contrast thresholds were also measured to "moving" sinusoidal grating patterns of low spatial frequencies ranging from 0.5 to 5 c/deg. These were generated by a 180 deg phase-reversal (with the positions of the light and dark bars of the grating being

continuously exchanged) at 5.5 Hz of a sinusoidal grating pattern giving rise to an apparent movement of the pattern (Kulikowski, 1971) known as the phi-phenomenon, which is an effective stimulus for movement detection (Kulikowski & Tolhurst, 1973). Thresholds were measured by an ascending method with the subject increasing the contrast of the grating until the temporal modulation or flicker was just seen, irrespective of whether or not the form of the pattern was perceived.

(2) Laser Display

The method used was that described by Morrison & McGrath (1985), based on that of Westheimer (1960) and Campbell & Green (1965). Vertical sinusoidal fringes were generated by the interference of two laser beams consisting of polarised coherent monochromatic light observed in the Maxwellian View (Fig. 6). Maxwellian viewing is the procedure of imaging a light source in the plane of the eye's pupil, instead of viewing it directly (Westheimer, 1960).

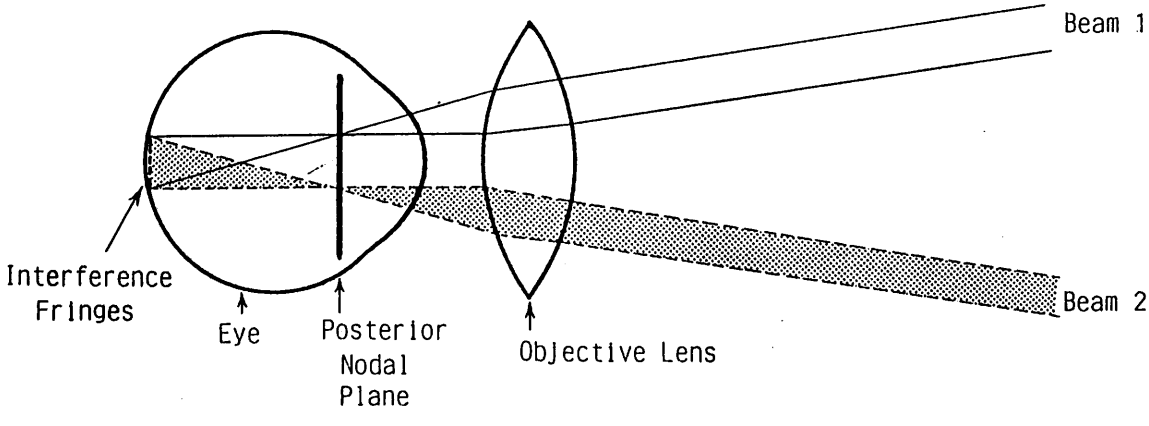


Fig. 6. Schematic representation of the formation of laser interference fringes on the retina from Morrison & McGrath (1985).

Normally, rays of light entering the eye are focused by the ocular media onto the retina. Instead, in the Maxwellian View, the rays of light are focused by a microscope objective onto a plane passing through the posterior nodal point of the eye which is about 17mm in front of the retina, approximately at the level of the pupil. As coherent rays pass unrefracted through this point the effects of standard geometrical aberrations associated with any optical system, viz. chromatic aberration (in the case of white light), spherical aberration, coma and cylindrical aberration are avoided. It should be noted that wide-angle scattering of light by small particles in the ocular media may still occur. Since the two beams overlap and interfere behind the posterior nodal point the exact position of the eye is not as critical as it may at first seem. One can move forward or backward a few millimetres without changing the spatial frequency of the pattern. Nevertheless, the head was kept as stable as possible by the subject placing his chin on a rest or a cushioned pad whichever was the more comfortable. Care was taken to reduce vibrations of the optical table on which the apparatus were mounted with four Barry Mount stabilisers as movement of the stimulus might influence the threshold being measured.

The vertical sinusoidal interference fringes were generated by a Mach-Zehnder interferometer (Born & Wolf, 1980) and their production has been given in detail in Morrison & McGrath (1985). Briefly, a beam of polarised coherent light from a He-Ne laser (wavelength 632 nm) is split into two and made to travel different paths before recombining and interfering so as to produce light maxima and minima. The two parameters of the interference fringes which were important to this study are spatial frequency and contrast.

The spatial frequency of the fringes is directly related to difference in path length of the two beams. The path length was altered

by translation of a front silvered mirror, mounted on a Beck rotation stage, placed in the path of one beam. The deflected beam was then realigned by rotation of the Beck stage until the beam was brought back onto the centre of the Maxwellian lens which consisted of a x2 Leitz objective. When the realigned beam was subsequently combined with the fixed beam, interference fringes of the required spatial frequency were obtained. The arrangement of the optical table was such that it allowed the formation of a second pair of laser beams which combined to form a second set of interference fringes (see Morrison & McGrath, 1985). These were used to measure the spatial frequency of the display in the x2 objective by counting the number of cycles within the phase ring of a x100 Vickers objective. This was multiplied by a correction factor of 0.75 which had been obtained by comparing the number of cycles in the x100 objective with those in the x2 objective lens obtained by photography of the image over the range of spatial frequencies studied (and counting the cycles on the negative under low power magnification). This correction factor remained constant despite dismantling the apparatus three times during the course of this work.

Variable contrast of the interference fringes was achieved by superimposing the laser beams on a diffuse beam from a tungsten source (Vickers microscope lamp), passed through a narrow band red interference filter with peak transmission 630 nm and bandwidth 5 nm (Barr & Stroud Ltd), but polarised at 90 deg. It was important that the two laser beams and the diffuse beam were of the same intensity and this was arranged using a photodiode operational amplifier unit (RS 305-462). First, the intensity of the two laser beams were measured separately and the brighter beam attenuated by placing the appropriate neutral density filter in its path. The recombined beams were then measured and matched for intensity with the diffuse beam. The laser beams and

diffuse beam all passed through a Glan-Thompson calcite polariser, with extinction circa 10^{-5} (J. Phillips Ltd) mounted within a Beck rotation stage. By rotating the polariser from 0 to 90 deg, the contrast of the interference fringes increases from zero (only diffuse beam passing) to 1.0 (only laser light passing). The angle of rotation θ is read directly from a scale on the rotation stage and as θ increases the contrast of the grating increases according to the sine-squared law (Westheimer, 1960). The intensity of the combined beams remained constant upon rotation of the polariser as determined photometrically and was determined psychophysically, in three observers, to be about 2.5 log units above foveal threshold. This intensity was chosen empirically for long term viewing as anything brighter was found to be uncomfortable. The field size used was 7 deg and the ambient background illumination was as for the CRT unless stated otherwise.

Thresholds were measured by an ascending method. In part 1, the experimenter slowly increased the contrast until the subject indicated that the grating was just visible in the centre of the field. In all other experiments in this study the subject increased the contrast himself until the grating was just visible and no more. For each threshold determination the rotation stage was placed at a different subthreshold position by the experimenter in order that the observer could not reach threshold by counting the number of turns of the dial. Five determinations of threshold were made at each spatial frequency and then averaged, similar to CRT measurements.

Other visual measurements

Throughout the study pupil diameter and near point accommodation were measured. Pupil diameter was measured by photography (Olympus OM-1 single lens reflex body with Tamron 80mm macro lens) under moderate

illumination (30 metre-candela). Both eyes were photographed, with a 10 mm scale bar placed just below one eye, from a distance of about 0.5m. Two frames were taken for each measurement and the pupil diameter in the horizontal plane averaged. Near point accommodation was measured, in dioptres, with a RAF Near Point Rule: the subject viewed the fine print chart and brought the slide towards himself to locate the point at which blurring was first detected. This was repeated five times and averaged.

The experiments were usually of a demanding nature and regular breaks were taken to avoid subject fatigue. No tea, coffee, drinks with additives or cigarettes were allowed, but refreshments were taken at appropriate intervals.

Subjects

The subjects who participated in this study were all volunteers (ages 18-42 years) recruited in the early part of the study from local Territorial Army units with some staff and post-graduate students from the Institute of Physiology. In later experiments volunteers were almost entirely post-graduate students and friends (ages 18-28 years). They each gave their written consent to the experiments after an explanation of the likely effects and hazards of the procedure to be used and were advised of their right to withdraw from the experiment at any time irrespective of having signed the consent form.

Exclusions

All subjects were to be of good health and those receiving any medication were excluded from the study. Undergraduate students receiving tuition within the Institute of Physiology were also excluded. In addition no subjects with over 2.5 D myopia were allowed to participate in the experiments involving physostigmine eyedrops (because

of the increased risk of retinal detachment in severely myopic patients from the use of miotic agents; Alpar, 1979).

Ethical consent

The work described in this thesis had received the prior approval of the Ethical Committee of The Greater Glasgow Health Board (Western District) which evaluates projects undertaken within the University of Glasgow.

Experiments performed

Prior to every experimental period each subject viewed the Snellen test and astigmatism fan and were optimally refracted to within 0.25 D (to within 0.12 D for part 1). Visual acuity was always 6/5 or 6/4 for parts 1 and 2. For the remainder of the experiments, visual acuity was measured from 5m and all subjects were corrected to 5/4. Additional lenses were worn if necessary for optimal acuity when viewing the CRT display but were not necessary for the laser display. For both stimulus displays the subject viewed monocularly with the same eye. Prior to the study proper, each subject underwent a familiarisation run consisting of spatial frequencies of 10, 20 and 30 c/deg. This process usually took longer with the laser display. It was found that once a subject had successfully practised the highest spatial frequency, he became attuned to the method and was reasonably consistent in judgements thereafter. The consistency of repeated contrast sensitivity measurements to the CRT was tested in a series of experiments described in part 4.

Part 1: A quantitative investigation of the effects of defocus, pupil diameter and spatial frequency on contrast sensitivity.

A total of 10 male and 2 female subjects ages 18-40 years participated in the first experiment in this section involving homatropine eyedrops. Of this group, eight also completed the second experiment for the natural eye.

CRT Display: The same standard protocol was followed by all subjects. Contrast thresholds for stationary sinusoidal gratings were measured at each of 10, 20, 30, 38, 35, 25, 15 and 10 c/deg viewed at 3m and 8, 3, 1, 0.5 and 5 c/deg at 1m, in this order. These were collected for the following conditions.

(1) Natural pupil diameter and optimal accommodation.
(2) Viewing with a 3.0mm artificial pupil and optimal accommodation.
(3) 2.0mm artificial pupil and optimal accommodation.
(4) 2.0mm artificial pupil and +2.0 D additional defocus.
(5) 3.0mm artificial pupil and +2.0 D additional defocus.
(6) 3.0mm artificial pupil and +4.0 D additional defocus.
(7) 3.0mm artificial pupil and +1.0 D additional defocus.
(8) Repeat measurements at 10, 20 and 30 c/deg with natural pupil and accommodation.

The artificial pupil was worn in a trial frame in the slot directly in front of the eye, additional lenses were also placed in this frame when required.

In the first set of experiments, one drop of 2% homatropine hydrobromide solution (Kirby-Warrick Pharmaceuticals Ltd.) was

instilled, prior to condition (2), which maximally dilated the pupil and paralysed accommodation after 20-30 minutes. The subject was then optimally refracted with a Snellen chart to within 0.12 D for viewing at 1m and 3m, as Charman (1979) has demonstrated the importance of accurate refraction for spatial frequencies of 10 c/deg and over (Charman found for a spatial frequency of 20 c/deg and a 3mm pupil that a defocus of only 0.5D caused a fall in relative contrast sensitivity by a factor of 0.6). These corrections were typically +0.75 D for 1m and +0.25 for 3m. Condition (8) was omitted but two repeat measurements were taken for the homatropinised pupil at 3 and 20 c/deg. These experiments were carried out by Dr. J.D. Morrison and subsequently analysed by myself.

In the second set of experiments the protocol was followed exactly as stated, thus repeating the measurements for the natural eye. The experimental measurements usually lasted for some 6 hours, with an additional 1-1.5 hours allowed for breaks.

Laser Display: Contrast thresholds were measured for a range of spatial frequencies from 1 to 50 c/deg. When setting the spatial frequency of the display it was not always possible to obtain exactly the intended spatial frequency without an undue amount of re-adjustment. Hence, the spatial frequencies measured do not always correspond exactly to those measured for the CRT display; however, the spatial frequency that was obtained was, of course, known very precisely. The spatial frequencies were studied in the following order: 9.5, 20.5, 30, 41, 45, 50, 33, 36, 26, 15, 9.5, 5, 1, 2.5, 7.5, 17.5 and 23 c/deg. The measurements were made in a darkened room in which the pupil would be dilated thus presenting no problem in viewing the two laser sources even at their widest separation. Even at 50 c/deg, the subjects were able to make reproducible measurements of contrast threshold without difficulty. In

cases of uncertainty, they were advised to look away from the display for a few moments and then review before making a decision, since high spatial frequency fringes readily disappear from view due to adaptation. Measurements usually lasted for 3 hours and were made on a different day from the CRT measurements.

Part 2: The effects of a single oral dose of pyridostigmine bromide on contrast sensitivity.

Thirteen male subjects, ages 18-40 years participated in this experiment. The effect of a single oral dose of 60mg pyridostigmine bromide on contrast sensitivity was investigated on a double-blind basis, with lactose as the control. Each subject made two visits to the laboratory at the same time of day. At least 1 hour, usually 2 hours after a light meal, the volunteer ingested one of two capsules, prepared by the Pharmacy, Western Infirmary Glasgow, which contained 60mg pyridostigmine bromide (Roche) or 60 mg lactose. Neither the subject nor myself nor Dr. Morrison was aware of the order in which the capsules were given until after the experiments had been completed and analysed.

The time course of the experiments was dictated by the pharmacokinetics of pyridostigmine absorption. At least 20% inhibition of whole blood cholinesterase is known to occur between 1 and 4.5 hours after ingestion of this dose (Dr. R.I. Gleadle, personal communication). The contrast sensitivity measurements were, therefore, made during this interval. Each volunteer underwent the following procedure after ingestion of the tablet.

(1) 0 - 1 h Rest period while pyridostigmine was absorbed.

(2) 1 - 1.5 h Familiarisation with laser and CRT displays, practice measurements at 10, 20 and 30 c/deg for each display.

(3) 1.5 - 2.25h Contrast sensitivity measurements to CRT display at 10, 20, 30, 35, 38, 25, 15, 8 and 10 c/deg in that order from 3m and 5, 3, 1, 6 and 8 c/deg at 1m.

(4) 2.25 - 2.5h Short break for refreshments (water/milk/decaffeinated coffee offered with the same on the second day).

(5) 2.5 - 4.25h Contrast sensitivity measurements to laser interference fringes at a range of spatial frequencies from 1 to 40 c/deg with approximately the same values as for the CRT display.

(6) 4.25 - 4.5h Repeat measurements were made for the CRT display at 10, 20 and 30 c/deg.

Throughout this study, starting with control measurements prior to ingestion of the tablets, pupil diameter was taken by photography, under dim illumination, every 30 min. After completion of the tests, and ascertaining that the subject was fit and well, he was allowed to go home, but requested not to drive until the following day.

Part 3: The effects of topical application of 0.25% physostigmine sulphate on visual performance

A total of 12 subjects completed this part of the study, 7 male and 5 female, ages 18 to 28 years. In this and all subsequent studies, subjects were first optimally refracted to within 0.25D by viewing an illuminated Snellen chart. As the increase in accommodation caused by physostigmine eyedrops is fairly transient (Rengstorff, 1970), the

visual tests consisted of short series of measurements which were repeated continuously for the first one and a half hours, and then once every half hour until 3 hours post-administration.

Each subject completed three separate experiments covering different aspects of visual function. Prior to the eyedrops two control series of measurements were taken on each of the three separate days. Following this on each occasion the subject received two drops of 0.25% physostigmine sulphate (Evans Medical Ltd.) into the lower conjunctival sac (of the same eye). The head was held back for 2 to 3 min so that the drug would be absorbed without being blinked away. Measurements then began at 5 min after instillation.

Every series of measurements commenced with a Snellen test and then the increase in accommodation caused by the eyedrops was measured by finding the strength of negative lens required to bring the bottom line of the Snellen chart back into sharp focus. Following this, near point accommodation was measured using the RAF rule for the natural pupil (and also for viewing through a 2mm artificial pupil in the initial control measurements). Contrast sensitivity was then measured in response to <u>one</u> of the following on a separate day.

(1) Stationary patterns of 10, 20, and 30 c/deg generated on a CRT measured from 2.86m and 3 c/deg from 1.43m. These measurements were repeated approximately every 15 min.

(2) Moving patterns (sinusoidal gratings phase-reversed at 5.5 Hz.) of 0.5, 1, 2 and 3 c/deg, from 1.43m. These measurements were repeated approximately every 20 min.

(3) Stationary patterns generated by the laser interferometer at 4, 15 and 25 c/deg. These measurements were preceded by a test for critical fusion frequency, which measures the lowest frequency of repetition at which a flickering object appears stationary. This was determined using

a U.S. Air Force Portable Visual Function Tester (Genco & Task, 1984). This is a hand-held device with which the subject views a target arranged to be at infinity at the centre of his visual field through a collimating eyepiece. Viewing was, of course, monocular and through an appropriate lens to bring an object at infinity into sharp focus as measured just prior to this test for the Snellen chart. Control measurements prior to administration of the eyedrops were made through a 2mm artificial pupil. This group of measurements was repeated every 30 min.

There may be some genetic factor involved in determining the strength of a person's response to physostigmine eyedrops. Two brothers were involved in the initial series of experiments just described, and so a third brother from this family was later tested with physostigmine eyedrops (and, in fact, went on to participate in part 5 of this study). Two siblings (twins, male and female) of another male member of the original 12 subjects were also tested with the same dose of physostigmine and their accommodation (near point and amplitude of defocus) and pupil diameter responses were assessed, and compared to that of their brother.

Part 4: The effect of an intramuscular dose of 2 mg atropine sulphate on visual performance

General Procedure

A total of 13 male subjects ages 21-40 years participated in this part of the study. In all experiments, following routine medical screening, the subject received an i.m. injection into the upper arm of 2mg atropine sulphate in 1 ml saline (McCarthys Ltd) from the Medical Officer in attendance. He also remained on-call for the duration of the

experiment and discharged the subject after the experiment had been completed. Two complications could arise from an injection of atropine. First, immediately after the injection the subject may become susceptible to a vaso-vagal attack resulting in fainting. Thus a rest period of 15 min was allowed before measurements commenced. During this period, heart rate was monitored continually by palpation until it started to rise; thereafter, heart rate was taken every half hour. Second, a possible risk existed in those subjects with an abnormality of the ventricular conducting system, e.g. with the accessory bundle of Kent when an increased risk of ventricular fibrillation existed. In the one case where some doubt of a subject's cardiac health existed, an electrocardiogram was taken prior to the experiment. In fact, no adverse effects other than those anticipated were encountered and all subjects were discharged as satisfactory after the experiment.

Throughout the study the subject was asked to describe any symptoms as they occurred. Viewing was binocular for this study and if additional lenses were necessary for optimal control acuity then they were worn throughout. Pupil diameter was measured every 30 min.

Two experiments were performed, the first was completed by 8 subjects and the second by 13 subjects.

Experiment 1

Prior to the injection, the subject was made familiar with the procedures to be used and two control runs were completed. Each test period consisted of the following seven procedures in the order given, and lasted for about 45 min. These were performed at hourly intervals up to 6 hours post-injection. The tests were performed under normal room lighting (30 metre-candela), except for tests 4,6 and 7, which required subdued lighting (0.5 metre-candela).

(1) Reaction time and choice reaction time

A simple reaction time unit was used to measure the time between the signal and the response. A Digitimer provided a pulse to illuminate a small light emitting diode (LED) and also start the counter (Digital Latency-meter) which was accurate to the nearest 0.1ms. The subject held a console with three coloured LEDs and three push-button switches and was asked to press the button adjacent to the illuminated LED. This stopped the counter and the reading was noted. Reaction time was measured as the time to respond to illumination of the red LED. An additional unit allowed the experimenter to select at random which of the three LEDs would be illuminated, requiring that a decision be made by the observer thus allowing a choice reaction time to be measured. The latencies of ten consecutive tests were averaged for each period.

(2) Extraocular muscle balance

These measurements were determined with the portable Visual Function Tester as used previously for critical fusion frequency measurements. For these determinations of extraocular muscle balance, the subject viewed the target at infinity binocularly through collimating eyepieces.

Lateral and vertical heterophoria. The relative positions of the eyes referenced to the X and Y axes were measured. The stimulus for the left eye was a short duration flash and for the right a grid calibrated vertically and horizontally in prism dioptres. On pressing a button the flash of light appeared in the left visual field and the subject gave the coordinates of the square in which the flash was seen. Thus perfect orthophoria would be coordinates H4.

Cyclophoria. This describes the relative position of the eyes along the Z axis, i.e. the rotation of one eye with respect to the other. The torsional phoria stimulus was an arrow in the left eye and a numbered scale calibrated in degrees in the right. Both eyes also viewed a bullseye pattern to allow fusion of the targets. The subject identified the position to which the arrow pointed. The amount of cyclophoria was given by the reading on the scale.

For both tests the mean of four readings were taken.

(3) Accommodation

Near Point Accommodation was measured with the RAF rule whilst viewing with both eyes and natural pupils.

(4) Colour-matching

A Pickford-Nicolson Anomaloscope was used to find the Red/Green matching range as described by Pickford and Lakowski (1960). The instrument is a simple colorimeter with two integrating boxes such that light from each box illuminates a separate half of the screen which measured 5cm in diameter. The light to one half passes through a yellow filter whilst that to the other half passes through red and green filters. The proportion of red and green was varied by adjusting a scale from 0 to 80 until the subject matched the hue with that on the other half of the screen. Brightness was always made equal before any judgement of colour was accepted. The test was carried out until a steady matching range was obtained, usually within four to six determinations. For normal subjects, the range lies between 33.6 - 41.2 (99% confidence limits).

(5) Stereoacuity

(i) Measurements to extended line stimuli based upon the method of Anderson and Weymouth (1923) were made. The subject, chin in rest, viewed three white vertical needles in an aperture from a distance of 2 metres. The needles were approximately 1mm thick, 30mm long and positioned 20mm apart. The central needle was mounted on a micrometer and could be positioned at varying distances in front and behind the two lateral needles: viz. 3.25mm in front and 3.75mm behind in 0.5mm steps. Each position was tested three times giving a total of 45 positions in all. The subject's stereoacuity was calculated as half the distance between the two 100% "hits" points (i.e. where 3 out of 3 correct scores were made) in front and behind and converted into visual angle. An overall assessment of performance on the test was also computed as the number of correct decisions expressed as a percentage of the 45 trials.

(ii) An alternative test of stereopsis based on a matrix of 4 dots was also performed on the portable Visual Function Tester. The subject viewed each test matrix in turn and identified which dot appeared closer to him than the others. The smallest stereoscopic disparity testable was 10 seconds of arc.

(6) Visual Acuity

Visual acuity was measured as the highest spatial frequency visible for a stationary sinusoidal grating generated on the CRT and viewed with both eyes from 2.86m. The subject was asked to say whether or not a high spatial frequency grating at full contrast was visible, giving him the option to turn the contrast down and then back up again to aid a decision. The cut-off frequency was taken as the highest spatial frequency detected on two or more of four trials. This is

related to Snellen acuity since 30 c/deg is equivalent to 6/6 vision, 36 c/deg to 6/5 vision and 45 c/deg to 6/4 vision.

(7) Contrast sensitivity to a moving stimulus

Contrast sensitivity was measured to sinusoidal grating patterns of 1, 2, 3 and 5 c/deg phase-reversed at 5.5 Hz, as described earlier.

Experiment 2

In this experiment each subject was tested on two separate days. On their first visit to the laboratory, each subject completed a full "dummy" run of the experiment consisting of four consecutive series of measurements, lasting for four hours, to determine the consistency of repeated contrast sensitivity measurements. The test day consisted of a further control run followed by the injection and three test runs.

Contrast sensitivity was measured monocularly to vertical sinusoidal grating patterns generated on both the CRT and the laser interferometer. Each series of measurements consisted of the following:

(1) CRT contrast sensitivity determinations for stationary patterns at 10, 20, 30, 25, 15, 8, 10, 5, 3, 1, and 8 c/deg in that order, of duration 30 min.

(2) Laser contrast sensitivity determinations at 8, 15, and 26 c/deg in variable order, of duration 15 min.

(3) Rest period during which heart rate, near point and pupil diameter were measured, of duration 15 min.

Part 5: The effectiveness of prior administration of an intramuscular injection of atropine sulphate or instillation of homatropine eyedrops against physostigmine eyedrops.

Those six subjects who were most markedly defocused by physostigmine eyedrops were then twice more tested with physostigmine, but this time preceded by 2mg of atropine sulphate (i.m.) at different intervals. Five of these subjects also completed a third experiment in which physostigmine eyedrops were preceded by homatropine hydrobromide eyedrops. Each subject first underwent two control series of tests of visual function before each of the following treatments.

(a) Intramuscular injection of 2mg atropine sulphate 8 min prior to two drops of 0.25% physostigmine sulphate eyedrops.

Following the atropine injection a rest period was allowed as the bradycardia experienced at this time may cause syncope. Two drops of 0.25% physostigmine sulphate were instilled into one eye as soon as the heart rate began to increase. Tests of visual function were recommenced 5 min after physostigmine and were made continuously until 1.5 hours after administration, thereafter one complete series was made every half hour until 3 hours.

(b) Intramuscular injection of 2mg atropine sulphate at 120 min prior to two drops of 0.25% physostigmine sulphate eyedrops.

Two series of tests of visual function were made following the atropine injection. After physostigmine, three series of tests were made in the first hour, followed by two further series, until 2 hours.

(c) Ocular instillation of three drops of 2% homatropine hydrobromide at 100 min prior to two drops of 0.25% physostigmine sulphate eyedrops.

Each subject first performed three control series of tests on this occasion. The subject then received three single drops of 2% homatropine hydrobromide (Kirby-Warrick Pharmaceuticals Ltd.) at 5 min intervals, instilled into the lower conjunctival sac. This dose is used clinically to induce cycloplegia. After an interval of approximately 30 min, the first series of tests was made, followed closely by a second series before a short break was taken for lunch at approximately 60 min after homatropine administration. A third series of tests was made, then at 100 min after homatropine administration the subject received two drops of 0.25% physostigmine sulphate into the same eye. The effects of which were followed for 3 hours as in part (a).

Each series of tests of visual function consisted of the following:

(1) Snellen test and measurement of the amount of myopia caused by the eyedrops as previously described for part 3.

(2) Near point accommodation and pupil diameter.

(3) Critical fusion frequency was measured using the portable Visual Function Tester (also as described for part 3) for the 8 min atropine pre-treatment only.

(4) Contrast sensitivity measurements were made for stationary sinusoidal grating patterns generated on a CRT at 10, 20, and 30 c/deg and at 3 c/deg for a grating pattern phase-reversed at 5.5 Hz.

Data Analysis

The statistical tests used in this thesis were made, in the main, with the Minitab package (Ryan, Joiner & Ryan, 1985) on the University Mainframe computer although some analysis was also performed on an Amstrad personal computer using the Amstat One package. Comparisons between test and control periods for the same set of subjects were made with paired t-tests wherever possible. Other tests performed were unpaired t-tests, analysis of variance and calculations of best-fitting linear regression equations. Further comparisons of the slopes and intercepts of the best fitting regression equations were made manually according to Draper & Smith (1981).

RESULTS

Part 1: A quantitative investigation of the effects of defocus, pupil diameter and spatial frequency on contrast sensitivity.

Contrast sensitivity to CRT display

The form of the CSF was the same for all individuals and, for the natural pupil (of some 6mm diameter), consisted of a peak at 3-5 c/deg with a decline at lower and higher spatial frequencies. A mean CSF was calculated for the 12 subjects and is shown in Fig. 7. The peak of this function occurred at 3 c/deg and had a mean value of 81 units (range 62-182). The high frequency fall-off was best fitted by a logarithmic-linear plot with a cut-off frequency of 35-38 c/deg. The standard deviation bars show that there was quite large variation between subjects in their absolute contrast sensitivity values.

Pupil Diameter

Homatropine eyedrops increased the pupil diameter from approximately 6mm (natural pupil under these illumination conditions) to 8mm without having an effect on contrast sensitivity after refraction for the two viewing distances. The regression equations for 3-38 c/deg for natural and homatropinised eyes did not differ significantly in intercept or slope ($P>0.25$). When paired t-tests were made for the highest spatial frequency detected, no significant difference was observed ($P>0.25$). Paired t-tests for the spatial frequencies less than peak, before and after homatropine, also failed to reveal a significant difference ($P>0.25$).

In the homatropinised eye, viewing with the 3mm artificial pupil marginally improved contrast sensitivity while the 2mm pupil had a tendency to slightly worsen it, compared with the fully dilated pupil (Fig. 8A). However, comparison of the regression equations of the 2mm

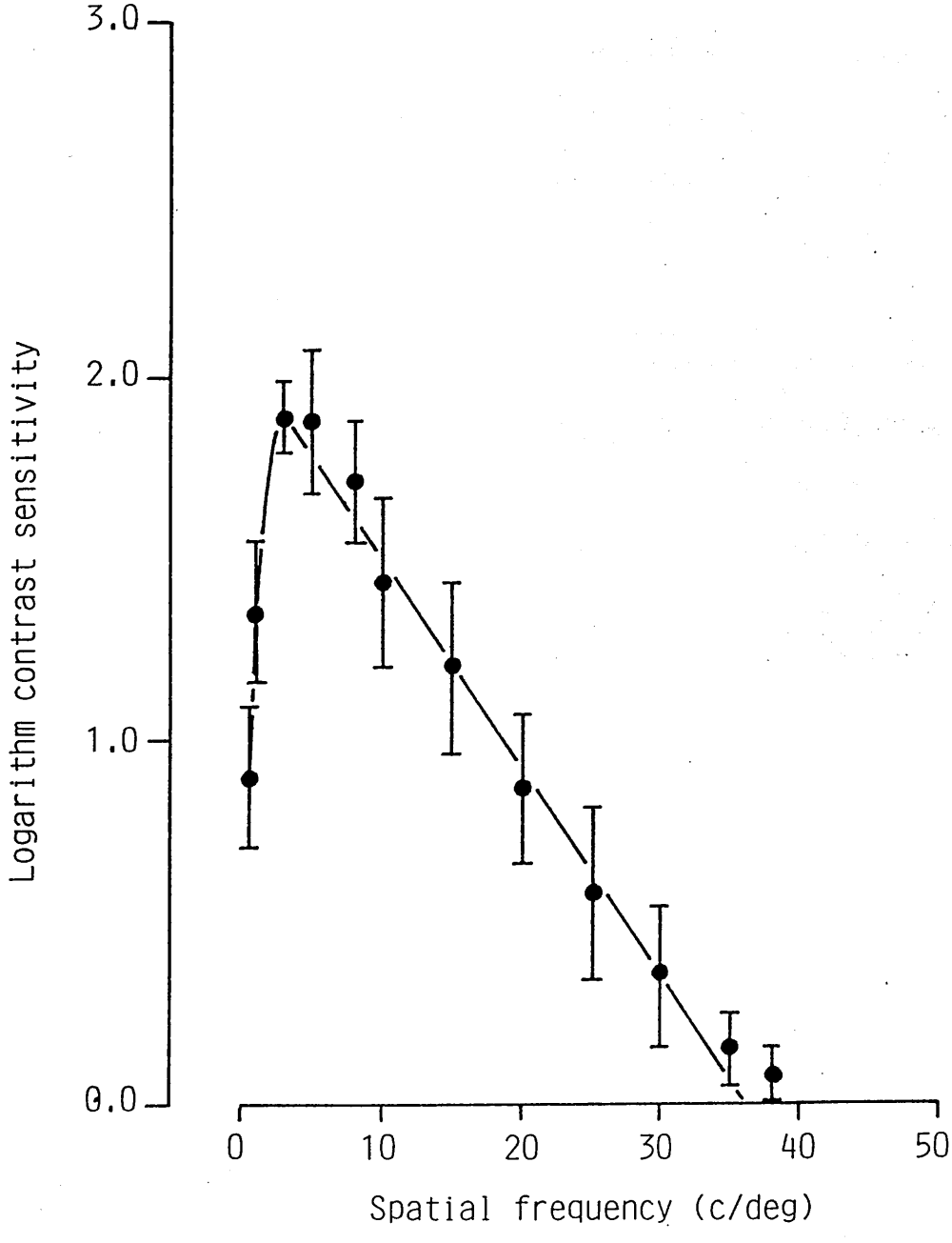


Fig. 7. Contrast sensitivities (on logarithmic scale) to CRT showing the mean ± S.D. of the logarithm contrast sensitivities (i.e. the geometric mean) for 12 subjects viewing with natural 6mm pupil.

and 3mm artificial pupils with that of the fully dilated pupil revealed that neither the slopes nor intercepts were significantly different ($P>0.25$). At 0.5 and 1.0 c/deg, the paired t-test showed that contrast sensitivities for the 3mm pupil were not significantly different from those for the dilated pupil ($P>0.25$); however those for the 2mm pupil were significantly reduced ($P<0.01$ for each spatial frequency).

In the repeat experiments for the natural eye without homatropine, similar results were obtained. The 3mm pupil marginally improved and the 2mm pupil marginally worsened contrast sensitivity compared with the natural 6mm pupil (Fig. 8B). The regression equations for 3-38 c/deg were not significantly different with regard to slopes and intercepts ($P>0.25$). Contrast sensitivity was unaffected at 0.5 c/deg with the 2mm and 3mm pupils (paired t-test, $P>0.25$) but a significant reduction did occur with both 2mm and 3mm pupils at 1 c/deg ($P<0.05$). The details of the regression equations for the different pupil diameters are listed in Table 2.

Defocus

When viewing with the 3mm artificial pupil with which optical quality is said to be optimal (Campbell & Green, 1965), increasing defocus by positive spherical lenses had an increasingly detrimental effect on contrast sensitivity over 3-38 c/deg in both homatropinised (Fig. 9A) and natural eyes (Fig. 9B). In both cases there is a step-wise parallel downward shift of the 3-38 c/deg segment of the CSF, with a smaller decrease for spatial frequencies below 3 c/deg. A similarly deleterious effect was caused by a +2.0D lens when viewing through the 2mm artificial pupil (Figs. 10A & B). The shifts in the CSF caused by defocus were examined by comparing the regression equations for 3-38 c/deg under each of the experimental conditions. These regression

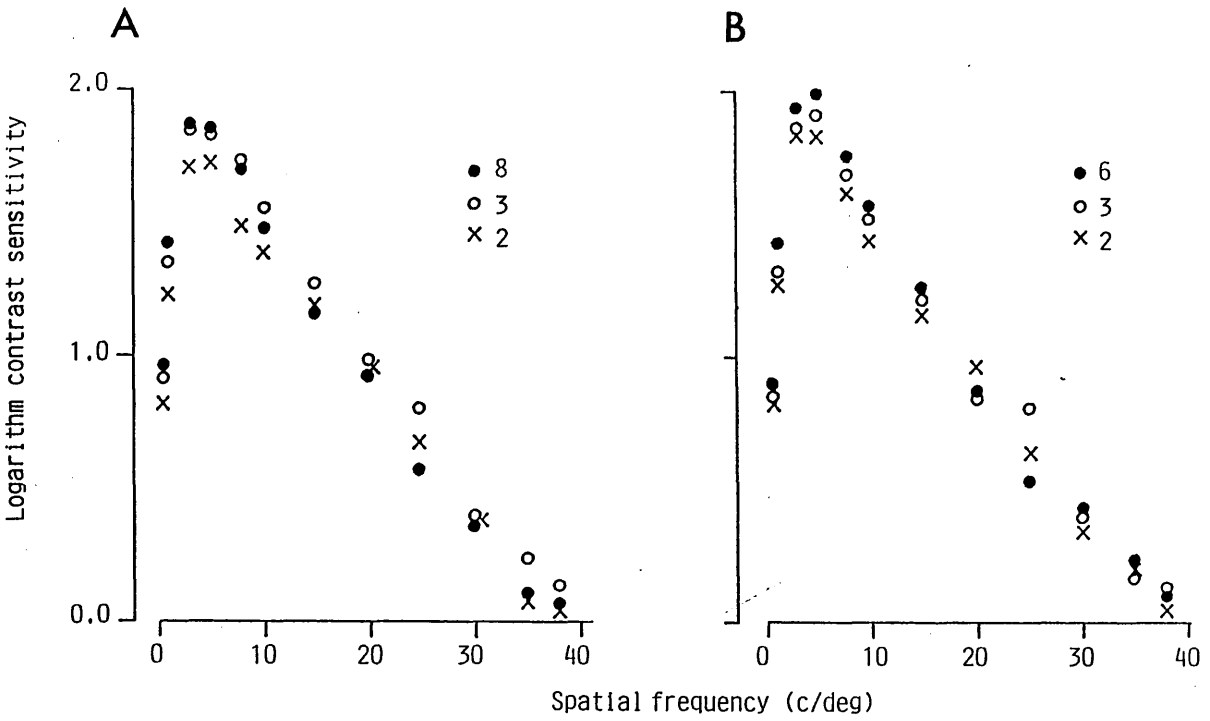


Fig. 8. Effects of pupil diameter on mean logarithmic contrast sensitivities in the homatropinised (A) and natural eye (B). Each point shows the mean for 12 (A) or 8 (B) subjects. Pupil diameter in mm is indicated.

Table 2. Effect of pupil diameter on the regression equation of mean log contrast sensitivity (y) against spatial frequency (x) in the range 3 - 38 c/deg.

Condition	Regression	SD of y	r	P
Pre-homatropine	$y = 2.08 - 0.056x$	0.08	−0.992	< 0.001
Homatropine/8 mm	$y = 2.07 - 0.058x$	0.05	−0.997	< 0.001
Homatropine/3 mm	$y = 2.06 - 0.052x$	0.06	−0.996	< 0.001
Homatropine/2 mm	$y = 1.94 - 0.052x$	0.05	−0.996	< 0.001
Natural/6 mm	$y = 2.15 - 0.057x$	0.12	−0.987	< 0.001
Natural/3 mm	$y = 2.07 - 0.053x$	0.08	−0.992	< 0.001
Natural/2 mm	$y = 2.00 - 0.052x$	0.05	−0.996	< 0.001

y is logarithm contrast sensitivity and x is spatial frequency.

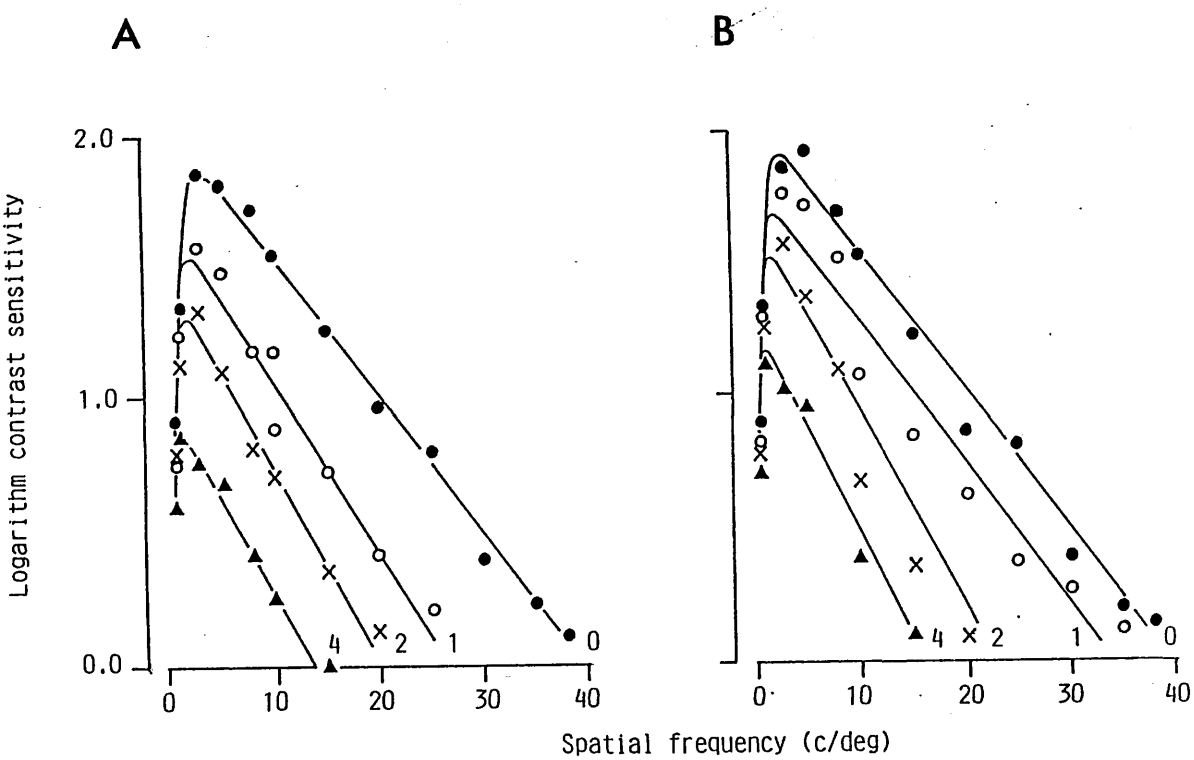


Fig. 9. Effects of positive defocusing lenses on mean logarithmic contrast sensitivities for viewing with the 3 mm artificial pupil in homatropinised (A) and natural (B) eyes. Each point shows the mean for 12 (A) or 8 (B) subjects. Defocus in dioptres is given alongside the appropriate contrast sensitivity curves.

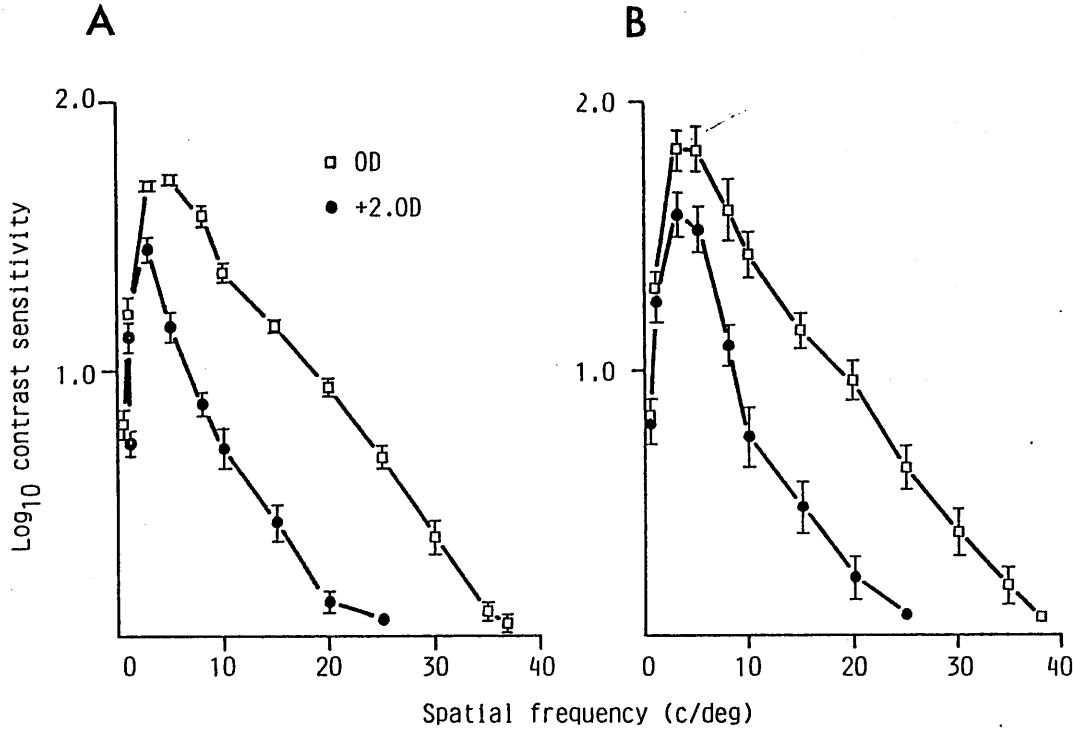


Fig. 10. Effects of a +2 D defocusing lens on mean logarithmic contrast sensitivities for viewing with the 2 mm artificial pupil in homatropinised (A) and natural (B) eyes. Each point shows the mean for 12 (A) or 8 (B) subjects.

equations are listed in Table 3. For 2D and 4D of defocus, there was a very marked reduction in the intercept of regression equations, which was significant when compared with optimal focus in the homatropinised and natural eyes ($P<0.001$). With the +1.0D lens, the decrease in intercept was significant only in the homatropinised eye ($P=0.05$) but not in the natural eye ($P>0.25$) where presumably relaxation of accommodation reduced the effects of defocus.

Inspection of the slopes of the regression lines shows an apparent increase in the negative slope with increasing defocus; however, statistical comparison of these slopes showed that the changes were not significant ($P>0.25$) in both homatropinised and natural eyes (Table 3). The same absence of a significant difference between slopes was also present in each one of the individual results listed in Table 4.

In the homatropinised eye, where defocus was precisely controlled, the subjects showed a range of effects with increasing defocus. Subjects 1 and 2 had slopes which were essentially unchanged with defocus. Subjects 10-12 had slopes which increased with increasing defocus in the same way as Campbell & Green's (1965) results, shown as "C and G" in Table 4. The remaining subjects (with the exception of subject 9) gave biphasic responses, in that the slope increased with +1.0D and then remained the same or decreased with greater defocus.

Viewing with the natural eye was subjectively reported as being difficult since continual shifts of focus occurred presumably as a result of the accommodation mechanism seeking optimal focus. The variability of the data was greater in the natural eye than in the homatropinised eye as shown for the mean data in Fig. 10B and Table 3. The individual results shown in Table 4 (right) again show a range of behaviour with regard to the slope of the regression equation. Some subjects showed an increase in slope with increasing defocus whilst

others showed an increase followed by a fall in slope at +4.0D. The data of Regan et al., (1977) shown as "R, S and M" also show an increase in slope for 1.0D. However, for all the data shown in Table 4 (right), including that of Regan et al., none of the regression equations for defocus were significantly different from optimal focus ($P>0.25$).

For spatial frequencies below peak of the CSF, the data were analysed using paired t-tests. Increased defocus in the homatropinised eye caused a significant reduction in contrast sensitivity at 0.5 and 1.0 c/deg with the 3mm pupil ($P<0.02$) but not with the 2mm pupil ($P>0.10$). Contrast sensitivity at 3 c/deg was significantly reduced with defocus for both 2 and 3mm pupils ($P<0.001$). In the natural eye, defocus reduced contrast sensitivity to a lesser extent, which was not significant for +1.0D ($P>0.1$), but with greater defocus, contrast sensitivity was significantly reduced with the 3mm pupil ($P<0.02$) but not with the 2mm pupil ($P>0.25$).

At the end of this long experiment (which usually lasted for 8 hours) repeat measurements were made for a range of spatial frequencies for the CRT display. For the homatropinised eye, repeat measurements were made at a low spatial frequency, 3 c/deg and at a high spatial frequency, 20 c/deg. When compared to the initial measurements for the homatropinised pupil and optimal focus, no significant differences were found for either 3 c/deg ($P>0.10$) or 20 c/deg ($P=0.89$), despite the arduous experiment just completed. For the natural eye repeat measurements were made at 10, 20 and 30 c/deg. At 10 c/deg a small but significant decline in contrast sensitivity was seen when compared to the first series of measurements taken for the natural pupil ($P=0.04$). Those measurements at 20 and 30 c/deg, however, were not significantly different from the initial measurements ($P=0.34$ and $P=0.51$ respectively).

Table 3. Effect of defocus with positive lenses on the regression equation of mean log contrast sensitivity (y) against spatial frequency (x) in the range 3 - 38 c/deg for the combinations of defocusing lens power and artificial pupil diameter indicated.

Condition	Regression	SD of y	r	P
After homatropine:				
0.0 D/3 mm	$y = 2.06 - 0.052x$	0.06	−0.996	< 0.001
+1.0 D/3 mm	$y = 1.69 - 0.063x$	0.10	−0.980	< 0.001
+2.0 D/3 mm	$y = 1.44 - 0.068x$	0.07	−0.986	< 0.001
+4.0 D/3 mm	$y = 0.96 - 0.068x$	0.04	−0.986	< 0.01
0.0 D/2 mm	$y = 1.94 - 0.052x$	0.05	−0.996	< 0.001
+2.0 D/2 mm	$y = 1.56 - 0.076x$	0.10	−0.979	< 0.001
Natural eye:				
0.0 D/3 mm	$y = 2.07 - 0.053x$	0.08	−0.992	< 0.001
+1.0 D/3 mm	$y = 1.79 - 0.053x$	0.15	−0.968	< 0.001
+2.0 D/3 mm	$y = 1.64 - 0.072x$	0.19	−0.950	< 0.001
+4.0 D/3 mm	$y = 1.26 - 0.077x$	0.07	−0.982	< 0.001
0.0 D/2 mm	$y = 2.00 - 0.052x$	0.05	−0.996	< 0.001
+2.0 D/2 mm	$y = 1.71 - 0.072x$	0.18	−0.956	< 0.001

Table 4. Slopes of regression equations of log contrast sensitivity against spatial frequency (c/deg) for 12 individual subjects with a 3 mm artificial pupil under the various conditions of defocus indicated. Also given are the slope values obtained from Campbell & Green (1965) - (C and G) and Regan et al., (1977) - (R, S and M).

	Homatropine				Natural			
	0.0 D	+1.0 D	+2.0 D	+4.0 D	0.0 D	+1.0 D	+2.0 D	+4.0 D
1. CDK	−0.054	−0.058	−0.059	−0.054	−0.063	−0.078	−0.095	−0.054
2. JDM	−0.049	−0.044	−0.042	−0.050	−0.063	−0.060	−0.048	−0.069
3. GW	−0.050	−0.061	−0.058	−0.067	−0.051	−0.052	−0.074	−0.124
4. AR	−0.048	−0.066	−0.058	−0.066	−0.048	−0.048	−0.069	−0.072
5. RO	−0.070	−0.085	−0.090	−0.083	−0.060	−0.075	−0.083	−0.077
6. JMI	−0.050	−0.061	−0.060	−0.059	−0.043	−0.069	−0.088	−0.076
7. HMD	−0.052	−0.073	−0.067	−0.059				
8. RR	−0.053	−0.074	−0.088	−0.080	−0.060	−0.067	−0.093	−0.069
9. DQ	−0.060	−0.043	−0.059	−0.081				
10. SON	−0.045	−0.059	−0.071	−0.085	−0.064	−0.068	−0.082	−0.075
11. LCC	−0.050	−0.058	−0.064	−0.073				
12. VK	−0.047	−0.055	−0.076	−0.093				
C and G	−0.048	−0.056	−0.060					
R, S and M					−0.064	−0.093		

Units: log units per cycle per degree.

Quantitative analysis

In order that these measurements might be of practical value in predicting contrast sensitivity at this CRT luminance of 1.4 cd/m^2, the best fitting multiple regression equations were derived taking into account pupil diameter, defocus and spatial frequency. To simplify the analysis the CSF was separated at the peak of 3 c/deg into low and high spatial frequency limbs.

The derived equation expressed logarithm contrast sensitivity (y) as a linear function of spatial frequency (x_F, in c/deg), pupil diameter (x_P, in mm) and defocus (x_D, in dioptres). The equations derived for the natural and homatropinised eyes are shown below.

Homatropine

0.5-3 c/deg, $y = 0.80 + 0.29x_F + 0.021x_P - 0.09x_D$,

$SD_y = 0.15$, $r = +0.924$ ($P < 0.001$)

3-38 c/deg, $y = 1.99 - 0.055x_F + 0.009\,x_P - 0.31x_D$,

$SD_y = 0.11$, $r = -0.981$ ($P < 0.001$).

Natural

0.5-3 c/deg, $y = 0.78 + 0.33x_F + 0.024x_P - 0.05x_D$,

$SD_y = 0.15$, $r = +0.926$ ($P < 0.001$)

3-38 c/deg, $y = 2.05 - 0.057x_F + 0.016x_P - 0.26x_D$,

$SD_y = 0.15$, $r = -0.972$ ($P < 0.001$).

Neither logarithmic nor square functions of the x variables in the equations resulted in a better fit with the exception of the natural eye at 3-38 c/deg where a very marginal improvement was obtained by taking the logarithm of the spatial frequency term. Analysis of the 0.5-1.0 c/deg data (i.e. excluding 3 c/deg) in the homatropinised eye gave coefficients for pupil diameter and defocus of +0.017 and -0.08,

respectively. These are similar to those obtained when 3 c/deg was included in the low spatial frequency limb and indicated that the 3 c/deg data had not biased these coefficients. To take account of a possible increase in negative slope with defocus, the latter term was incorporated as a function of spatial frequency. The best fitting regression for the homatropinised eye over 3-38 c/deg thus obtained was

$$y = 1.93 - 0.052x_F + 0.006x_P - 0.15x_D\log_{10}x_F$$

$$SD_y = 0.10,\ r = -0.980\ (P< 0.001)$$

As the correlation coefficient was almost exactly the same for this equation there was no reason to prefer this equation over the more simple form originally computed.

To summarise the findings of these computations, at all spatial frequencies, changes in pupil diameter had only very marginal effects, tending to increase contrast sensitivity as pupil diameter increased. The effects were somewhat greater at 0.5-3 c/deg than over the 3-38 c/deg segment of the CSF. By contrast, the effects of defocus were profound, being greater in the homatropinised eye than in the natural eye. At 0.5-3 c/deg, contrast sensitivity was reduced by 19% and 11% per dioptre in the homatropinised and natural eye respectively while at 3-38 c/deg, the reduction was 51% and 45% per dioptre respectively.

Contrast sensitivity to the laser display

The shape of the CSF for the laser display was generally more flattened at the peak than that for the CRT display. Attenuation of contrast sensitivity did occur at low and high spatial frequencies as shown for the mean CSF for all 12 subjects (Fig. 11). The peak contrast sensitivity was 148 units (range 43-692) and also occurred at 3 c/deg as for the CRT display but the peak was more noticeably flattened. Spatial frequencies as high as 50 c/deg were observed without difficulty. The

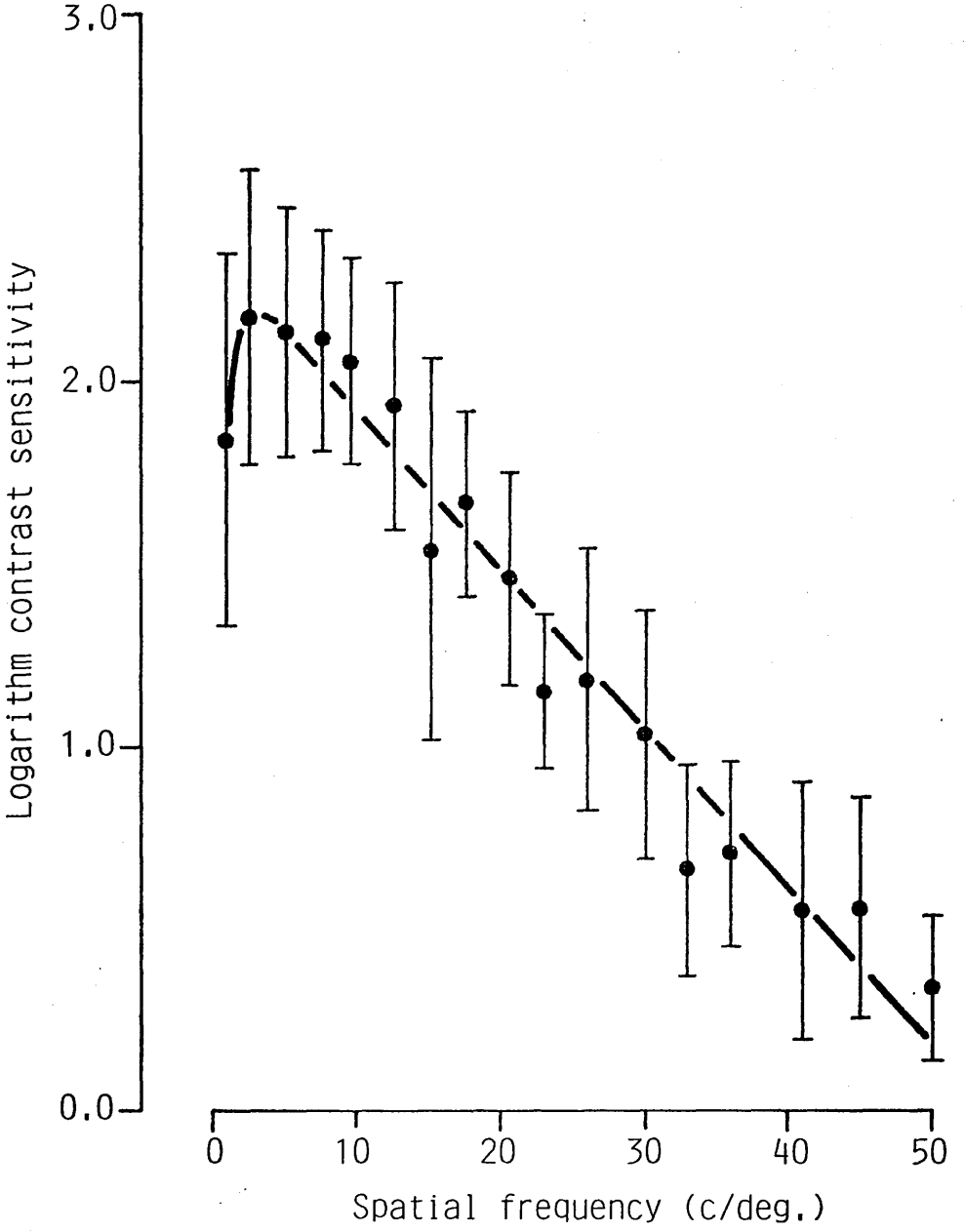


Fig. 11. Contrast sensitivities (on logarithmic scale) to laser interference fringes showing the geometric mean ± S.D. for 12 subjects for viewing with natural 6 mm pupil.

high frequency fall-off was fitted by a logarithmic-linear plot as shown in Fig. 11 and the regression equation obtained is given below. However, the relationship may be more curvilinear at very high spatial frequencies for omitting the 45 and 50 c/deg points from the regression resulted in a slightly better fit to the regression line as also shown below.

The regression for the laser display obtained over 2.5-50 c/deg was

$$y = 2.34 - 0.043x$$

$$SD_y = 0.12,\ r = -0.983\ (P < 0.001)$$

The best fitting regression obtained over 2.5-41.5 c/deg was

$$y = 2.41 - 0.047x$$

$$SD_y = 0.10,\ r = -0.986\ (P < 0.001)$$

The standard deviation bars of Fig. 11 show that there was a great deal of variation between subjects in the absolute contrast sensitivity values, nevertheless the general shape of the CSF was the same for all subjects. The contrast sensitivity measurements to the laser interference fringes were always greater than those for the CRT at corresponding spatial frequencies, since the contrast of the fringes is not degraded by the ocular media. The ratio of CRT to laser contrast sensitivities should represent the modulation transfer of the ocular media. The continuous line uppermost in Fig. 12 which has been obtained from the two best-fitting lines for CRT and laser displays represents the derived MTF. From a peak value of 0.5 (which represents 50% transmission of contrast) at 3 c/deg, the modulation transfer declines to 0.3 at 10 c/deg above which it remains fairly level over the range of spatial frequencies studied. The mean slope of the MTF was less steep than that for the laser display, indicating, for the higher spatial frequencies, that greater loss of contrast sensitivity arises in the

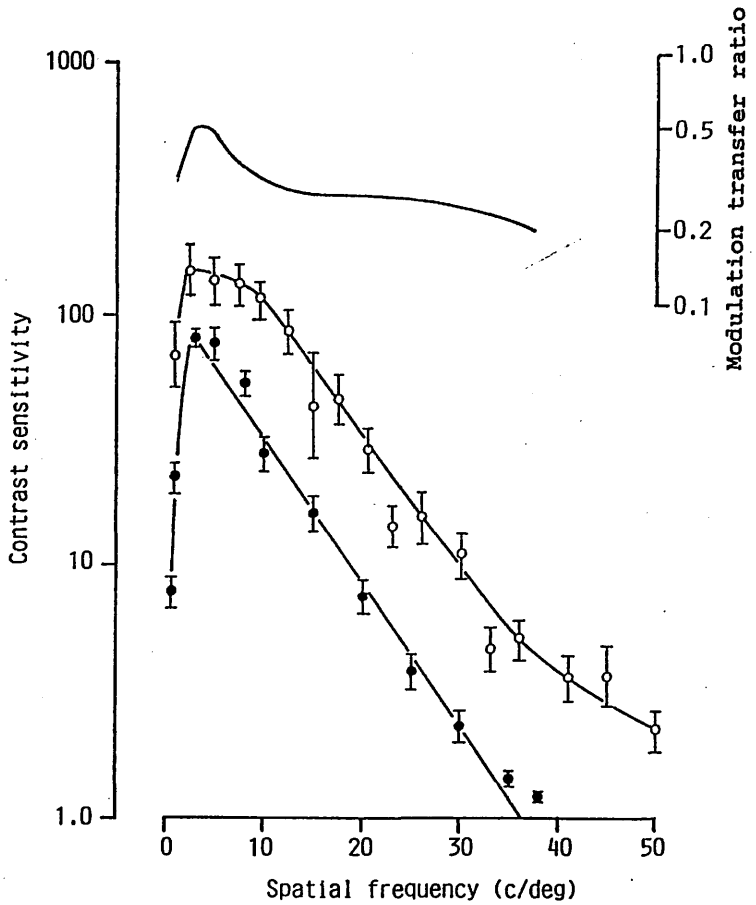


Fig. 12. Contrast sensitivities to CRT (filled circles) and laser displays (open circles), showing the mean ± S.E. for 12 subjects (as in Figs. 7 and 11 respectively). Uppermost continuous curve is the MTF derived from the two lower curves.

retina-brain which must ultimately set the limit to resolution. Below 3 c/deg, the fall in the MTF which arose in all subjects, is clearly anomalous because theoretical considerations predict an increase in the transmission to unity as spatial frequency decreases (Hopkins, 1955).

This relationship was further investigated by an honours student working in our laboratory. By carefully matching field size and luminance of the two displays she found that for very low spatial frequencies the MTF did in fact increase from 0.2 to 0.6 over the range of 3 to 0.2 c/deg, but never approached unity as predicted for a simple optical lens. Her results are reproduced in Fig. 13. On the left are the results obtained for a typical subject and on the right those of a group of 7 subjects others showing means and range of values. The mean values reflect the general shape of the individual CSFs, although the numerical range of values was fairly wide. The MTF obtained from these results is shown at the top of the figure.

Part 2: The effects of a single oral dose of pyridostigmine bromide on contrast sensitivity

Contrast sensitivity to CRT and laser displays

Individual results

The CSF was of the same general form for each subject both for each experimental day (pyridostigmine and lactose control) and for each display (CRT and laser). Contrast sensitivities to the laser display were generally greater than to the CRT display; however there was some variation between subjects and Fig. 14 shows some representative results. Subject 7 is one of a group of five subjects for whom contrast sensitivity to the laser display always exceeded that to the CRT display. Subject 8 is one of a group of six subjects in whom laser

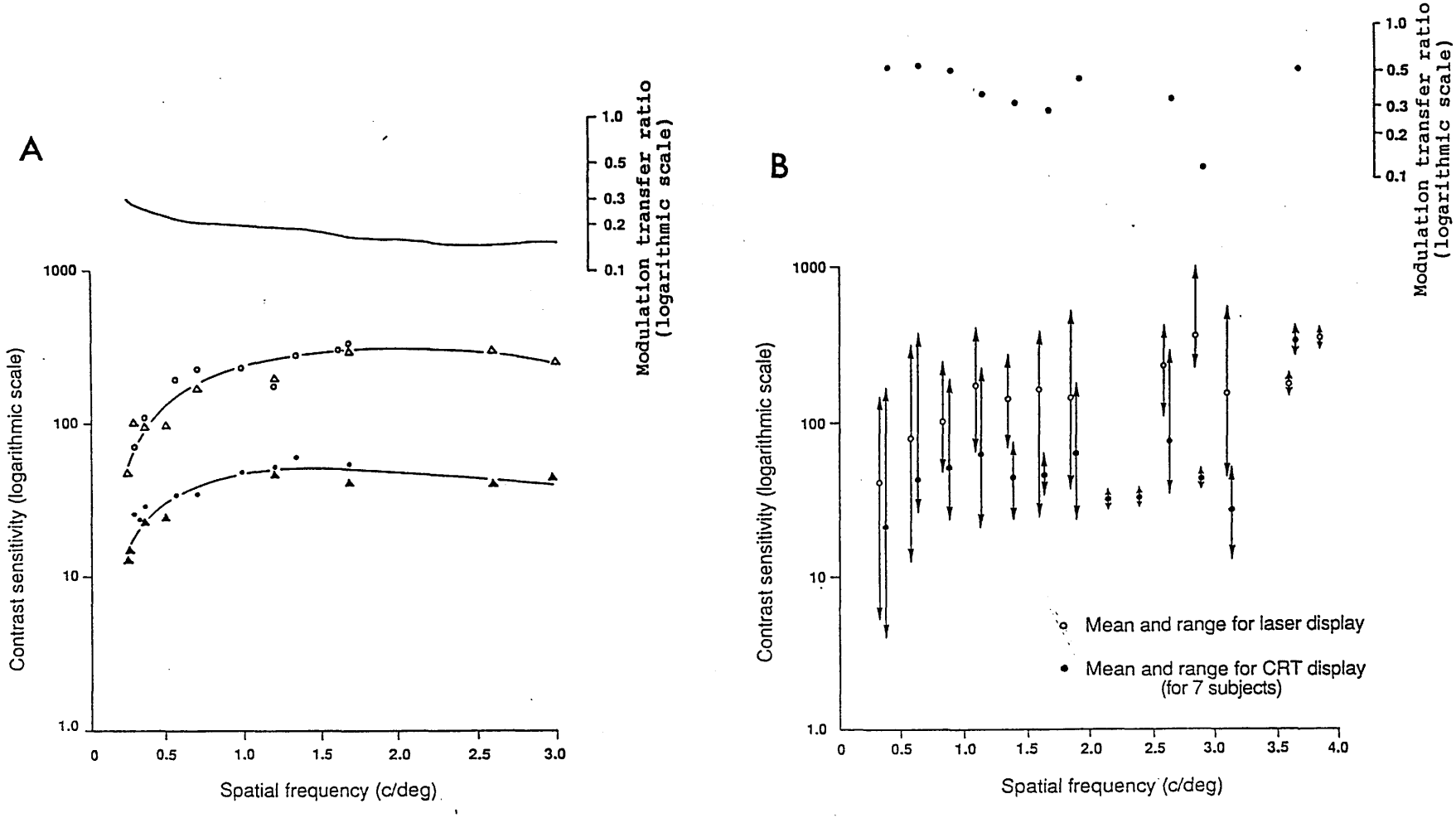


Fig. 13. Contrast sensitivities (logarithmic scale) to CRT display (closed symbols) and laser display (open symbols) with the derived MTF shown uppermost. Results for one subject for two sessions (circles and triangles) (A). Mean results and numerical range of values for 7 subjects (B). Reproduced with permission from (A), Fraser & Morrison (1987) and (B), Fraser (1987).

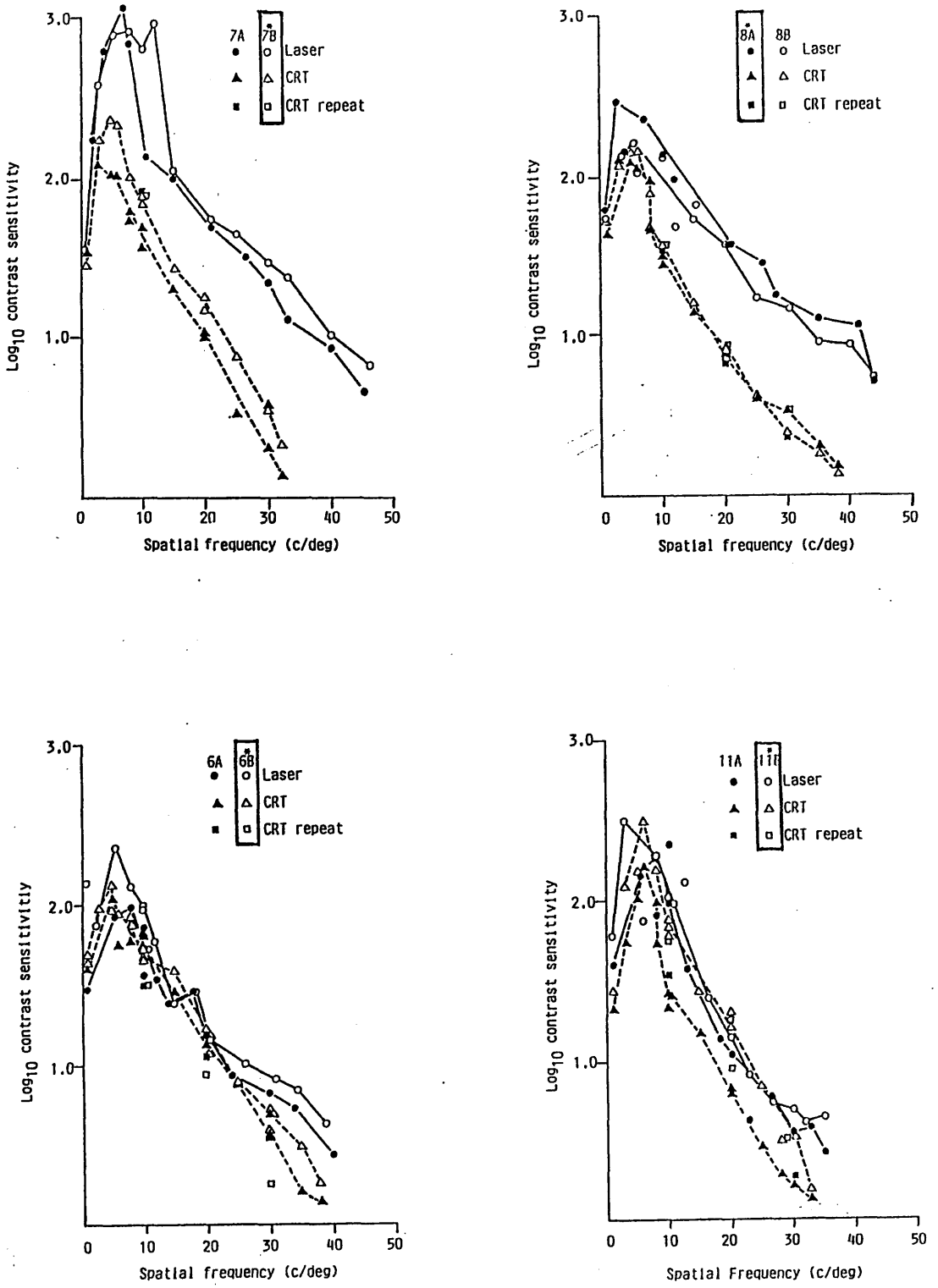


Fig. 14. Effects of ingestion of 60 mg of pyridostigmine bromide (boxed symbols) compared with 60 mg lactose on contrast sensitivity (logarithmic scale) measured to CRT and laser displays. The results of four individual subjects are shown, repeat measurements for the CRT display made at the end of the experiment are indicated.

contrast sensitivity generally exceeded that to the CRT at most spatial frequencies except for those at the peak of the CSF where much closer overlap occurred. This may have been due, in part, to an overestimation of threshold to the laser display as the precision of measurement was poorer at small angles of rotation than at larger angles. The responses of the two other subjects tested in this experiment are shown in the bottom half of this figure as they did not fit into either of the categories just described. Subject 6 only showed a greater sensitivity to the laser display at high spatial frequencies above 25 c/deg. Subject 11 showed greater sensitivity to the laser display than to the CRT on the first day he was tested (11A), but on the second day (11B) which was after pyridostigmine, the laser and CRT results overlapped over the complete range of spatial frequencies tested. This seemed to be due to an increase in performance to the CRT display (when comparing the results to those in 11A). While both subjects 6 and 11 were naive subjects, five others were also being tested for the first time in this study and they did not produce any unusual results.

By inspection of the individual CSFs following physostigmine, three subjects showed slightly greater, three showed slightly reduced and five showed no change in contrast sensitivity for the CRT display. Similarly, for the laser display, three subjects showed slightly greater, two showed slightly reduced and six showed no change in contrast sensitivity following pyridostigmine.

The CSF for both stimulus displays consisted of a peak at 3-5 c/deg with a decline at lower and higher spatial frequencies. Each individual's high frequency fall-off (3 c/deg and above) was best fitted by a logarithmic-linear plot, the correlation coefficient of which was always better than $r = -0.92$ ($P<0.001$). Since it is highly improbable that any one spatial frequency would be affected in isolation, these

regression equations were statistically compared between pyridostigmine and lactose control for each individual. No significant differences were present between the slopes of the pyridostigmine and control results, or between their intercepts to either stimulus display. Statistical comparisons at low spatial frequencies for each subject were not feasible since this involved comparing the five contrast sensitivity measurements used to compute the mean value. However, at 1 c/deg and 3 c/deg it was apparent by inspection that overlap occurred between pyridostigmine and control results for both stimulus displays, indicating the absence of a discernible change.

Repeat measurements were made for the CRT display at 10, 20 and 30 c/deg at the end of the experimental period and were compared with those obtained for the same spatial frequencies at the start of the experiment. Paired t-tests revealed no significant changes between the two sets of measurements for either the pyridostigmine or control experiment. The significance levels are given in Table 5 below. Thus, no change in the subject's criterion of threshold had occurred during the course of the experiment.

Table 5. Comparison of contrast sensitivity measurements to CRT display at start and end of the experiment by paired t-test for 13 subjects.

Spatial Frequency (c/deg)	Pyridostigmine	Control
10	P = 0.87	P = 0.81
20	P = 0.68	P = 0.23
30	P = 0.54	P = 1.00

Mean Results

A mean CSF was calculated for the 13 subjects under the two different experimental conditions for the two displays used. The mean value and standard error of the mean for each spatial frequency are shown in Fig. 15. For the CRT display, the peak of the CSF had a mean value of 93 units after pyridostigmine and a mean value of 91 units for the control experiment (Fig. 15A). The high frequency fall-off was best fitted by a logarithmic-linear plot with a cut-off frequency of 39.8 c/deg for the pyridostigmine results and 39.6 c/deg for the control results. The laser display CSF had a mean value of 126 units at peak for both the pyridostigmine and control experiments (Fig. 15B). The regression of the linear part of the CSF gave a predicted cut-off frequency of 53.5 c/deg for the pyridostigmine and 53.4 c/deg for the control. The regression equations are given below, where y is logarithm_{10} contrast sensitivity and x is the spatial frequency (c/deg).

CRT display

Control (lactose) $y = 2.06 - 0.052x$

$SD_y = 0.11$, $r = -0.983$ ($P < 0.001$)

Pyridostigmine $y = 2.11 - 0.053x$

$SD_y = 0.09$, $r = -0.988$ ($P < 0.001$)

Laser display

Control (lactose) $y = 2.19 - 0.041x$

$SD_y = 0.08$, $r = -0.988$ ($P < 0.001$)

Pyridostigmine $y = 2.30 - 0.043x$

$SD_y = 0.12$, $r = -0.978$ ($P < 0.001$)

Comparison of the regression equations for the mean results for each display for the pyridostigmine and control experiments revealed that neither the slopes nor the intercepts were significantly different

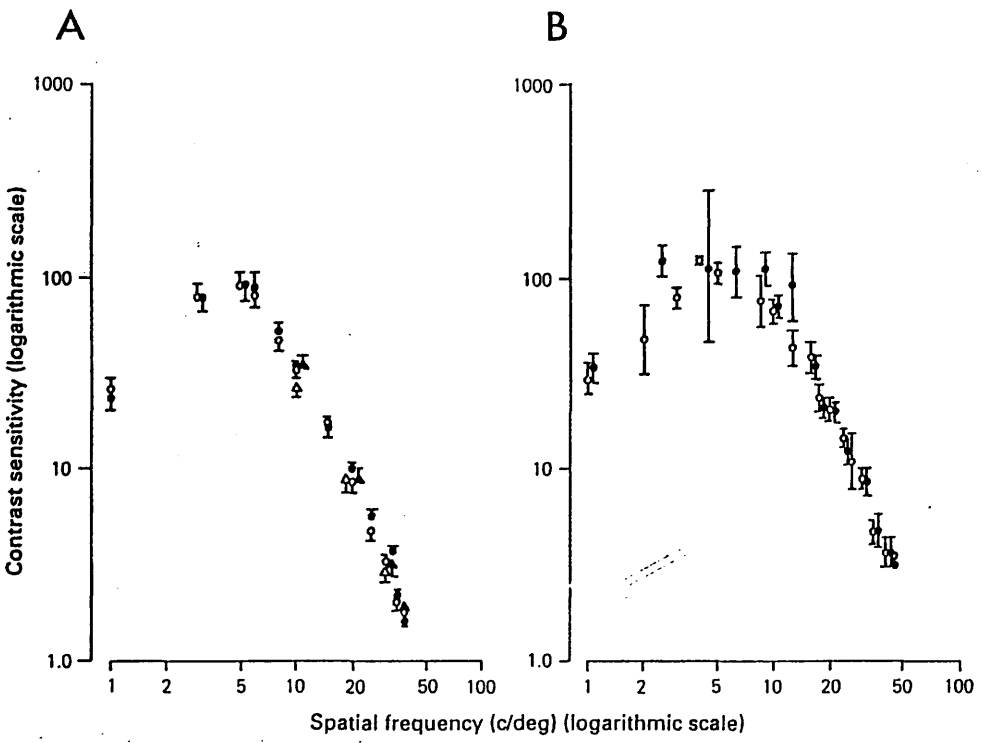


Fig. 15. Effects of ingestion of 60 mg of pyridostigmine bromide compared with 60 mg lactose on contrast sensitivity (logarithmic scale) to CRT display (A) and laser display (B). Each point shows the mean ± S.E. for 13 subjects. ○, lactose; △, lactose repeat; ●, pyridostigmine; ▲, pyridostigmine repeat.

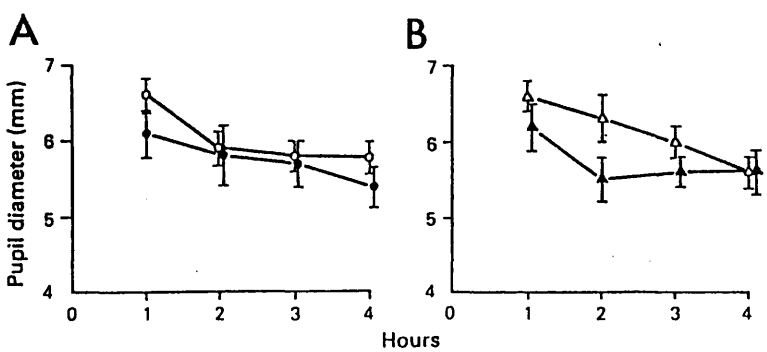


Fig. 16. Effects of ingestion of 60 mg pyridostigmine bromide on pupil diameter compared with 60 mg lactose, ●, pyridostigmine; ○, lactose (A). For comparison, pupil diameter measured on subject's first or second visit to the laboratory, △, first visit; ▲, second visit (B). These are the mean results ± S.E. of 10 subjects.

(P>0.25). Paired t-tests were also performed for the mean logarithm contrast sensitivity data. Contrast sensitivity for all spatial frequencies above peak showed a mean improvement of 0.031 log units, S.D., 0.042 (viz. a 7% improvement) after pyridostigmine for the CRT display only (P=0.02). Below the peak spatial frequency no significant differences occurred with either display (CRT, P>0.25; laser, P>0.10).

Pupil diameter was also measured during the course of this experiment for 10 subjects for whom satisfactory photographs had been obtained. The mean values and standard errors are shown in Fig. 16. A small reduction in pupil diameter occurred after pyridostigmine (Fig. 16A) though this was rather less than the decrease occurring between first and second visits to the laboratory (Fig. 16 B).

Part 3: The effects of topical application of 0.25% physostigmine sulphate on visual performance.

Twelve subjects (18-28 years) completed the experiments in this part of the study. They comprised of 7 males and 5 females, though no differences in performance were seen (by inspection) between the two sexes. Each subject completed 3 experimental trials on well spaced days, so that no cumulative effects would be possible. Application of the eyedrops produced a stinging sensation and stimulated some tear formation. The amount of tears produced was quite variable and was noted for each subject. Twitching of the eyelids also occurred in several subjects during the first few minutes after administration.

All 12 subjects showed a marked and long lasting pupillary constriction in response to the eyedrops. This usually began at about 10 min and was maximal after 30 min, by which time the pupil had constricted from a mean of 5.7mm to 2.2mm in diameter. The pupil

remained constricted for the duration of the experiment, though the mean pupil diameter increased slightly to 2.7mm at the end of the 3 hour period. Pupil diameter then slowly returned to normal by 24-36 hours. The mean pupil diameters, calculated for each of the three experimental trials are shown in Fig. 17. The control eye did not show any evidence of constriction from possible systemic absorption of physostigmine, or any consensual response and remained fairly constant at a diameter of circa 6mm throughout.

The physostigmine eyedrops caused an increase in amplitude of voluntary near point accommodation (i.e. brought the near point closer) and also an increase of involuntary accommodation which caused a defocus of all distant objects (in fact all objects beyond the near-point distance). The increase in accommodation began between 5 and 10 min after administration and the resulting defocus was maximal by 30 min and then subsided by some 2 hours. The mean defocus for all 12 subjects is shown in Fig. 18. There was, however, a great deal of variation in the magnitude of this response as shown by the peak values for all subjects given in Table 6. Following physostigmine eyedrops, on Trial 1 three groups of responses were observed. Subjects 1-6 were all severely defocused by some 7-9D, and Snellen acuity was reduced to less than 5/60. Three subjects (subjects 7,8 & 9) were only moderately defocused (1.5-4.5D) with Snellen acuity being reduced to 5/35, 5/18 and 5/6 respectively. The remaining three subjects (10-12) were only slightly defocused (0.5-0.75D) with Snellen acuity at worst being 5/5. Subject 12, in fact, maintained 5/4 acuity throughout. This large variation in response was not associated with eye colour or to the amount of tears produced.

To demonstrate the two extremes in response seen for each of the parameters measured, the individual results for subjects 1 and 12 are

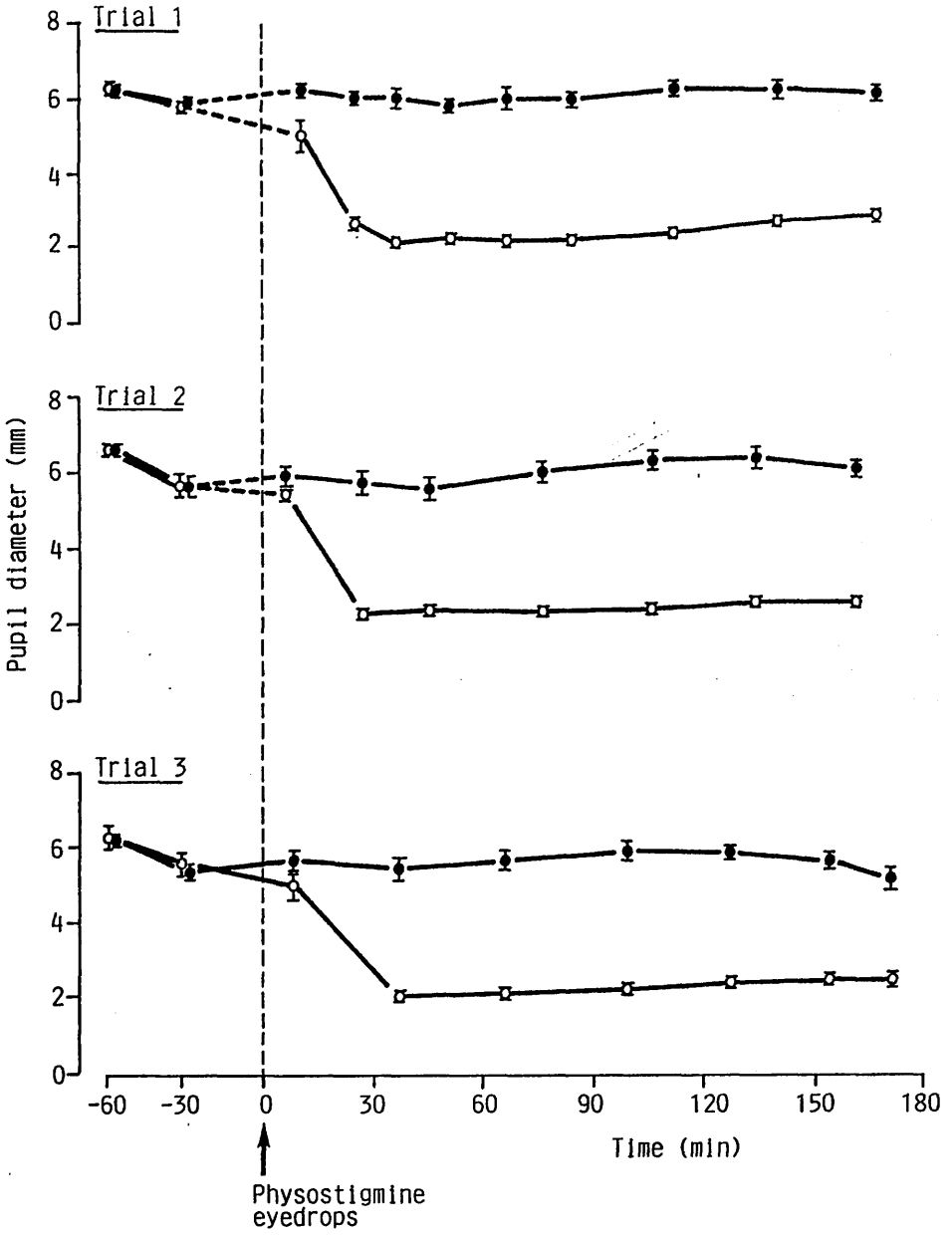


Fig. 17. Effect of 0.25% physostigmine eyedrops on pupil diameter for three trials on the same 12 subjects. Each point shows the mean ± S.E., ● control eye; o test eye.

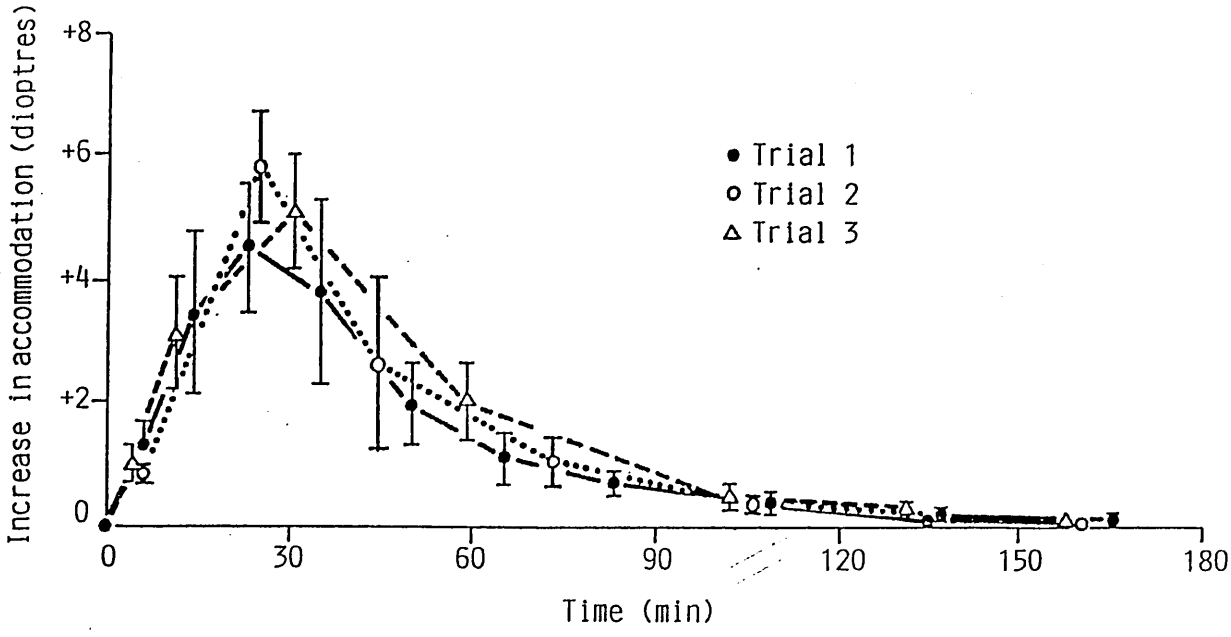


Fig. 18. Effect of 0.25% physostigmine eyedrops on amplitude of involuntary accommodation for three trials. Mean ± S.E. for 12 subjects.

Table 6. Effect of 0.25% physostigmine eyedrops on the amplitude of involuntary accommodation.

Subject	Sex	Age (yr)	Peak increase in accommodation (dioptres) Trial 1	Trial 2	Trial 3
1. SW	M	28	9.0	10.0	6.0
2. DO	M	20	9.0	8.5	7.5
3. CK	F	24	8.0	9.0	8.0
4. GM	M	24	8.0	8.5	7.5
5. AB	F	25	7.0	8.5	9.0
6. SO	M	23	7.0	1.25	6.0
7. AM	F	20	4.5	4.0	6.0
8. LM	F	22	3.0	3.5	-
9. CH	M	23	1.5	6.0	4.5
10. JG	F	20	0.75	1.5	3.5
11. JR	M	24	0.5	4.0	1.5
12. MF	M	22	0.5	0.75	0.5

shown along with the mean results. Fig. 19A & B shows that the pupil constricted equally for subjects 1 and 12 whilst the increase in accommodation shown in Fig. 20A & B was quite different, being 9.0D for subject 1 at the peak of the response compared to only 0.75D for subject 12.

Those subjects who were most severely defocused on the first experimental trial generally were so on each of the two repeats (Table 6 and shown in Fig. 20A for subject 1). The only exception to this was for subject 6 who showed a very much smaller response on the second trial. This may however have been due to poor delivery of the drug for excessive twitching of the eyelids was noted (which did not occur for this subject on either Trial 1 or 3). If excessive drug was absorbed through the skin, a smaller amount may have been available for absorption through the cornea. However, the drug was still reaching the iris in sufficient concentration to constrict the pupil to a similar extent as Trials 1 and 3. Those subjects who were moderately defocused gave somewhat variable responses as did two of the subjects (10 & 11) who were only slightly defocused on the first trial. Subject 12 was consistently not defocused to any great extent (Fig. 20B).

The increase in accommodation caused a certain amount of discomfort with pain felt in and around the eye which led to headaches in some cases. The severity of the pain seemed related to the degree of defocus response and increased when an accommodative effort was made to focus on a close object, as in near point measurements. One subject, on the second occasion tested, experienced a delayed reaction to the eyedrops in that severe headache and nausea were experienced later that day. She was therefore not asked to complete the third trial.

An increase in voluntary near point accommodation was seen which peaked at 30 minutes after the eyedrops were given and then gradually

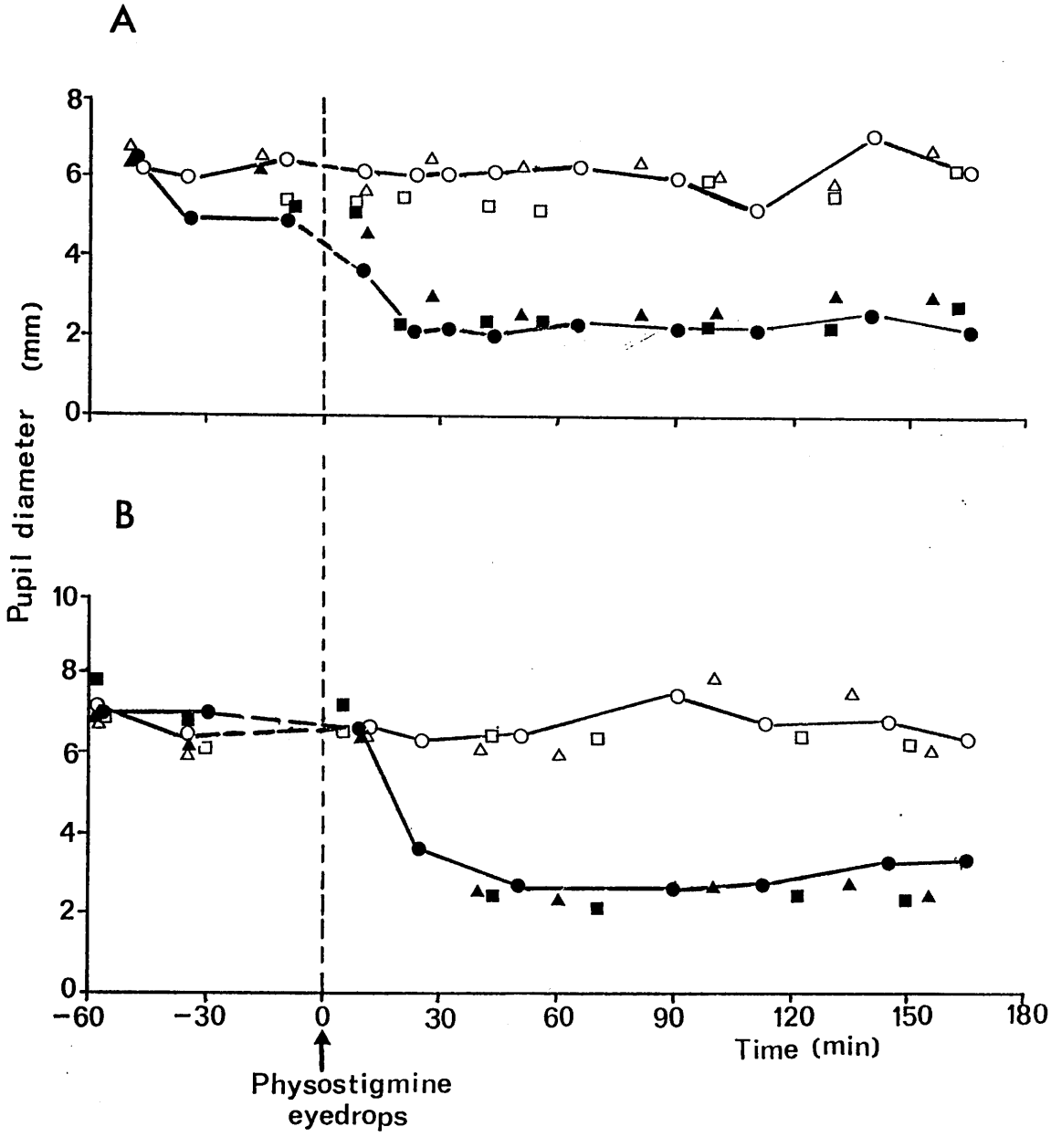


Fig. 19. Effect of 0.25% physostigmine eyedrops on pupil diameter of test (closed symbols) and companion eye (open symbols) for three trials. Individual results of subject 1 (A) and subject 12 (B).

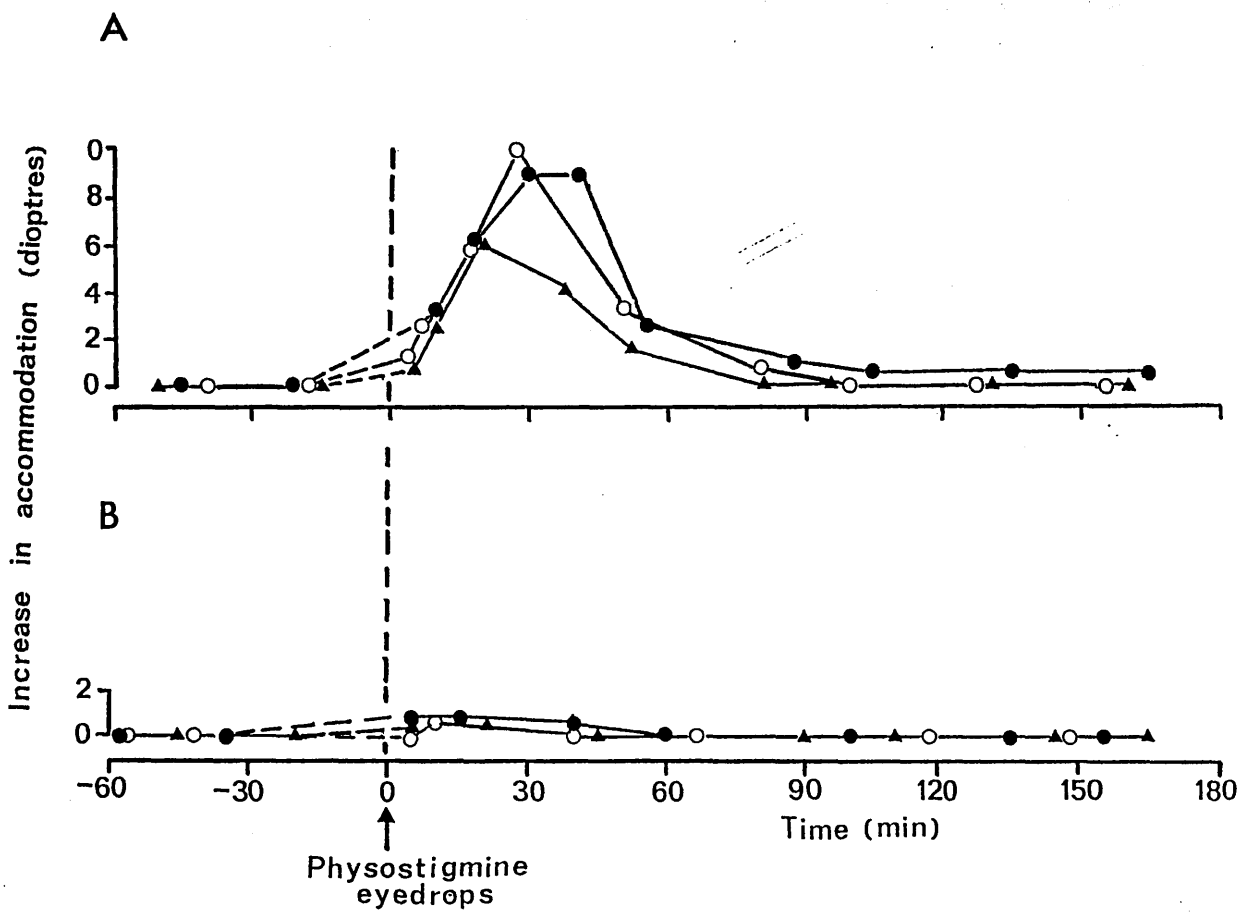


Fig. 20. Effect of 0.25% physostigmine eyedrops on amplitude of accommodation. Individual results of subject 1 (A) and subject 12 (B).

declined, but an additional 2D of accommodation remained at the end of the experiment (Fig. 21). Some of this increase in accommodative amplitude would be due to the increased depth-of-focus obtained from the small pupil. To take account of this, control measurements prior to physostigmine were also made viewing through a 2mm artificial pupil - these data are shown in Fig. 22. This approach had varying degrees of success due to the problem which subjects experienced in alignment of the artificial pupil with the chart of the RAF rule. In the case of Subject 1, no difference in near point accommodation occurred (Fig. 22A) while Subject 12 showed an increase of 2D in near point accommodation viewing through the 2mm artificial pupil in control measurements. This was similar to the increase measured for the constricted pupil following physostigmine (Fig. 22B).

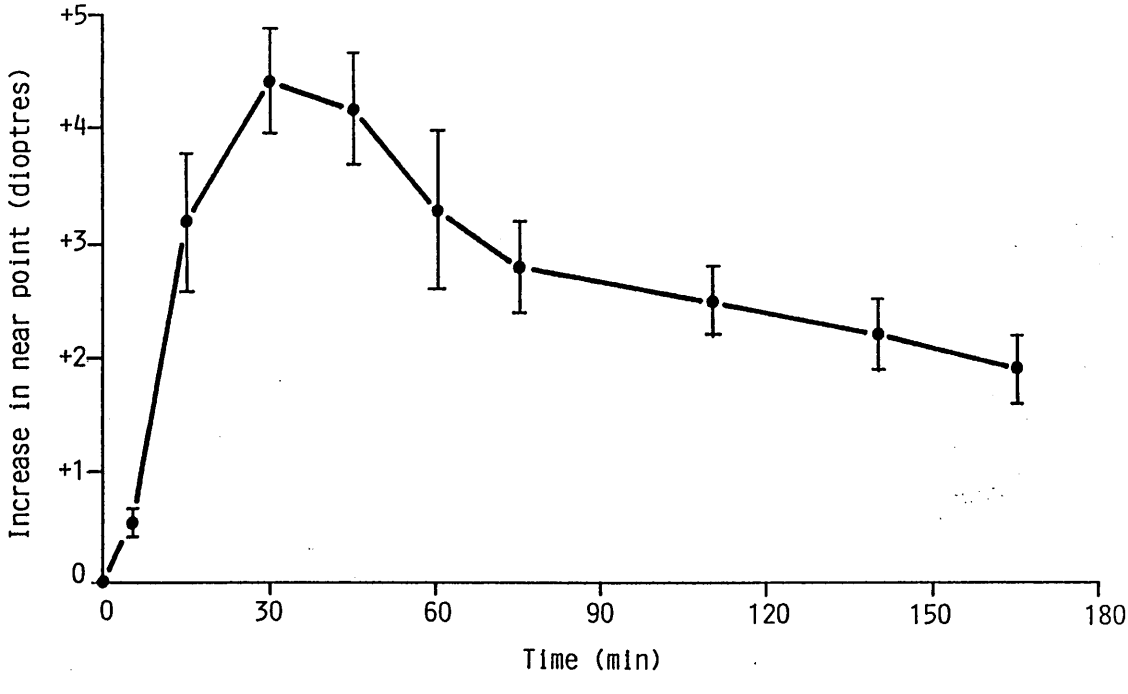


Fig. 21. Effect of 0.25% physostigmine eyedrops in causing an increase in near point accommodation. Mean ± S.E. for 12 subjects.

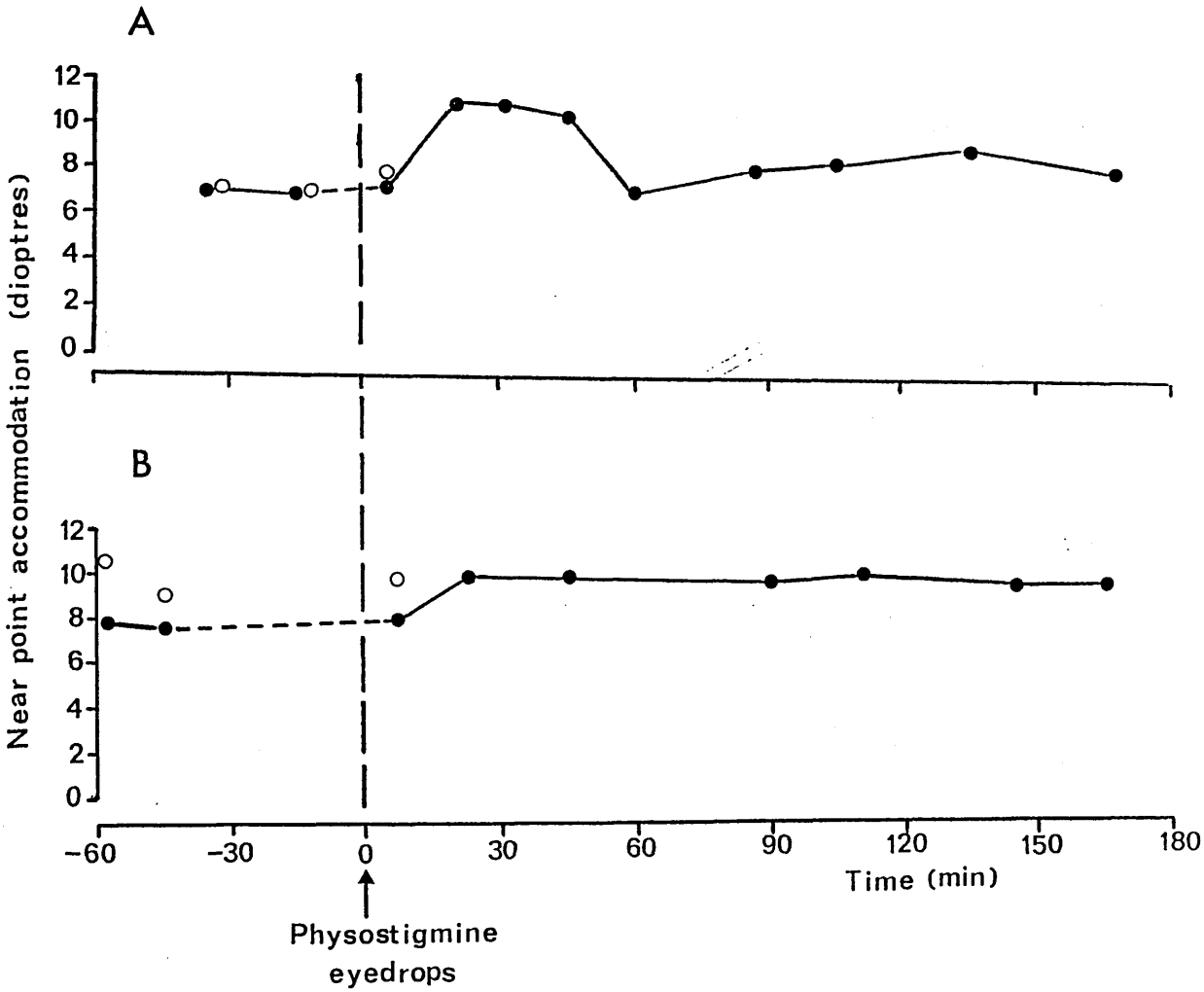


Fig. 22. Effect of 0.25% physostigmine eyedrops on the amplitude of near point accommodation. Individual results of subject 1 (A) and subject 12 (B). ○, near point accommodation viewing through 2mm artificial pupil.

Contrast sensitivity to stationary grating patterns

The mean contrast sensitivity to stationary gratings of 3, 10, 20 and 30 c/deg also declined with a time course similar to that of defocus. The curves in Fig. 23A show logarithm contrast sensitivity plotted against time for each spatial frequency measured. At each spatial frequency, there was a large fall in contrast sensitivity which peaked at about 30 min and then subsided by 2-2.5 hours. The curves in Fig. 23B show the change in contrast sensitivity expressed as a percentage of the second control value (at -30 min). The time course and minimum contrast sensitivity were approximately the same for each spatial frequency, though the contrast sensitivity at 3 c/deg remained at only 75% control at the end of the experiment.

The peak decline in contrast sensitivity, for the grouped data, was proportional to the degree of defocus as shown in Fig. 24. At 3 c/deg a clear correlation exists between the decline in contrast sensitivity (expressed as logarithm of test/control value) and the amount of defocus ($r=-0.71$, $P=0.009$). A similar relationship was seen at 10 c/deg ($r=-0.74$, $P=0.006$). At both 20 and 30 c/deg, many observers were unable to detect the pattern at all when the drug's effect was maximal (represented as open triangles in Fig. 24). However, the relationship between peak decline in contrast sensitivity and defocus still applies at 20 and 30 c/deg as the same downward trend is apparent.

While the time course of reduction in contrast sensitivity shown in Fig. 23 generally applied to all subjects, the actual amplitude of change showed considerable variation between individuals. The extreme range is illustrated in Fig. 25 in which Subject 1 showed the usual transient fall in contrast sensitivity while Subject 12 instead showed a small improvement which was attributed to improved ocular quality following the physostigmine induced miosis.

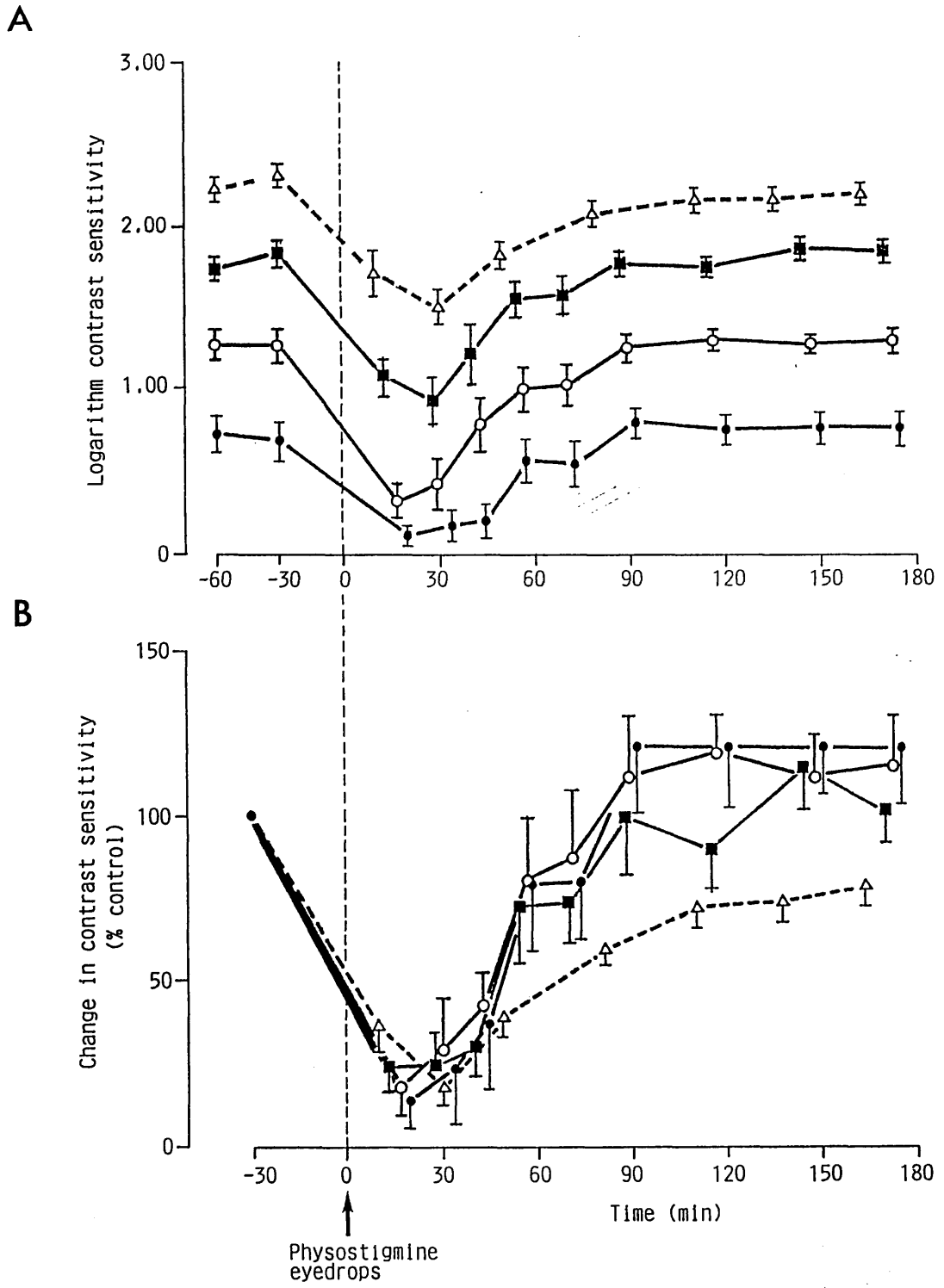


Fig. 23. Effect of 0.25% physostigmine eyedrops on log contrast sensitivity to stationary grating patterns generated on CRT display (A) and the change in contrast sensitivity (linear scale) from control value C2 (B). Each point shows the mean ± S.E. of 12 subjects for spatial frequencies of 3 △; 10 ■; 20 ○ and 30 ● c/deg.

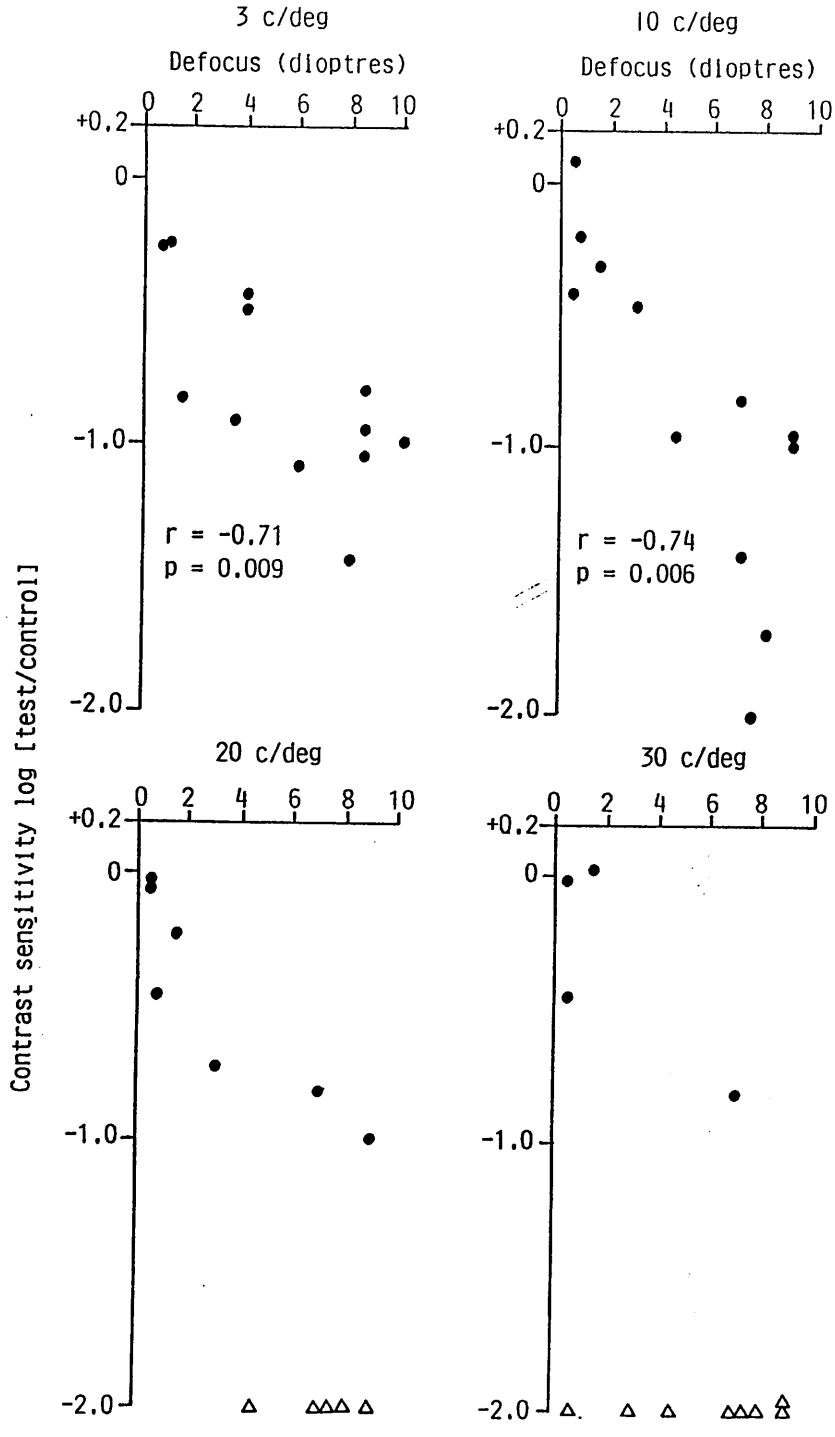


Fig. 24. Maximum reduction in contrast sensitivity from control value C2 (logarithmic scale) for stationary grating patterns plotted against maximum defocus caused by 0.25% physostigmine eyedrops, for the spatial frequency indicated. Correlation coefficient (r) and significance level (p) of the linear regression are shown. Readings which were off-scale are shown by open triangles; statistical analysis was not performed in these cases.

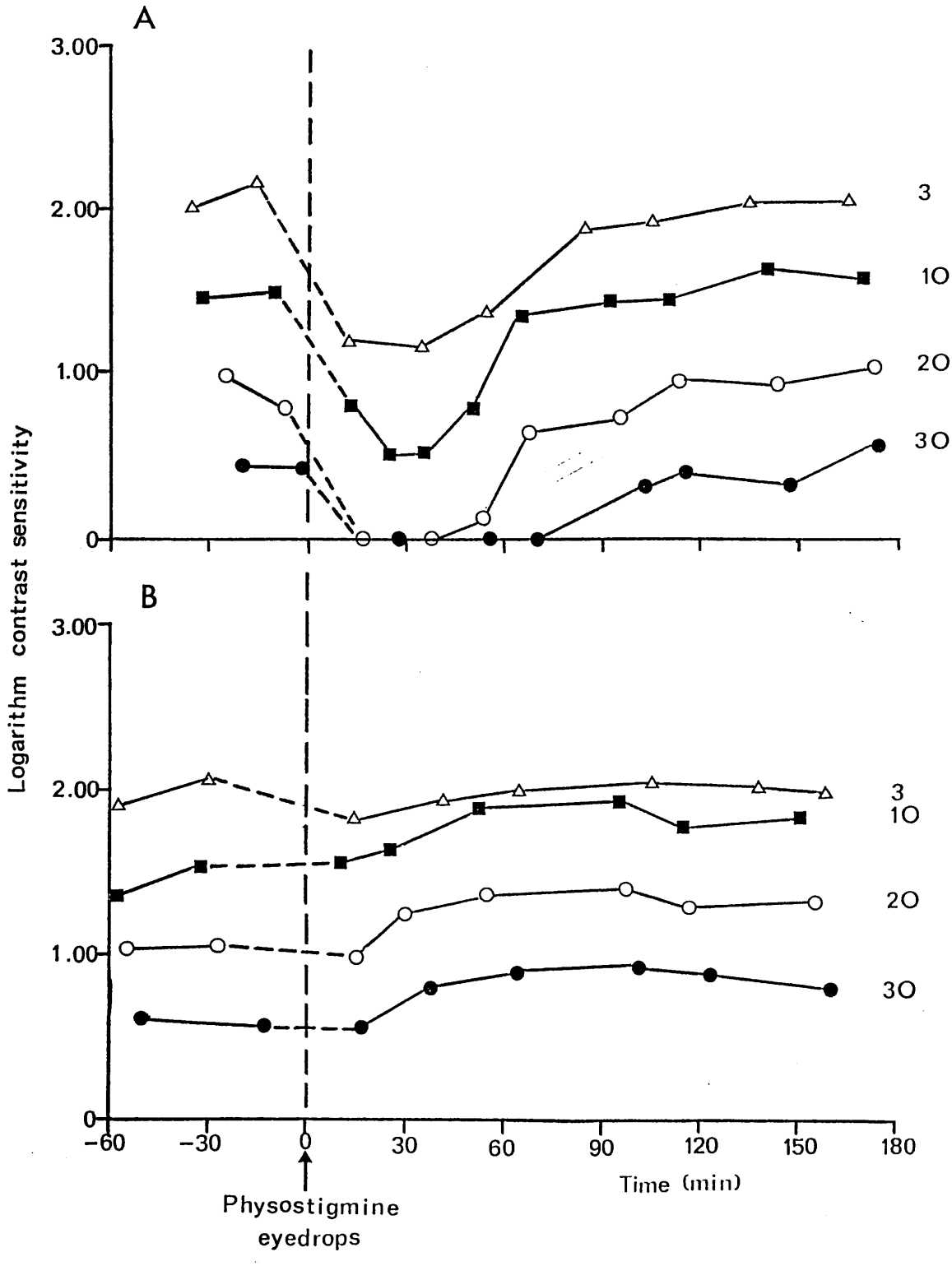


Fig. 25. Effect of 0.25% physostigmine eyedrops on log contrast sensitivity to stationary grating patterns generated on CRT display for spatial frequencies of 3, 10, 20 and 30 c/deg. Individual results of subject 1 (A) and subject 12 (B).

Contrast sensitivity to "moving" grating patterns

In Fig. 26A an appreciable decline in contrast sensitivity at 2 and 3 c/deg with a somewhat smaller change at 1 and 0.5 c/deg is shown. This variation in response is more clearly seen in Fig. 26B, with the lower spatial frequencies being less affected. The contrast sensitivities to these moving gratings were again related to the degree of defocus and the relationship between the peak decline in contrast sensitivity and the degree of defocus for the mean results is shown in Fig. 27. The decline in contrast sensitivity seen for a 3 c/deg moving grating was very similar to that for a 3 c/deg stationary grating, and indeed paired t-tests for each series of measurements after physostigmine revealed no significant differences ($P>0.14$). Thus, the fact the grating was moving does not seem to be important. A point to note, was that contrast sensitivity to each of these low spatial frequency moving gratings remained significantly reduced (at approximately 70 % of control) at the end of the experimental session (Fig. 26B, $P<0.05$). The amplitude of the change in contrast sensitivity to the moving grating patterns also varied between subjects. The extreme range is illustrated in Fig. 28 in which Subject 1 showed the usual fall in contrast sensitivity whereas Subject 12 showed no change at all.

Contrast sensitivity to laser interference fringes

The mean contrast sensitivities to laser interference fringes of spatial frequencies 4, 15 and 25 c/deg were calculated for the 11 subjects who completed this part of the study (Fig. 29A). At 4 c/deg there was a small though significant decline in contrast sensitivity ($P=0.02$, paired t-test) for the first measurement (on average 17 min) after physostigmine and a small but significant increase in contrast sensitivity ($P=0.05$) by the third measurement (some 70 min after

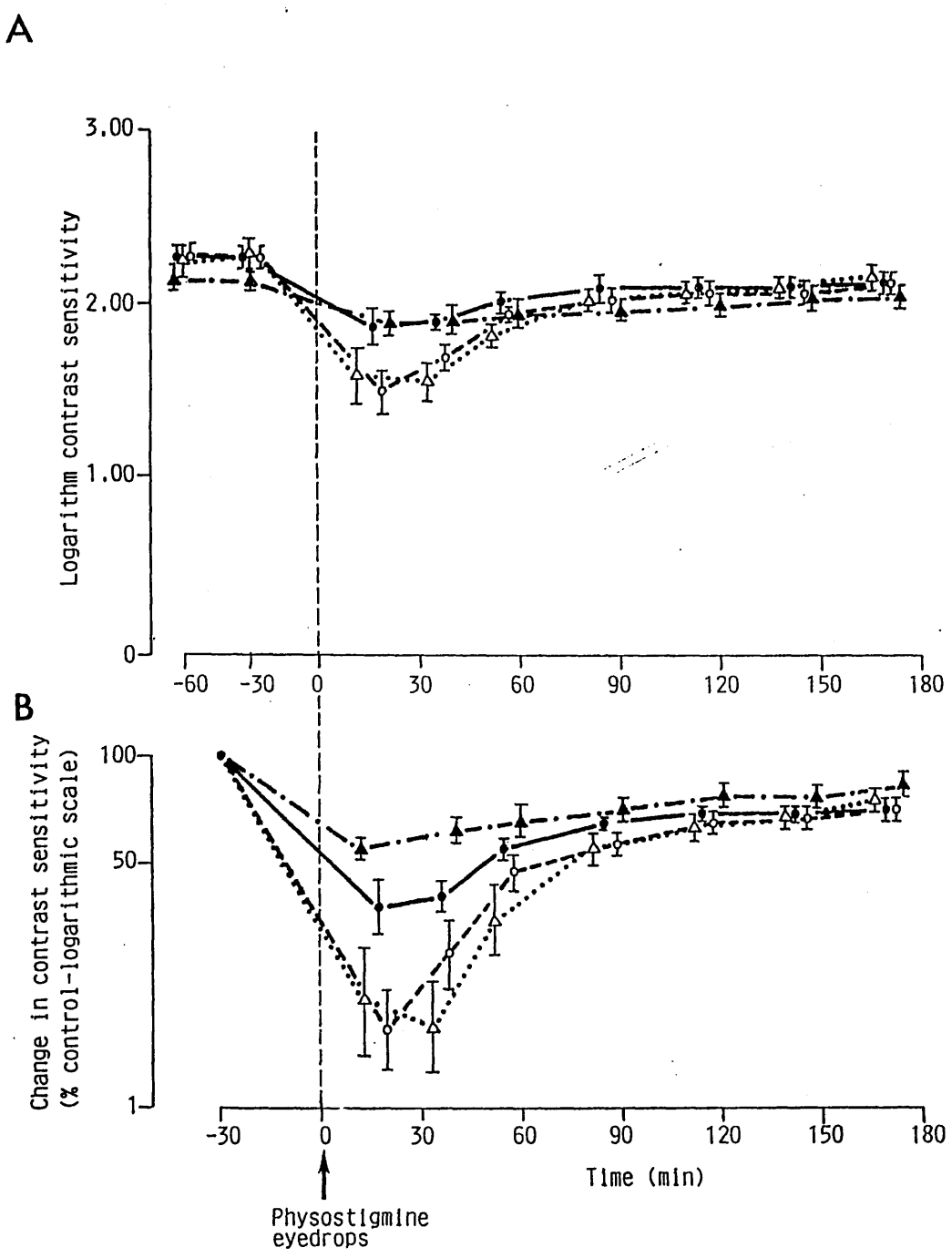


Fig. 26. Effect of 0.25% physostigmine eyedrops on log contrast sensitivity for grating patterns generated on CRT display with contrast phase-reversed at 5.5z (A) and the change in contrast sensitivity (logarithmic scale) from control value C2 (B). The contrast of the gratings was phase-reversed at 5.5 Hz. Each point shows the mean ± S.E. for 12 subjects at spatial frequencies of 0.5 ▲ ; 1 ● ; 2 o and 3 △ c/deg.

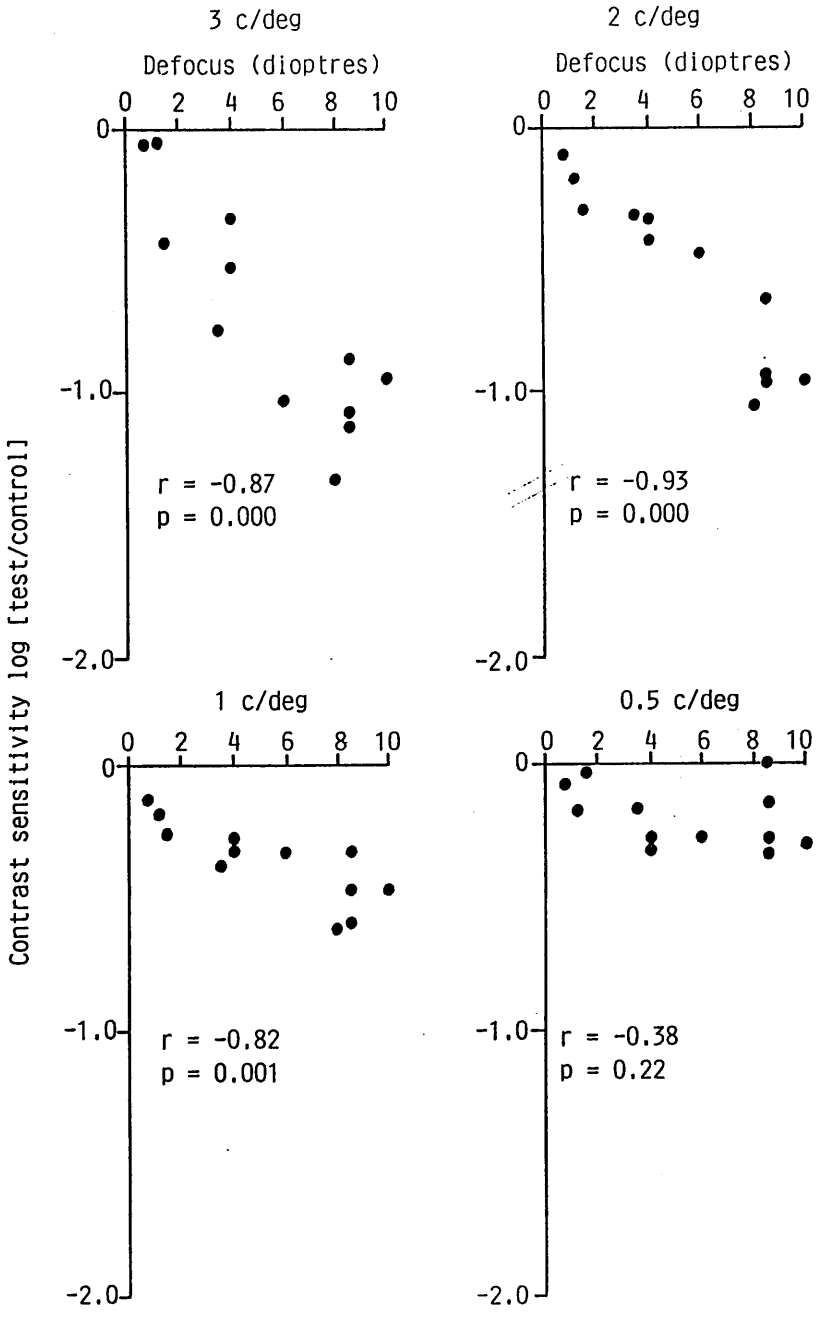


Fig. 27. Maximum reduction in contrast sensitivity from control value C2 (logarithmic scale) for moving grating patterns plotted against maximum defocus caused by 0.25% physostigmine eyedrops, for the spatial frequency indicated. The contrast of the grating was phase-reversed at 5.5 Hz. Correlation coefficient (r) and significance level (p) of the linear regression are shown.

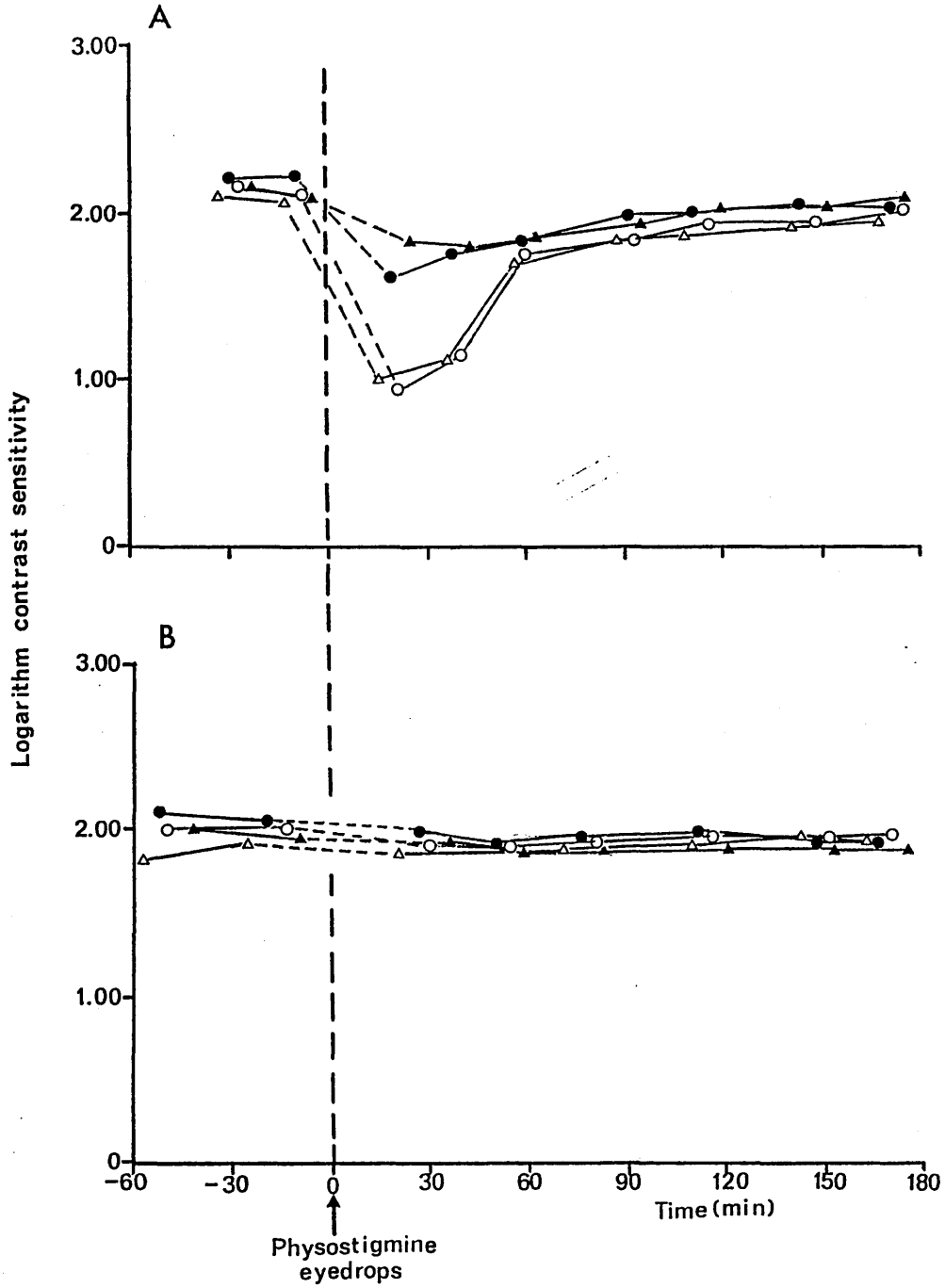


Fig. 28. Effect of 0.25% physostigmine eyedrops on log contrast sensitivity for phase-reversed (at 5.5 Hz) grating patterns generated on CRT display at 0.5 ▲ ; 1 ● ; 2 O and 3 Δ c/deg. Individual results of subject 1 (A) and subject 12 (B).

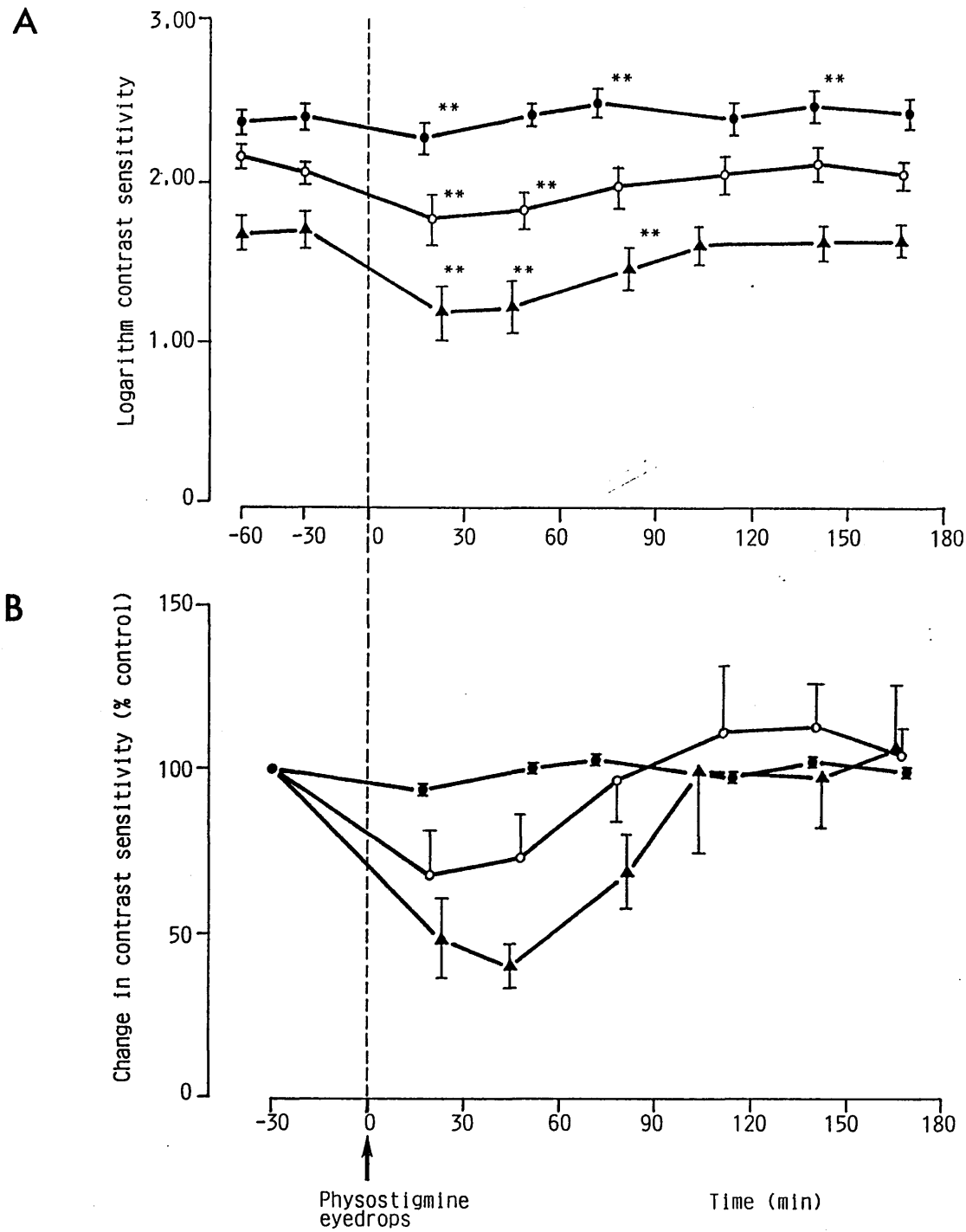


Fig. 29. Effect of 0.25% physostigmine eyedrops on log contrast sensitivity to laser interference fringes (A) and the change in contrast sensitivity (linear units) from control value C2 (B). Mean data ± S.E. for 11 subjects at spatial frequencies of 4 ● ; 15 O and 25 ▲ c/deg. ** denotes significant reduction from control value ($p < 0.05$).

physostigmine). At 15 and 25 c/deg a larger, more consistent, decline in contrast sensitivity was seen with a similar time course to defocus. At 15 c/deg, this fall in contrast sensitivity to 75% control had recovered by the third series of measurements some 78 min after physostigmine whereas the fall to 50% control at 25 c/deg recovery was not complete until some 90 min (Fig. 29B). The two extremes of response to physostigmine as shown by the individual results for subjects 1 and 12 are presented in Fig. 30A and B.

These results therefore indicate that physostigmine was adversely effecting retinal contrast sensitivity. Since the loss in contrast sensitivity followed the time course of defocus but not that of pupillary constriction which was much more sustained, an effect of defocus as the cause of this loss was investigated. One possibility was while the interference fringes themselves would not be affected by the increase in accommodation, the aperture surrounding the fringes would be defocused. To investigate this contrast sensitivity was measured to 4, 14 and 25 c/deg interference fringes for both optimal focus and defocused vision. This was achieved by a myopic subject viewing the fringes wearing his normal correcting lens or with his unaided eye (i.e. 7.0D defocus). The mean threshold was measured twice for each spatial frequency for both optimal focus and defocused vision, in a random order: no significant differences were found between the two sets of mean contrast thresholds ($P>0.20$), which closely overlapped as shown in Fig. 31. This therefore, excludes defocus of the aperture as the cause of the decline in contrast sensitivity after physostigmine. By exclusion, physostigmine appeared to be exerting its deleterious effect through a direct action on the retina.

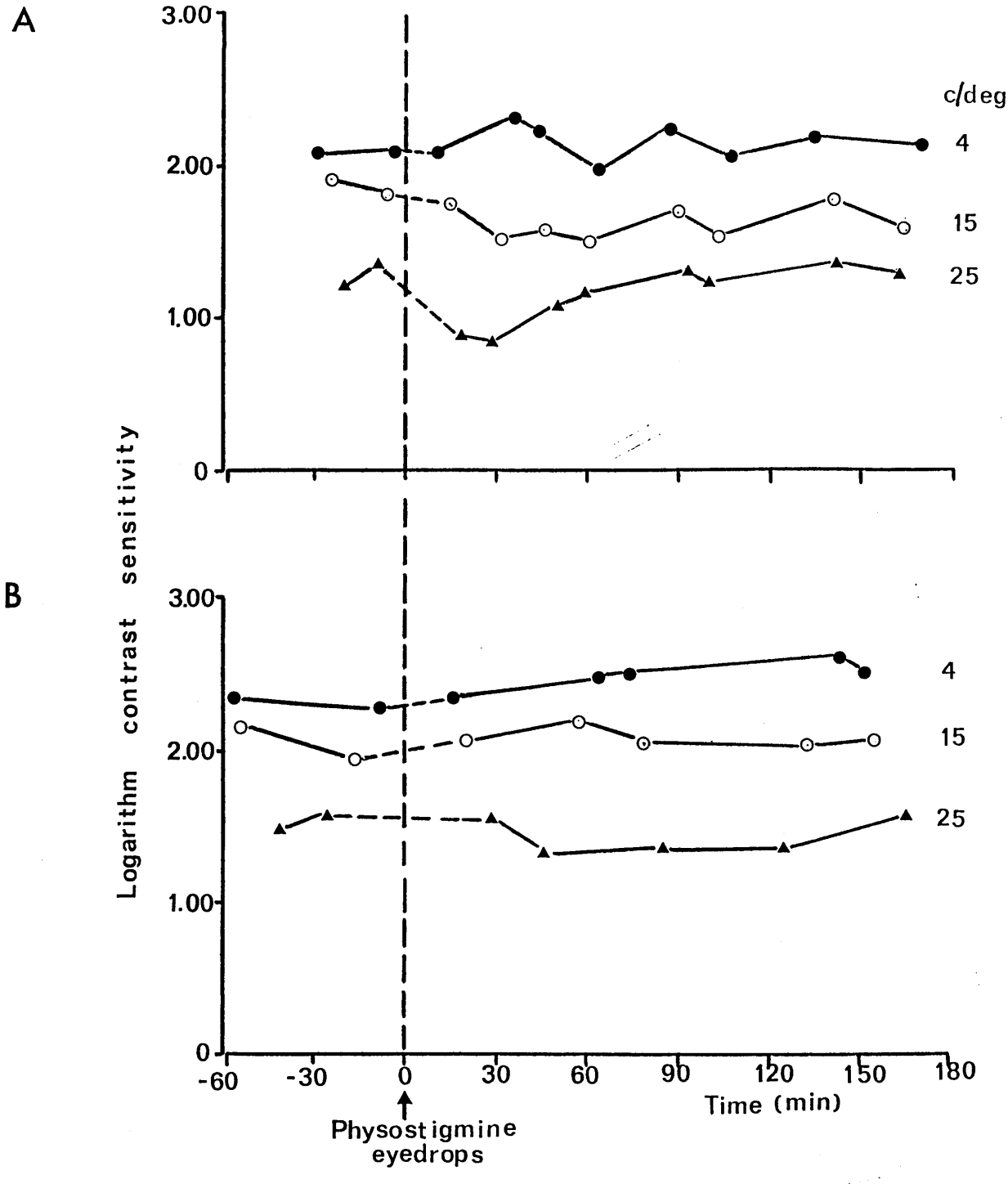


Fig. 30. Effects of 0.25% physostigmine eyedrops on log contrast sensitivity to laser interference fringes at spatial frequencies indicated. Individual results of subject 1 (A) and subject 12 (B).

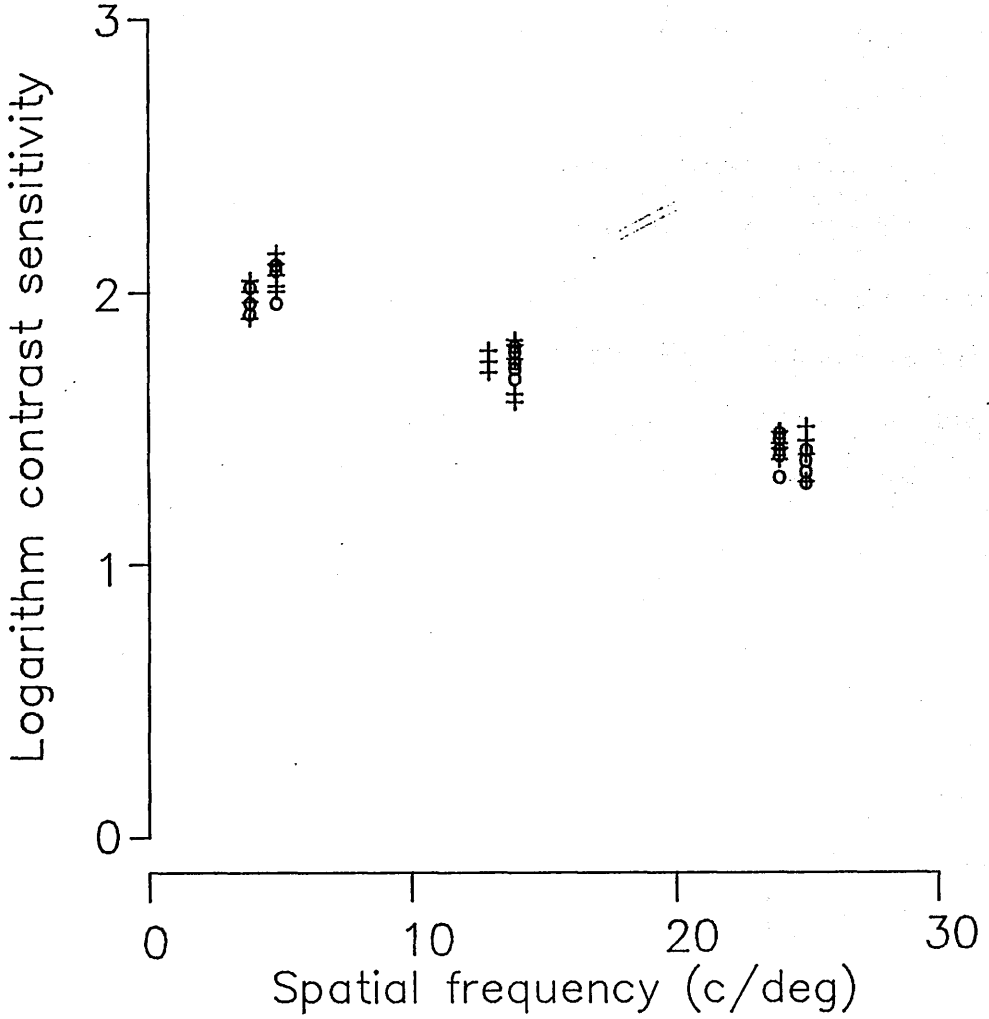


Fig. 31. Contrast sensitivity to laser interference fringes of spatial frequencies 4, 14 and 25 c/deg measured for one observer; +, viewed with optimal focus and O, viewed with unaided eye, -7.0 D myopia.

Further evidence to support a retinal effect of physostigmine is that there was also a transient fall in the mean critical fusion frequency which was also measured for 11 subjects (Fig. 32). The small fall in fusion frequency after 10 min was not significant ($P>0.10$) but the reduction from 44 to 42 Hz attained significance after 37 min ($P=0.03$). Subsequent measurements were not significantly different from the control value ($P>0.50$). The individual results for subjects 1 and 12 however, do not show any marked differences in response (Fig. 33).

At the end of the experimental period most subjects reported tiredness although the unpleasant side-effects such as headaches and tension had subsided. Subjects generally did not go back to normal work immediately after the experiment and usually went home to rest. All were fit and well the following day, though in some cases partial constriction of the pupil was still noted.

Possible genetic factors involved in sensitivity of accommodation to physostigmine

Two groups of siblings were involved in this part of the study (Table 7). Family 1 consisted of two of the original subjects who received physostigmine (subjects 1 and 3 in this table) and a third brother. All three members of Family 1 were markedly defocused on their first exposure to 2 drops of 0.25% physostigmine sulphate eyedrops and the maximal increase in accommodation ranged from 7 to 12 D. Pupil diameter was constricted to less than 2mm for each subject. Family 2 consisted of one of the original subjects (Subject 3 in this table) who was only slightly affected by physostigmine eyedrops of his first exposure and two of his siblings. These were twins, male and female and they both experienced an increase in involuntary accommodation of 4.5 D which although larger than that of their brother's response (0.5D) on

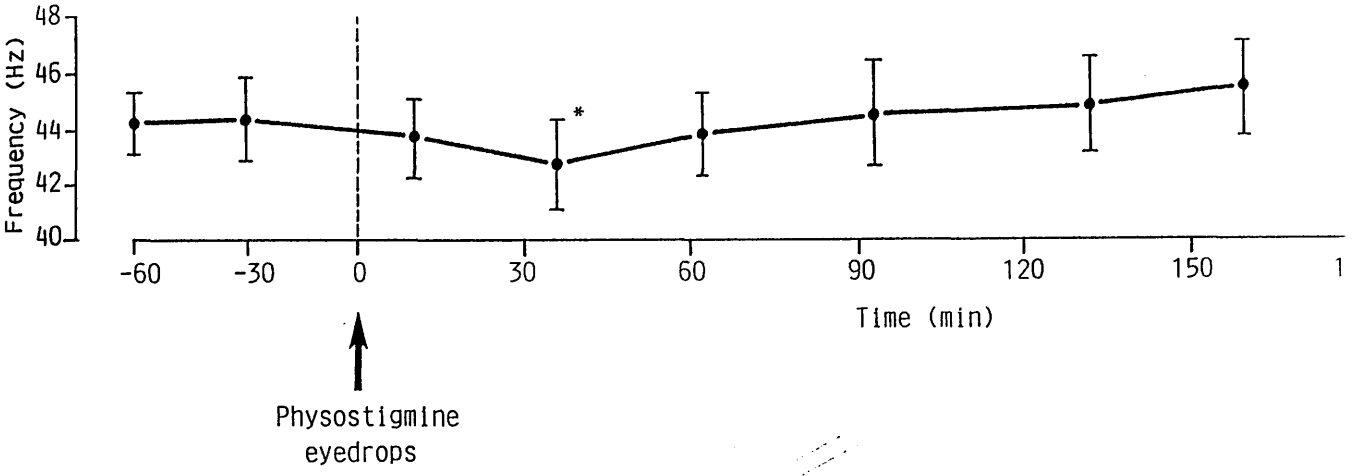


Fig. 32. Effect of 0.25% physostigmine eyedrops on critical fusion frequency. * denotes a significant reduction from control value at -30 min ($p < 0.05$). Each point is the mean $\pm$ S.E. for 11 subjects.

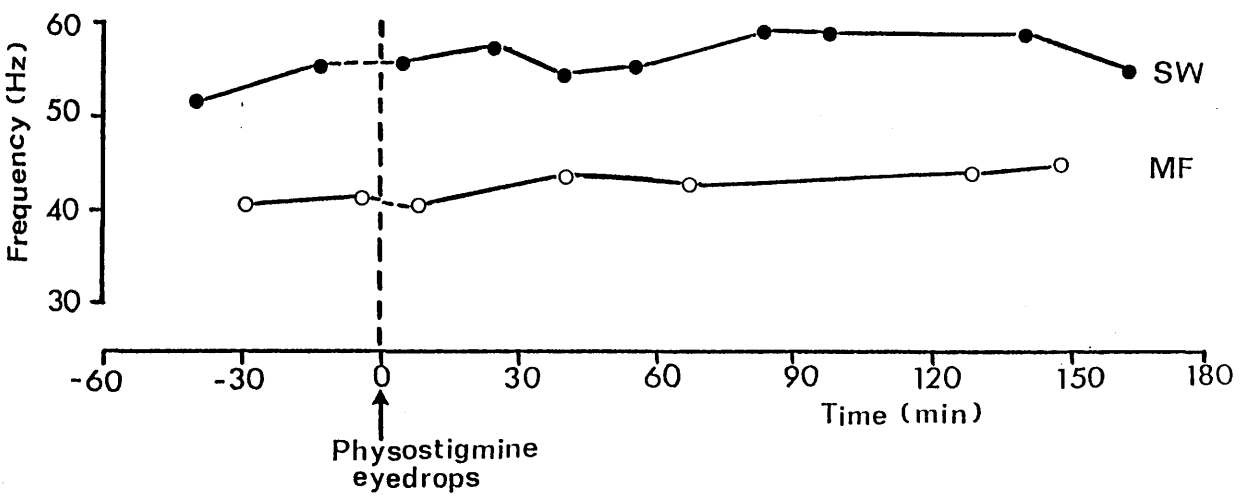


Fig. 33. Effect of 0.25% physostigmine eyedrops on critical fusion frequency. Individual results of subject 1, S.W. and subject 12, M.F.

his first exposure to physostigmine was similar to the 4.0D response observed for this subject on his second exposure to physostigmine. Hence there is some reason to suppose that there may be a genetic disposition to the action of physostigmine on accommodation but not pupil diameter. Further investigations would, thus, be desirable.

Table 7. Comparisons between families.

	Family 1					Family 2				
	Sex	Age	Minimum pupil diameter (mm)	Peak defocus		Sex	Age	Minimum pupil diameter (mm)	Peak defocus
1. DO	M	20	1.8	9.0	SR	M	17	1.9	4.5
2. KO	M	21	1.6	12.0	DR	F	17	1.9	4.5
3. SO	M	23	1.9	7.0	JR	M	25	1.8	0.5

Results are for first exposure to 2 drops of 0.25% physostigmine sulphate eyedrops.

Part 4: The effect of an intramuscular dose of 2mg atropine sulphate on visual performance

Experiment 1, which assessed the effects of atropine on a variety of visual measurements, was completed by 7 male volunteers, aged 19-24 years and one volunteer age 35 years. Experiment 2, which looked specifically at the effects of atropine on contrast sensitivity measured for both CRT and laser displays, was completed by 13 male volunteers aged 22-42 years, 3 of whom had also completed Experiment 1. In each person, Snellen acuity was 5/4, wearing spectacles if necessary. The results were examined on both an individual and group basis. The significance of any changes in measured values was assessed by paired t-tests between control measurements prior to the atropine injection and test measurements. The convention used throughout is that control periods are denoted as C and post-atropine periods as A followed by the hourly interval.

General Effects of Atropine

The most common effect of the i.m. injection of 2mg atropine sulphate was dryness of the mouth with difficulty in swallowing. The degree of discomfort varied from person to person. For three subjects who completed both parts of the study, less discomfort was felt on the second occasion. Two subjects expressed some difficulty in micturition. Several subjects reported dry hands, as sweating was inhibited, and one subject described the blocking of sweating even on a long training run later the same day. Central effects in the form of lightheadedness, slight ataxia (unsteadiness in walking) and difficulty in concentrating were reported in 10 out of the 21 experiments. Two subjects reported a headache during the course of the experiment, which

had disappeared before completion of the tests. All subjects expressed tiredness by the end of the session.

A transient bradycardia occurred in 17 of 21 experiments within the first five minutes, followed by a more pronounced tachycardia, rising to within pre-injection levels by 10 min (Fig. 34A). Heart rate increased significantly above control levels by 15 min post-injection ($P<0.05$). The tachycardia became pronounced by 20-40 min post-injection, consisting of an approximately 50% increase in resting heart rate. For Experiment 1, the peak increase in heart rate averaged 35.4 beats/min and 28.4 beats/min for Experiment 2, thereafter declining slowly to within control levels by 4.5 hours post-injection. Heart rate was still significantly elevated ($P<0.01$) at the end of Experiment 2. The most extreme case in an individual was an initial slowing from 60 to 46 beats/min, followed by an increase to 120 beats/min at 60 min post-injection.

Pupil diameter and accommodation

The ocular effects followed a slower time course than the heart rate response. In Experiment 1, atropine produced a gradual mydriasis of 0.3mm at 30 min (not significant), 1.2mm at 2 hours ($P<0.05$) and a maximum dilation of 1.5mm at 2.5 hours ($P<0.01$) (Fig. 34B). After 5 hours, the mean increase in pupil diameter was 1.0mm, but was no longer significantly different from control levels ($P>0.10$). In Experiment 2, similar results were obtained though the maximum dilation of 1.5mm was reached after between 1 and 2 hours ($P<0.001$).

Following atropine, the amplitude of accommodation, measured as the near point, had declined by 0.7D after 1 hour ($P<0.04$), with a maximum fall of 1.8D after 4 hours ($P<0.001$) (Fig. 34C). Some recovery of accommodation had occurred by 6 hours post-injection, though the

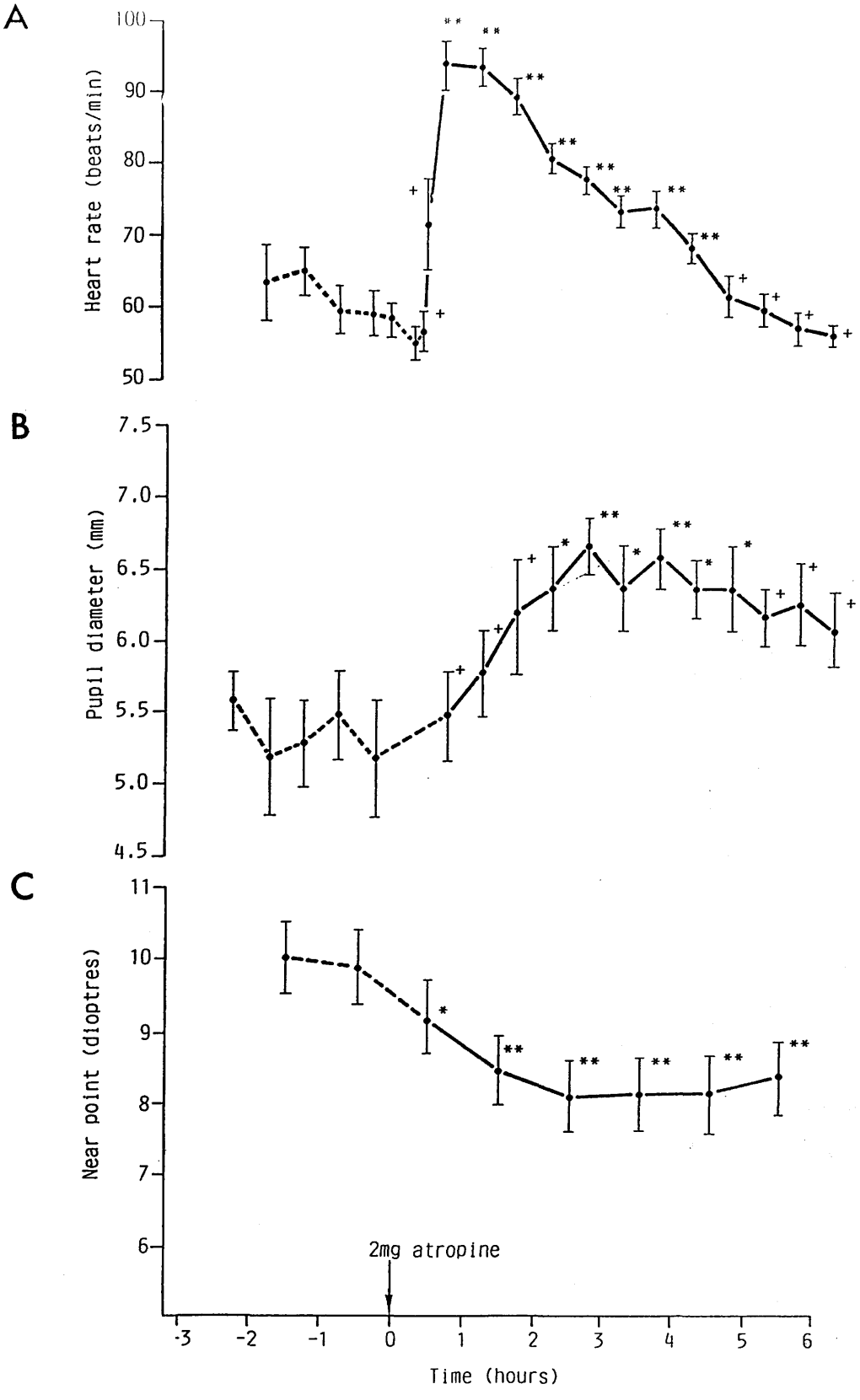


Fig. 34. Effects of injection of atropine sulphate (2 mg, i.m.) on (A) heart rate, (B) pupil diameter and (C) near point accommodation. Each point shows the mean ± S.E. for 8 subjects measured in Experiment 1. The significance levels indicated are for paired t-tests between the test period and the period immediately preceding the injection. ** $P < 0.01$; * $p < 0.05$; + N.S.

deficit of 1.5D was still significant ($P<0.01$ Experiment 1). Similarly in Experiment 2, accommodation was reduced on average by 1.2D after 1 hour ($P<0.01$), 1.3D after 2 hours ($P<0.001$) and 1.2D after 3 hours ($P<0.01$).

Experiment 1

Reaction time and choice reaction time

Atropine did not significantly affect either reaction time or choice reaction time to a visual stimulus (Table 8), as no significant differences were present between any given test period and the second control period (C2) ($P>0.20$). This experiment was completed in 7 subjects.

Extraocular muscle balance

The results of these tests, performed on the Visual Function Tester are shown in Figure 35. For the grouped data, horizontal heterophoria changed significantly from a mean of 3.7 prism dioptres (C2) to 2.8 prism dioptres at 1 hour ($P<0.05$, Fig. 35A). Seven out of 8 subjects became less esophoric after atropine while the remaining subject became more exophoric, i.e. the common direction of change of alignment was towards exophoria by some 0.8 prism dioptres. Vertical heterophoria remained unchanged ($P>0.05$, Fig. 35B). Cyclophoria declined significantly from a mean of 1.94 deg (C2) to 1.06 deg at 2 hours ($P<0.05$, Fig. 35C).

Colour-matching

Colour-matching was not affected by atropine as the red-green matching range remained constant throughout (Table 9). The readings which are the positions of the right hand slide of the anomaloscope give

Table 8. Effect of atropine on reaction time and choice reaction time to a visual stimulus. Mean ± S.E. for 7 subjects.

Reaction Time (msec)							
C1	C2	A1	A2	A3	A4	A5	A6
491.3	452.1	467.5	452.8	472.8	464.4	469.7	452.9
±40.0	±43.3	±39.4	±41.7	±43.4	±51.6	±39.2	±40.0
Choice Reaction Time (msec)							
C1	C2	A1	A2	A3	A4	A5	A6
651.2	590.7	622.7	630.1	629.1	635.4	601.1	591.3
±39.1	±46.4	±32.4	±29.9	±38.8	±35.8	±45.8	±45.9

Table 9. Effect of atropine on red-green colour perception.

Interval	Mean Red-Green Matching Range ± S.E. (n=8)
C1	38.5 ± 0.3 - 39.4 ± 0.2
C2	39.0 ± 0.3 - 39.7 ± 0.2
A1	38.9 ± 0.2 - 39.5 ± 0.2
A2	38.7 ± 0.2 - 39.6 ± 0.3
A3	38.7 ± 0.4 - 39.2 ± 0.3
A4	38.9 ± 0.3 - 39.6 ± 0.3
A5	38.7 ± 0.2 - 39.4 ± 0.3
A6	38.6 ± 0.4 - 39.2 ± 0.3

(Normal range 33.6 to 41.2, with 99% confidence limits)

C: control measurements, A: measurements after atropine at hourly intervals.

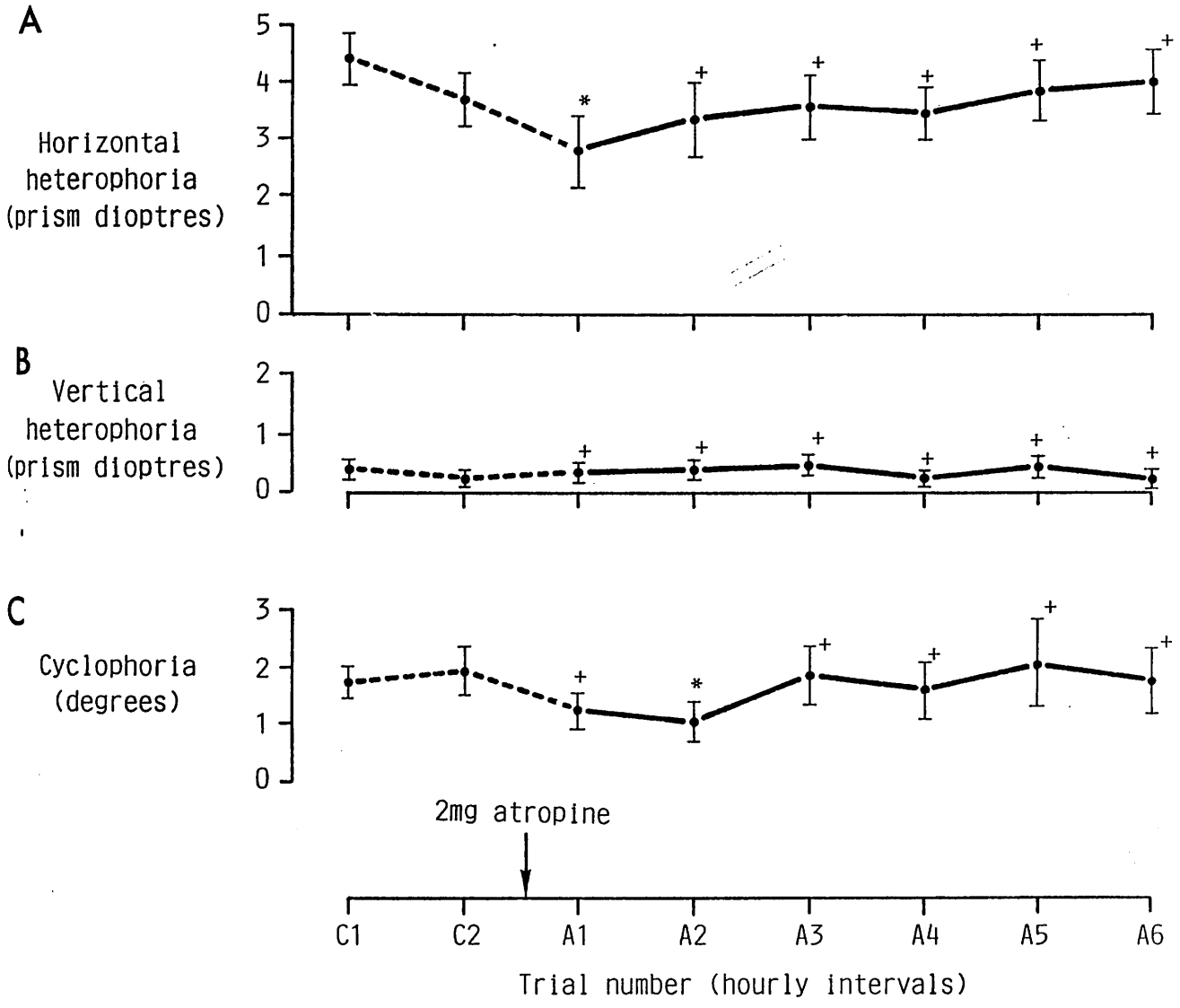


Fig. 35. Extraocular muscle balance as measured with the Visual Function Tester (see text for details). Mean ± S.E. for 8 subjects. Significance levels are for paired t-tests against the control measurement C2: * $p < 0.05$ and + N.S.

the proportion of red and green needed for matching with the yellow reference half-field. In all test periods there were no significant differences from the second control period in either the individual or the grouped results ($P>0.10$).

Stereoacuity

(i) Stereoacuity was measured to extended line stimuli in a demanding series of determinations which involved many judgements of exceedingly fine discrimination. These judgements were not significantly affected by atropine since line stereoacuity remained at around 3 seconds of arc throughout the experiment ($P>0.18$). A small decline in overall performance of the test which represented the percentage of correct responses, occurred but was not significant ($P>0.28$; Table 10).

(ii) A measurement of stereoacuity based on discrimination of the disparate dot out of a pattern of 4 dots was made with the Visual Function Tester. This represents a different measure to that in part (i) since it assesses global stereopsis and in fact gives a value of stereoacuity appreciably less than in the above method. The smallest disparity available on the Visual Function Tester was 10 seconds of arc. Subjects were always able to identify the disparate dot before and after atropine.

These experiments were completed in 8 subjects but one subject experienced such difficulty in performing both parts of the test that his results were discarded.

Visual acuity.

Individually, 2 subjects showed a slight fall in cut-off frequency after atropine, whereas the other 5 individuals showed a slight increase or no change. Examination of the mean data showed that atropine did

not significantly affect visual acuity, as the cut-off frequency remained within normal limits throughout the experiment (P>0.20) (Table 11). This experiment was completed in 7 subjects.

Table 10. Effect of atropine on line stereoacuity and overall performance on the test. Mean ± S.E. for 7 subjects.

Stereoacuity							
Visual Angle (seconds of arc)							
C1	C2	A1	A2	A3	A4	A5	A6
2.9	2.8	2.6	2.6	2.4	3.1	2.9	2.2
±0.4	±0.3	±0.5	±0.2	±0.2	±0.1	±0.3	±0.2
% Overall Performance							
C1	C2	A1	A2	A3	A4	A5	A6
71.1	71.1	70.7	67.0	69.6	65.9	69.2	68.9
±4.7	±4.5	±4.1	±1.9	±2.3	±3.1	±2.3	±1.9

Table 11. Effect of atropine on visual acuity. Mean ± S.E. for 7 subjects.

Visual Acuity (c/deg)							
C1	C2	A1	A2	A3	A4	A5	A6
42.4	43.7	43.9	43.5	42.3	42.7	43.3	43.1
±1.5	±1.3	±0.9	±1.1	±1.0	±1.0	±0.8	±0.8

C: control measurements, A: measurements after atropine at hourly intervals.

Contrast sensitivity to a moving grating pattern.

Atropine caused a decline in contrast sensitivity to a grating pattern which was phase-reversed at 5.5 Hz (Fig. 36). In 4 out of the 8 subjects tested this decline in sensitivity occurred at the 4 spatial frequencies tested and 3 subjects showed a decline in sensitivity for 3 of the 4 spatial frequencies tested. The eighth subject showed no change at any spatial frequency.

The mean contrast sensitivity values at 1, 2 and 3 c/deg were significantly reduced by atropine by 20-35% at 1-2 hours post-injection which persisted at 5-6 hours post-injection (Table 12 and Fig. 36). At 5 c/deg, a smaller reduction was observed which was significant only at 5 and 6 hours post-injection. Thus, the detection of moving grating patterns was adversely affected by atropine with a time course which was more sustained than that of either heart rate or pupil diameter.

Experiment 2

Contrast sensitivity to a stationary grating pattern; CRT display

The contrast sensitivity curve was of the same form for each subject. There was a peak at 3-5 c/deg with a linear fall in logarithm contrast sensitivity with increasing spatial frequency. First, the data were examined against time for each spatial frequency on both an individual and combined basis (Fig. 37). Two factors had to be considered: first, an appraisal of the reproducibility of results in the 'dummy' run of 4 hours without atropine; and second, the effects of atropine with respect to the preceding control measurement.

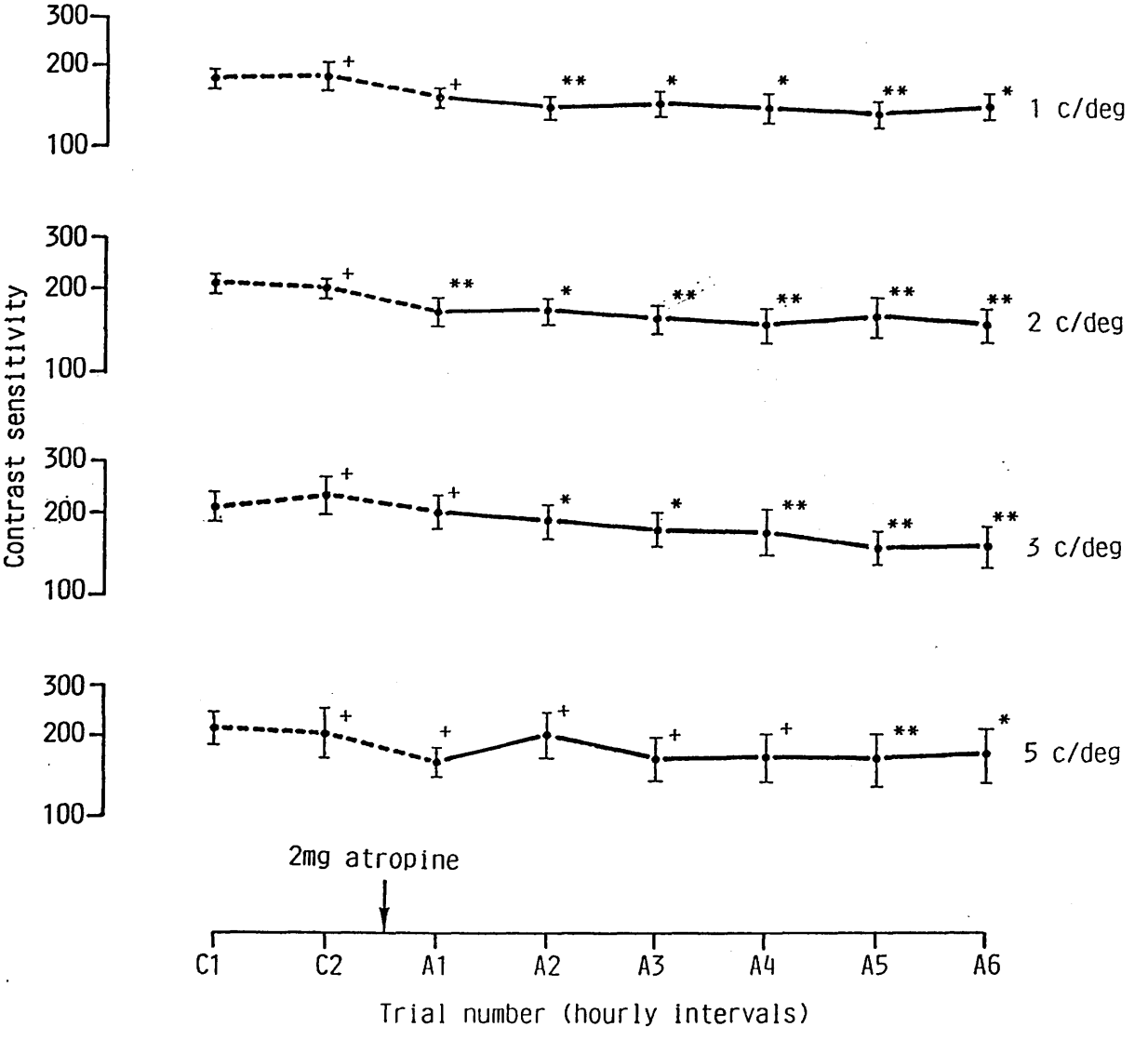


Fig. 36 Effects of atropine on contrast sensitivity (logarithmic scale) to sinusoidal grating patterns phase-reversed at 5.5 Hz at the spatial frequencies indicated. Each point shows the mean ± S.E. for 8 subjects. Significance levels are for paired t-tests between the test period and the control period C2: ** $p < 0.01$; * $p < 0.05$; + N.S.

Table 12. Effect of atropine on contrast sensitivity to sinusoidal gratings phase reversed at 5.5 Hz for the spatial frequencies stated. Change in contrast sensitivity at the periods indicated is expressed as a percentage of C2. Significance levels are for paired t-tests.

	1c/deg		2c/deg		3c/deg		5c/deg	
	change	P	change	P	change	P	change	P
C1	+2%	0.97	-4%	0.63	+9%	0.46	-4%	0.41
A1	-17%	0.05	-17%	0.009	-13%	0.08	-22%	0.12
A2	-22%	<0.001	-17%	0.01	-19%	0.04	-2%	0.81
A3	-19%	0.07	-22%	0.005	-24%	0.02	-19%	0.09
A4	-22%	0.02	-26%	<0.001	-26%	0.003	-19%	0.17
A5	-26%	<0.001	-21%	0.01	-35%	<0.001	-21%	0.006
A6	-21%	0.05	-26%	<0.001	-34%	<0.001	-17%	0.04

C: control measurements A: measurements after atropine at hourly intervals.

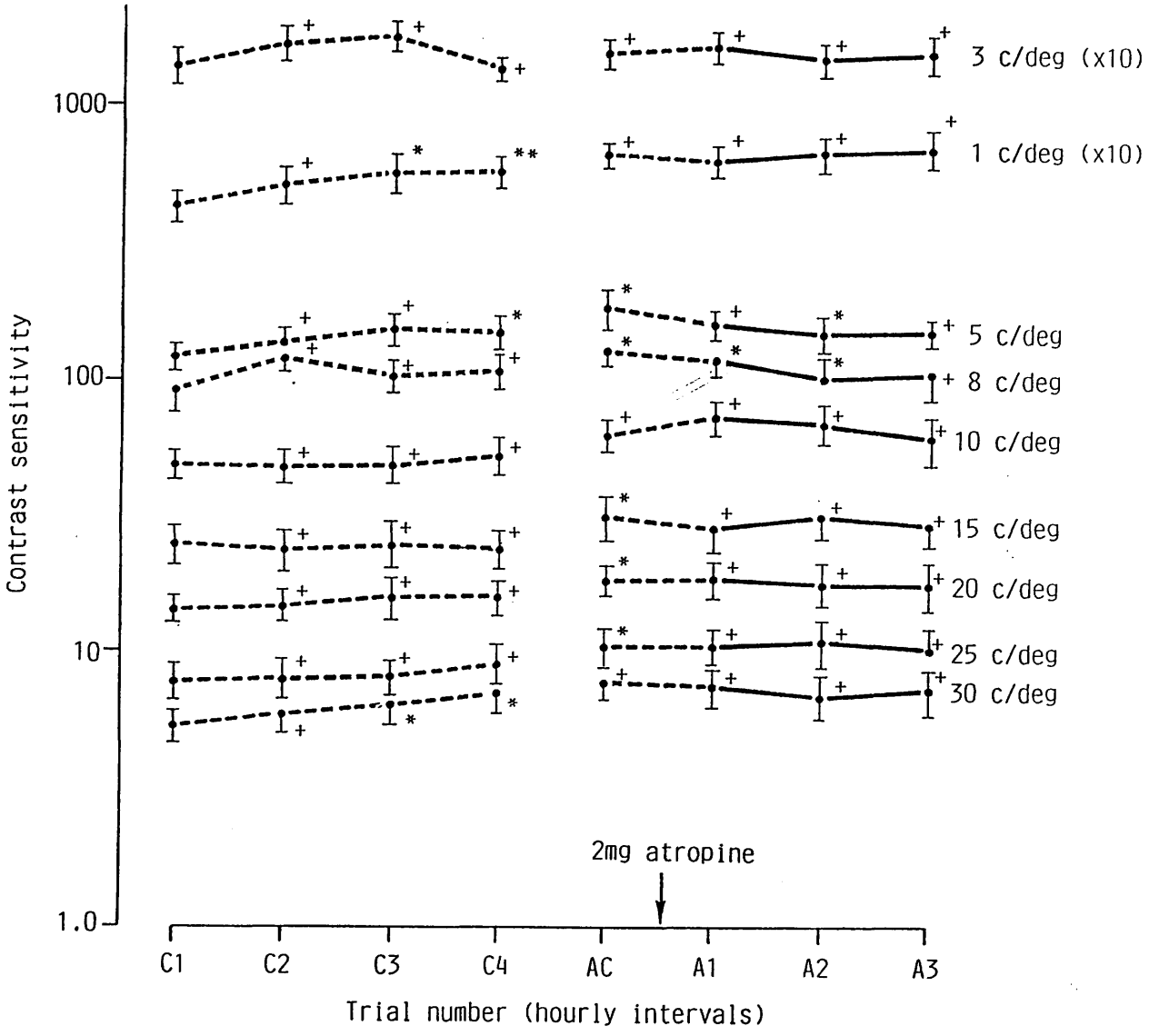


Fig. 37. Effects of atropine on contrast sensitivity (logarithmic scale) at spatial frequency stated. Left, a series of four control measurements (C1-C4) repeated at hourly intervals: each point shows the mean ± S.E. for 13 subjects. Right, one control measurement (AC) followed by post-atropine measurements (continuous line). Contrast sensitivity at 1 and 3 c/deg are displaced upwards (x 10) for clarity. Significance levels are for paired t-test against the first measurement in each series (C1 or AC). ** $P < 0.01$; * $P < 0.05$; + N.S.

Reproducibility of measurements.

Reproducibility was expressed as a percentage of the first control run for all spatial frequencies for 13 subjects. (Fig. 38). Overall, a 7% improvement in sensitivity occurred on the first repeat, a further 4% on the second, and a further 5% on the third. However, when shown on the conventional logarithmic scale, these changes are minimal (Fig. 38).

Paired t-tests between periods C2-C4 with respect to C1 generally revealed no significant differences except at 1 and 30 c/deg at C3 and C4 and 5 c/deg at C4 when a significant improvement occurred (Fig. 37). However on day 2, the control prior to atropine (CA) was significantly higher than C4 of the previous day at 5, 8, 15, 20 and 25 c/deg ($P<0.05$) (Fig. 37).

Thus, measurements of contrast sensitivity are generally reproducible on the same day, with a small time-related improvement in performance. There is a likelihood of a much larger increase in contrast sensitivity on a second day perhaps representing a resetting of criterion.

Effect of atropine.

Given that the expected response in a series of repeated measurements should lead to a gradual improvement in contrast sensitivity with testing, only three or possibly four of the 13 subjects showed a deleterious effect following atropine administration. In the remaining 9 subjects either no effect or a marked improvement occurred. For the grouped data for all 13 subjects, there was generally a lack of effect of atropine. Most post-atropine contrast sensitivity values were not significantly different from C2 ($P>0.10$); however for 5 c/deg a significant reduction occurred at 2 hours post-atropine ($P<0.05$) and for 8c/deg at 1 and 2 hours post-atropine ($P<0.05$). After 3 hours, however,

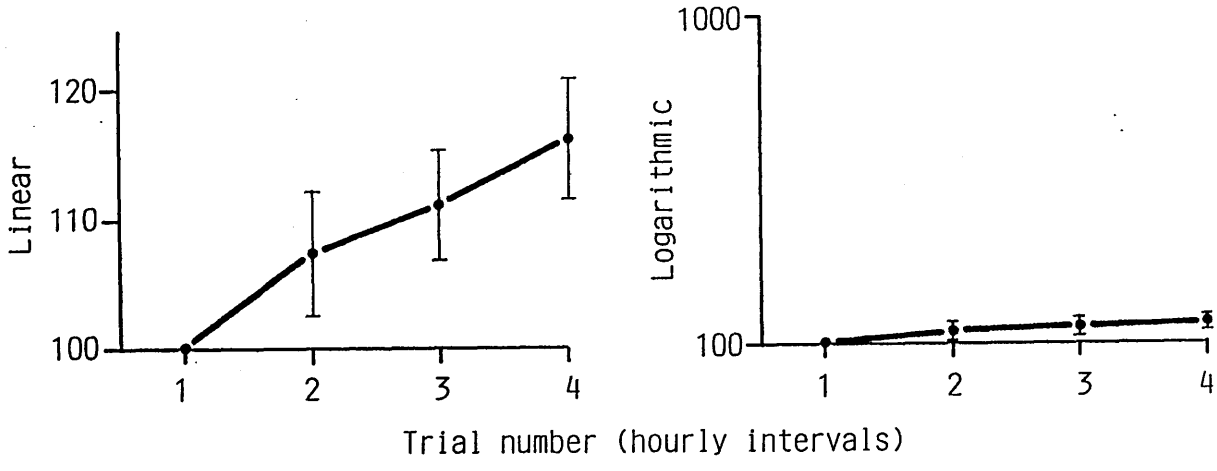


Fig. 38. Consistency of contrast sensitivity measurements to the CRT display for all spatial frequencies expressed as a percentage of the first reading. Each point is the mean ± S.E. for 13 subjects at 10 spatial frequencies.

contrast sensitivity at both 5 and 8 c/deg was not significantly different from control levels ($P>0.05$).

It is unlikely that any one spatial frequency would be depressed in isolation while adjacent spatial frequencies were unaffected since spatial frequency channels have a finite bandwidth of about an octave (Blakemore & Campbell, 1969). The contrast sensitivity data were, therefore, collectively tested by regression analysis. As mentioned earlier, contrast sensitivity (logarithmic scale) was inversely linearly related to spatial frequency over the range 3-30 c/deg. For the pre-atropine control and the three post-atropine periods (Fig. 37, right), the correlation coefficients were at least -0.985 with significance of $P<0.001$. Comparison between pre- and post-atropine regression equations for each subject showed an absence of a significant difference in both intercept and slope ($P>0.25$). A one-way analysis of variance also showed that atropine had no effect ($P>0.10$). These results are consistent with the lack of effect of atropine on visual acuity which remained at 42-43 c/deg (Table 11).

Contrast sensitivity to the laser display.

Contrast sensitivity was measured at three spatial frequencies on the linear part of the CSF, corresponding to medium and fine object detail. First, the data were examined against time on an individual basis and on a group basis as shown in Figure 39. Individually, 2 out of the 13 subjects showed an increase in sensitivity as they repeated the measurements in the control experiment whilst all other observers showed no effects at all. Likewise the grouped data generally showed no significant changes between C1 and any of the repeat measurements C2-C4 ($P>0.10$) except at 7-9 c/deg where a gradual improvement in contrast sensitivity occurred which became significant at C4 ($P<0.01$). When the

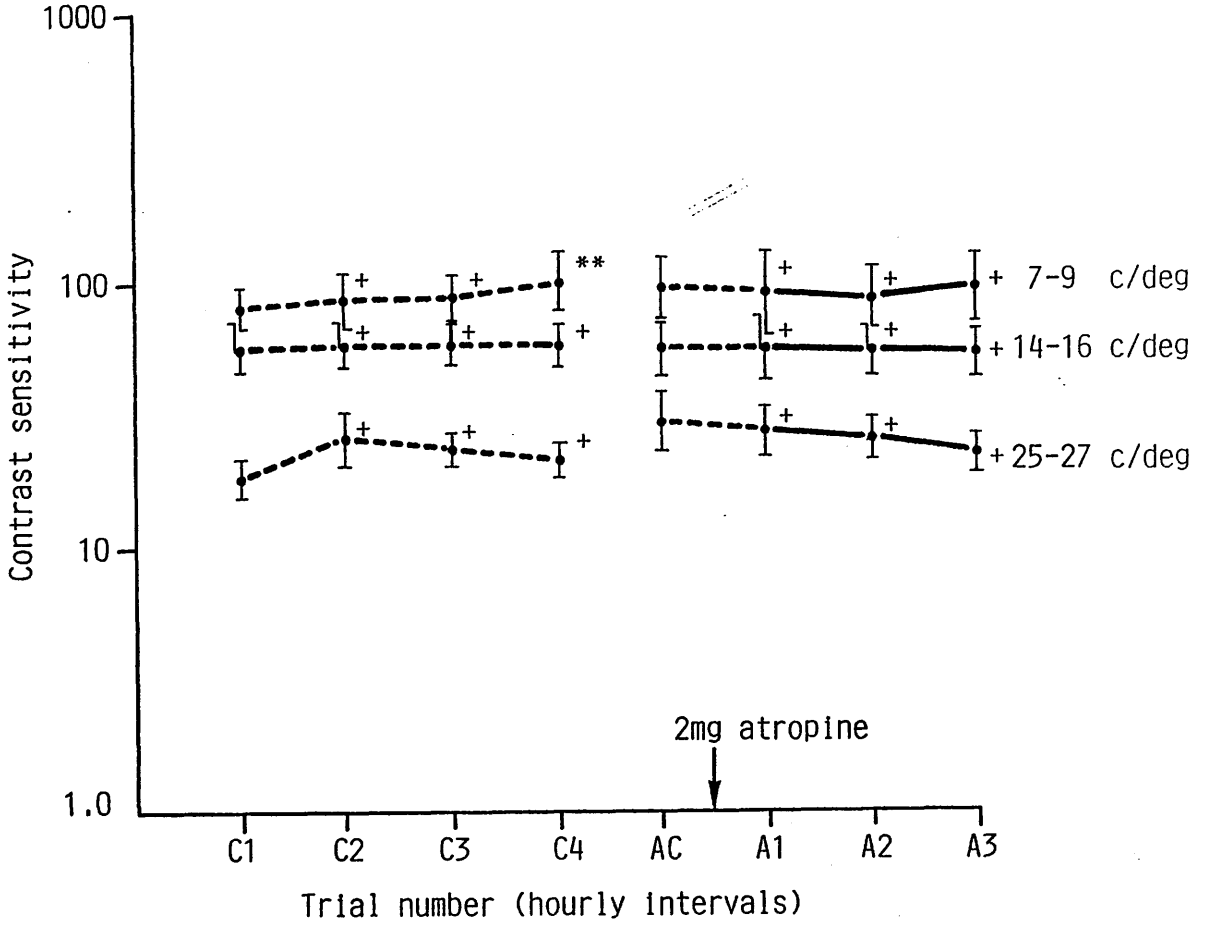


Fig. 39. Effects of atropine on contrast sensitivity (logarithmic scale) to laser interference fringes of the spatial frequency stated. Left, a series of four control measurements (C1 - C4) repeated at hourly intervals: each point shows the mean ± S.E. for 13 subjects. Right, one control measurement (AC) followed by post-atropine measurements (continuous line). Significance levels are for paired t-tests against the first measurement in each series (C1 or AC). ** $p < 0.01$; + N.S.

effects of atropine were considered with respect to the preceding control measurement, 5 subjects showed a deleterious effect following atropine administration, three showed an increase in contrast sensitivity following atropine and the remaining five showed no change at all. The grouped data showed no significant changes between the pre-atropine control and any of the test measurements after atropine ($P>0.10$).

It is, therefore, concluded that atropine had no measurable effect on the detection of stationary grating patterns over the range of spatial vision studied.

Part 5: The effectiveness of prior administration of intramuscular injection of atropine sulphate or instillation of homatropine eyedrops against physostigmine eyedrops.

Pre-treatment with atropine sulphate

Those 6 subjects whose accommodation was most markedly increased by physostigmine in Part 3 of this study (subjects 1-6 of Table 6) participated in the first of these experiments. In the second, subject 5 was unavailable and was replaced by another subject. This subject first underwent a single control experiment with physostigmine eyedrops alone prior to the atropine experiment. This necessitated the calculation of two different sets of control data for physostigmine alone (from part 3) for comparison with the results of prior injection of atropine, which was made with the paired t-test.

In the first set of experiments an i.m. injection of 2mg atropine sulphate was given approximately 2 hours prior to physostigmine eyedrops (mean time interval = 124 min) to allow the ocular effects to develop fully (Fig. 34). The effects of physostigmine eyedrops were followed

for only 2 hours post-administration because of the long duration of the control measurements. In the second set of experiments the injection was given just prior to the eyedrops (mean time interval = 8 min) so that the peak plasma atropine as reflected by the peak heart rate (Fig. 34) would coincide with the peak effect of physostigmine (Fig. 18). The effects of physostigmine were then followed for 3 hours.

The data were first analysed on an individual basis and then on a group basis. As all subjects showed a qualitatively similar response, the mean data shown are representative of the individual results. Most of the figures shown in this part of the study are of the same format. Each measurement made is plotted against time with the administration of 2 drops of 0.25% physostigmine sulphate shown as time 0. Injection of the pre-treatment of 2mg atropine sulphate (i.m.) is indicated by an arrow. Control measurements obtained prior to the administration of atropine are also shown. For comparison, the mean results (for the same 6 subjects) for physostigmine alone are shown. The upper portion of each figure (A) shows the results obtained for a pre-treatment of atropine sulphate given 8 min prior to the physostigmine eyedrops. The lower portion of each figure (B) shows the results obtained for a pre-treatment of atropine sulphate given 124 min prior to physostigmine eyedrops.

Pupil diameter

Pre-treatment with atropine sulphate did not prevent the marked pupil constriction following physostigmine eyedrops. The mean changes in pupil diameter are shown in Fig. 40 along with those previously measured for physostigmine alone.

Following atropine 8 min prior to physostigmine, the pupil of the test eye which received the eyedrops constricted to 1.9mm by 30 min and

then gradually dilated to 2.4mm at the end of the 3 hour experimental period. By comparison, in the experiment in which the subjects received only physostigmine, the pupil of the test eye constricted to 2.1 mm by 24 min after physostigmine. The maximal constriction of 1.9mm was seen after 50 min thereafter the pupil gradually dilated to 2.7mm by 3 hours (Fig. 40A). Thus, the maximal pupil constriction was the same following physostigmine whether preceded by atropine or not and the constriction appeared slightly longer lasting after the pre-treatment. Paired t-tests, however, at each time period showed no significant difference in the time course or extent of pupil constriction between atropine pre-treatment and physostigmine alone ($P>0.08$). The pupil of the control eye which did not receive physostigmine, gradually dilated from 5.3mm to 7.1mm by 3 hours post-atropine.

Atropine given 124 min prior to physostigmine caused a dilatation of both pupils from 5.0 to 7.0mm before the physostigmine was administered. After physostigmine, the pupil of the test eye constricted to 2.1mm by 30 min, dilating to 2.2mm by 2 hours (Fig. 40B). The mean results for these subjects for physostigmine alone showed a constriction of the pupil to 2.1mm by 37 min and to 2.0mm by 51 min then a gradual dilatation to 2.4mm by 2 hours (and to 2.7mm by 3 hours). Once again the pre-treatment seemed to slightly prolong the pupillary constriction. However, paired t-tests again showed no difference in time course or extent of pupil constriction ($P>0.16$) except at the first time interval after the eyedrops ($P=0.01$) which may be attributable to a difference in sampling times (6 and 10min). The pupil of the control eye remained dilated at <u>circa</u> 7mm throughout.

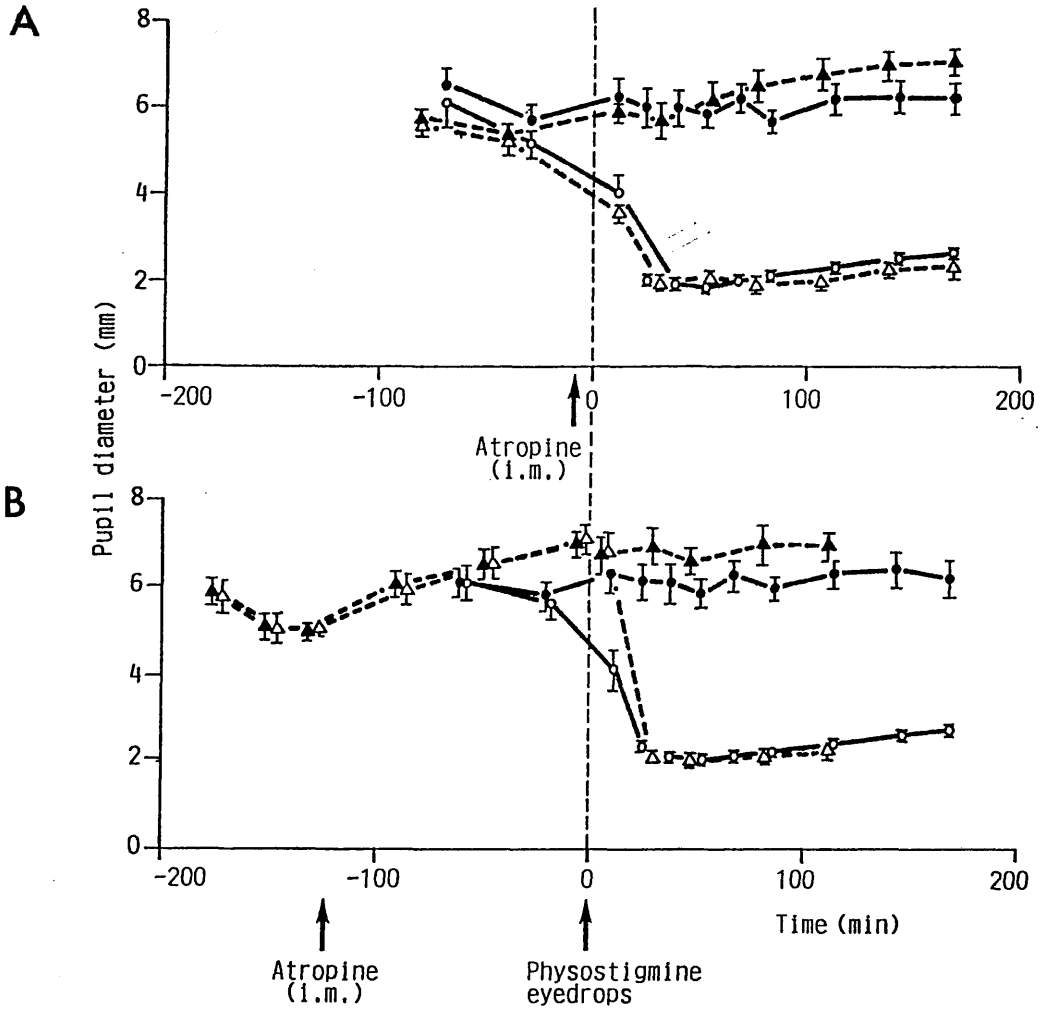


Fig. 40. Effect of injection of atropine sulphate (2 mg i.m.) at 8 min (A) and 124 min (B), prior to 0.25% physostigmine eyedrops (broken lines) on pupil diameter for test eye △ and companion eye ▲. Control data for physostigmine eyedrops alone are also shown (continuous line) for test eye ○ and companion eye ● . Mean results ± S.E. for 6 subjects.

Accommodation

Physostigmine eyedrops produced a large increase in involuntary accommodation despite administration of atropine 8 min earlier (Fig. 41A). The increase in accommodation amounted to 1.8 D after 7 min and was maximal at 6.5 D after 30 min, then declining gradually over 2 hours. Physostigmine alone had caused a somewhat larger increase in accommodation of 4.5 D after 5 min but the peak effect after 30 min was similar at 7.1 D and the response had also declined by 2 hours. Indeed, no significant difference was detected between the increase in accommodation caused by physostigmine alone or when preceded by atropine, at any of the time periods measured ($P>0.10$).

Atropine given 124 min prior to physostigmine slightly reduced the increase in involuntary accommodation measured after physostigmine eyedrops (Fig. 41B). An increase of 1.1 D was seen after 5 min, rising to a maximum increase of 6.0 D after 26 min then declining to 0.3 D by 105 min after physostigmine. In comparison, physostigmine alone had caused an increase of 2.1 D after 5 min, which rose to a maximum increase of 7.6 D after 29 min and declined to 0.8 D by 110 min, with some effect still detected until 3 hours after physostigmine. However, paired t-tests showed that the peak increase in accommodation was not significantly different from that for physostigmine alone ($P>0.10$). The increase in accommodation was less after 5 and 45 min although this was only of marginal significance ($0.05<P<0.10$) and significantly less than that obtained for physostigmine alone after 105 min ($P<0.05$).

The change in near point accommodation (i.e. the mean control value for near-point accommodation subtracted from each test value) is shown in Fig. 42. Pre-treatment with atropine 8 min prior to physostigmine resulted in, if anything, a slightly larger increase in near point accommodation after physostigmine (Fig. 42A). A peak

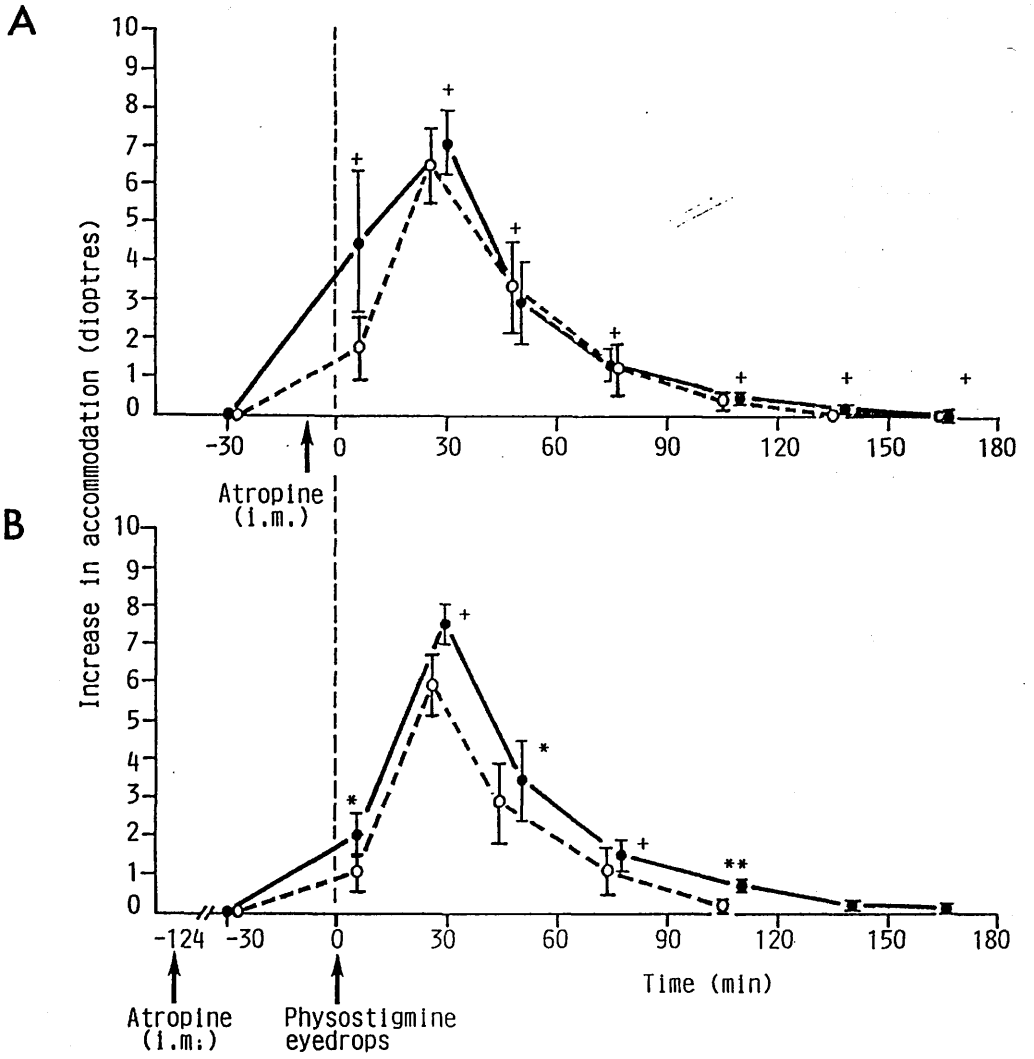


Fig. 41. Effect of injection of atropine (2 mg i.m.) at 8 min (A) and 124 min (B) prior to 0.25% physostigmine eyedrops (O, broken lines) on amplitude of accommodation. Significance of differences from control data for physostigmine eyedrops alone (●, continuous line) are shown: ** $p < 0.05$; * $0.05 < p < 0.1$; + N.S., $p > 0.1$. Mean results $\pm$ S.E. for 6 subjects.

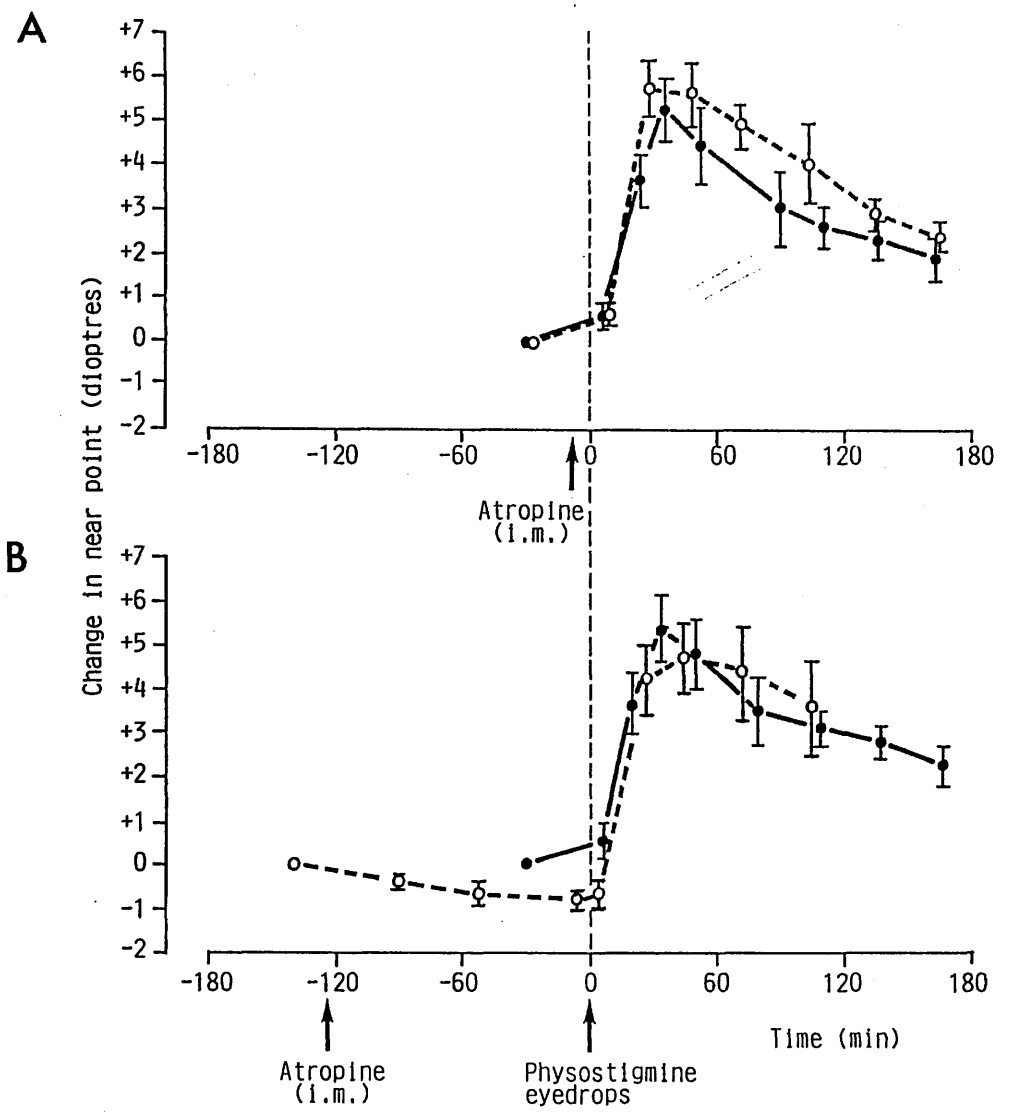


Fig. 42. Effect of injection of atropine (2 mg i.m.) at 8 min (A) and 124 min (B) prior to 0.25% physostigmine eyedrops (O, broken lines) on near point accommodation. Control data for physostigmine eyedrops alone also shown (●, continuous line). Mean results ± S.E. for 6 subjects.

increase of 5.8 D was measured 30 min after physostigmine which then gradually declined to 2.5 D at the end of the experiment (3 hours); physostigmine alone had previously caused a peak increase of 5.4 D after 34 min which had declined to 2.2 D by 3 hours. However, these small differences were not significant at any time interval ($P>0.10$).

Atropine given 124 min prior to physostigmine resulted in a loss of near point accommodation of 0.8 D immediately prior to physostigmine administration. Following physostigmine, a peak increase of 4.7 D was observed at 45 min after physostigmine and an increase of 3.6 D remained at the end of this experiment (105 min; Fig. 42B). Physostigmine alone had previously caused a peak increase of 5.3 D after 34 min and an increase of 2.7 D had remained at 110 min after physostigmine. Again however, prior administration of atropine made no significant difference at any time period ($P>0.35$) to the effect of physostigmine on near point accommodation.

Critical fusion frequency

A transient fall in the mean critical fusion frequency occurred at 36 min for physostigmine alone ($P=0.03$). After the pre-treatment with atropine 8 min prior to physostigmine, this effect was no longer seen and no significant differences were found between any of the test measurements and the second control measurement C2 prior to atropine administration ($P>0.20$; Fig. 43). The critical fusion frequency was not measured for the 124 min atropine pre-treatment.

Contrast sensitivity

Irrespective of atropine given 8 min or 124 min beforehand, application of physostigmine still resulted in a large decline in contrast sensitivity at 10, 20 and 30 c/deg for a stationary pattern and

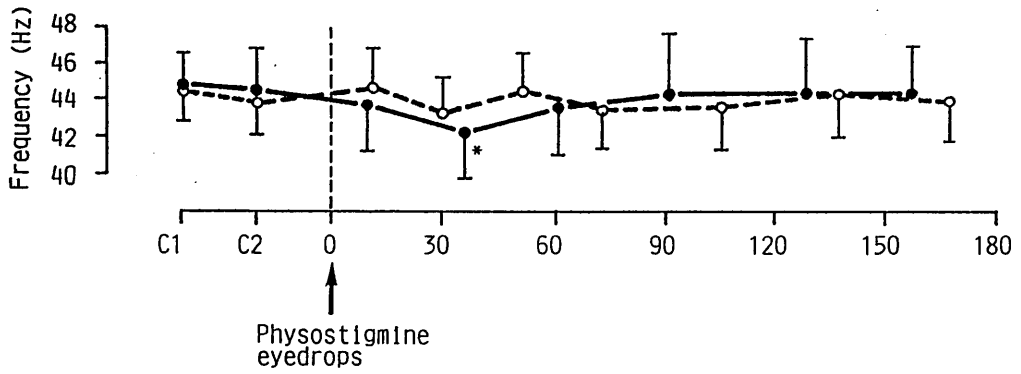


Fig. 43. Effect of injection of atropine (2 mg i.m.) at 8 min prior to 0.25% physostigmine eyedrops (O, broken line) on critical fusion frequency. Control data for physostigmine eyedrops alone also shown (●, continuous line). Significance of difference from control period C2; * $p = 0.03$; all other periods N.S., $p > 0.20$. Mean results $\pm$ S.E. for 6 subjects.

at 3 c/deg for a phase-reversed pattern. First, the data were examined against time on an individual basis and then as a group. All 6 subjects showed a transient fall in contrast sensitivity at each spatial frequency measured which peaked at about 30 min and then subsided by some 2 hours after physostigmine, despite both atropine pre-treatments.

(a) Atropine 8 min prior to physostigmine

The grouped data for contrast sensitivity for atropine at 8 min + physostigmine are shown in Fig. 44. Paired t-tests between the two control measurements prior to atropine showed no significant differences at any of the spatial frequencies measured ($P>0.09$). The test measurements following physostigmine were then compared to the second control measurement (C2). After physostigmine there was a transient fall in contrast sensitivity at each spatial frequency measured. Contrast sensitivity to a 10 c/deg grating pattern was significantly depressed until 108 min after physostigmine, contrast sensitivity to a 20 c/deg grating pattern had recovered to within control levels by 81 min and that to a 30 c/deg grating pattern by 143 min after physostigmine. However, contrast sensitivity to a 3 c/deg phase-reversed grating pattern remained significantly depressed at the end of the experimental period ($P<0.05$). The significance levels for the t-tests are shown in Table 13.

The factor to be considered was whether the pre-treatment was having any effect at all. In order to compare the contrast sensitivity measurements obtained for the same subject on different days each individual's results were normalised by expressing contrast thresholds as a percentage of C2. Paired t-tests were then performed at corresponding time periods between the physostigmine + atropine and physostigmine alone data.

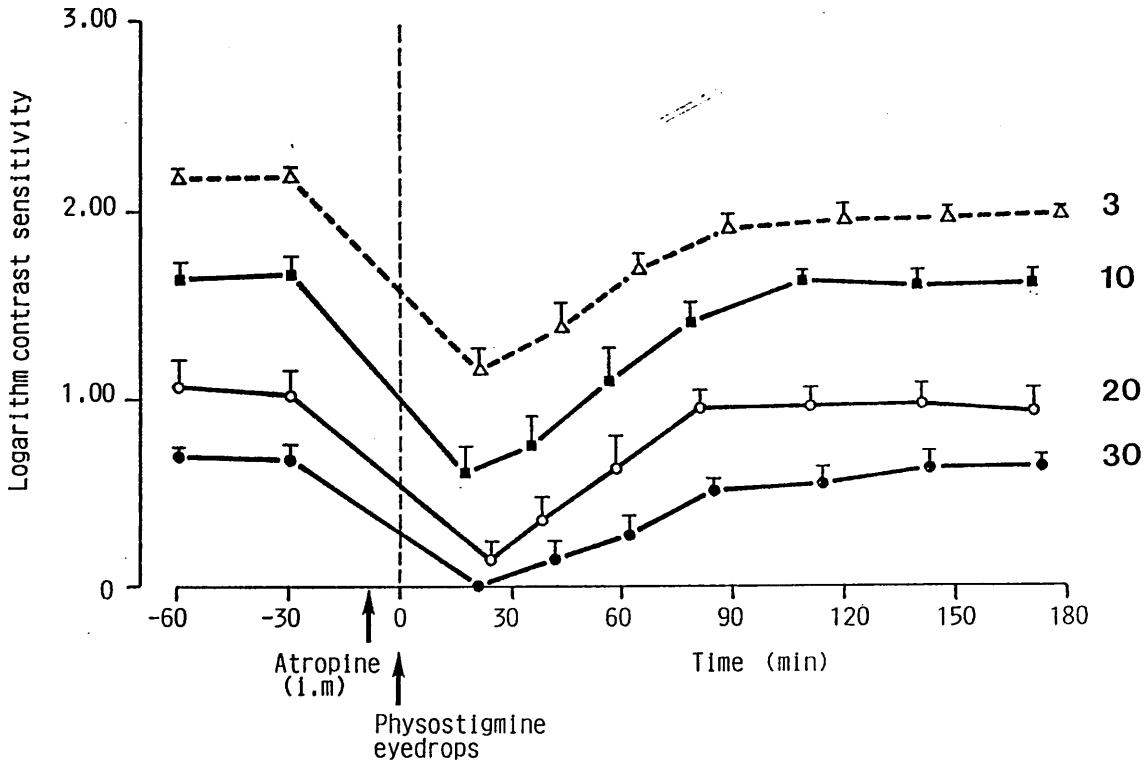


Fig. 44. Effect of injection of atropine (2 mg i.m.) at 8 min prior to 0.25% physostigmine eyedrops on contrast sensitivity (logarithmic scale) to stationary sinusoidal grating patterns of 10, 20 and 30 c/deg and a 3 c/deg grating phase-reversed at 5.5 Hz. Mean data $\pm$ S.E. for 6 subjects.

The reduction in contrast sensitivity following physostigmine for a 10 c/deg grating pattern is shown in Fig. 45A. Pre-treatment with atropine 8 min prior to physostigmine resulted in a fall to 11% of control after 17 min which was not significantly different from a fall to 13% of control after 13 min for physostigmine alone ($P>0.80$). By 170 min contrast sensitivity had recovered to 92% of control compared to 105% of control after 170 min for physostigmine alone (which again was not significantly different, $P>0.20$). Indeed, there was no significant difference between the physostigmine + atropine and physostigmine alone data at any time period measured ($P>0.10$; Fig. 45A)

The reduction in contrast sensitivity at 20 and 30 c/deg after physostigmine is shown in Figs. 46A and 47A respectively. In general, there was no significant difference in the reduction of contrast sensitivity seen after the 8 min atropine pre-treatment compared with that for physostigmine alone, nor in the recovery of contrast sensitivity ($P>0.16$). The only exception was for 20 c/deg at 170 min after physostigmine ($P<0.05$).

For the 3 c/deg phase-reversed grating pattern, the loss of contrast sensitivity after physostigmine was slightly greater with the 8 min atropine pre-treatment where the maximal reduction was to 13% of control compared to 26% of control after physostigmine alone (Fig. 48A). However this difference was not significant ($P>0.50$). As with the physostigmine alone experiment contrast sensitivity to the 3 c/deg moving grating pattern returned to only 70% of the control level by the end of the experimental period.

Table 13. Atropine 8min prior to physostigmine. Significance levels (paired t-tests) of the difference in logarithm contrast sensitivity between the test period and the control period C2, at the spatial frequencies indicated.

Period	C1	P1	P2	P3	P4	P5	P6	P7
Time (min)	-	(21)	(39)	(60)	(83)	(113)	(114)	(173)
10 c/deg	0.09	<0.001	0.001	0.02	0.05	0.61	0.63	0.63
20 c/deg	0.35	0.001	0.006	0.05	0.54	0.30	0.56	0.17
30 c/deg	0.88	<0.001	0.01	0.04	0.02	0.009	0.26	0.51
3c/deg *	0.94	<0.001	0.002	0.001	<0.001	0.01	0.008	0.01

C: control measurements and P: measurements after physostigmine at the mean time indicated. * grating pattern phase-reversed at 5.5 Hz.

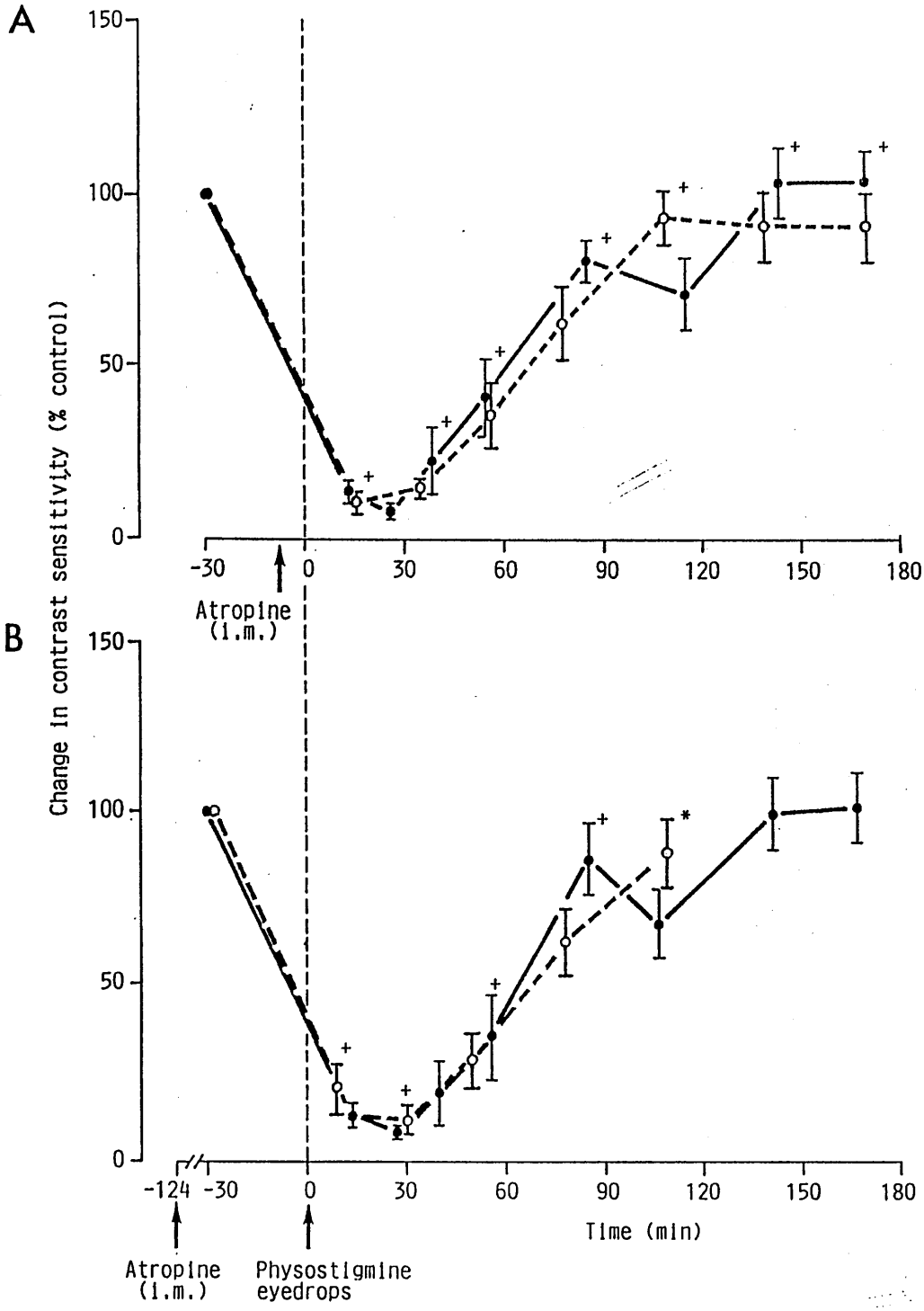


Fig. 45. 10 c/deg stationary grating pattern: effects of injection of atropine sulphate (2 mg i.m.) at 8 min (A) and 124 min (B) prior to 0.25% physostigmine eyedrops (○, broken line) compared with physostigmine alone (●, continuous line) on contrast sensitivity. None of the values was significantly different; + $p > 0.1$; * $0.05 < p < 0.1$. Mean ± S.E. for 6 subjects.

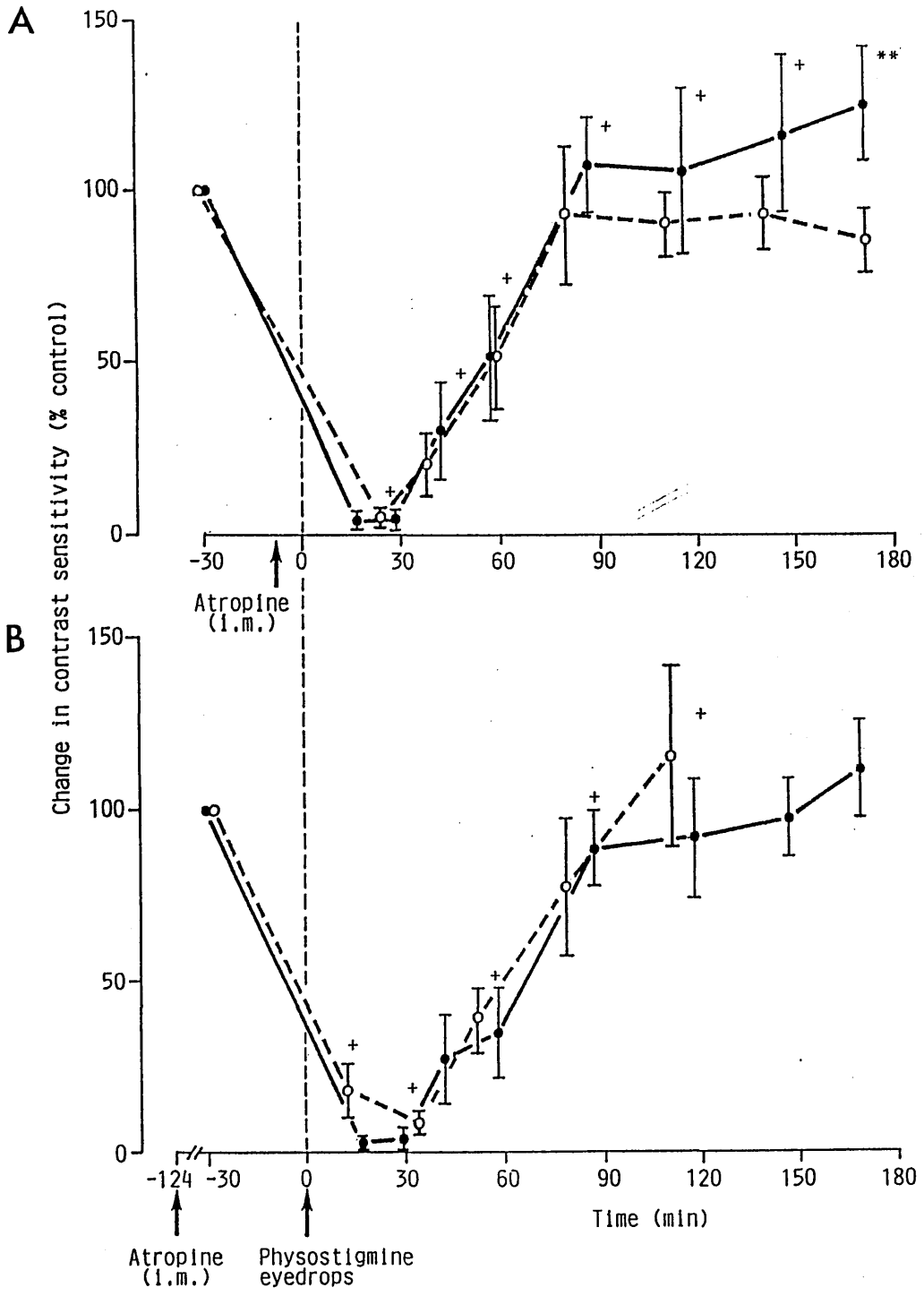


Fig. 46. 20 c/deg stationary grating pattern: effects of injection of atropine sulphate (2 mg i.m.) at 8 min (A) and 124 min (B) prior to 0.25% physostigmine eyedrops (O, broken line) compared with physostigmine alone (●, continuous line) on contrast sensitivity. Significance levels of difference are indicated next to the points compared; + N.S., $p > 0.1$; ** $p < 0.05$. Mean ± S.E. for 6 subjects.

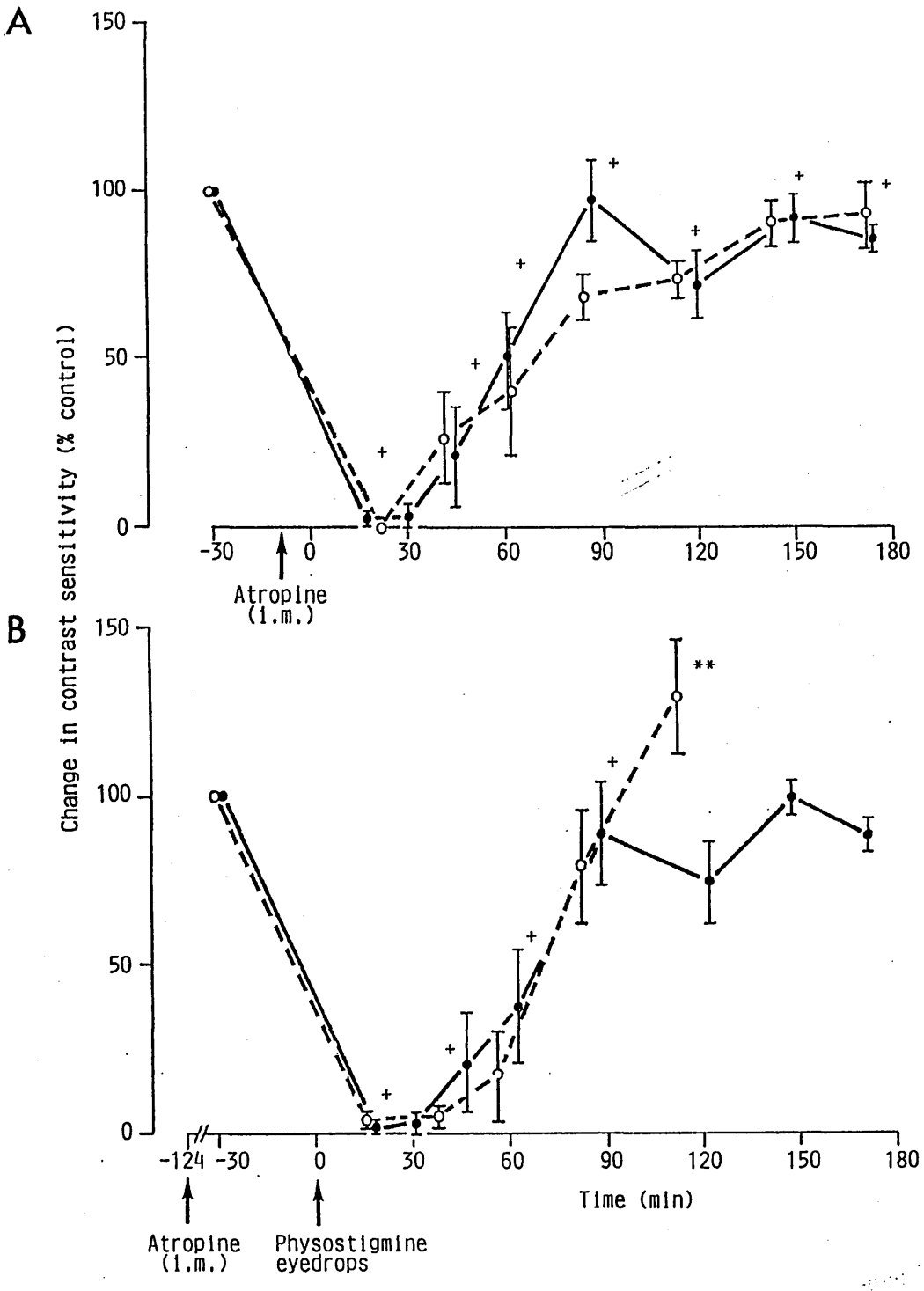


Fig. 47. 30 c/deg stationary grating pattern: effects of injection of atropine sulphate (2 mg i.m.) at 8 min (A) and 124 min (B) prior to 0.25% physostigmine eyedrops (O, broken line) compared with physostigmine alone (●, continuous line) on contrast sensitivity. Significance levels of difference are indicated next to the points compared; + N.S., $p > 0.1$; ** $p < 0.05$. Mean ± S.E. for 6 subjects.

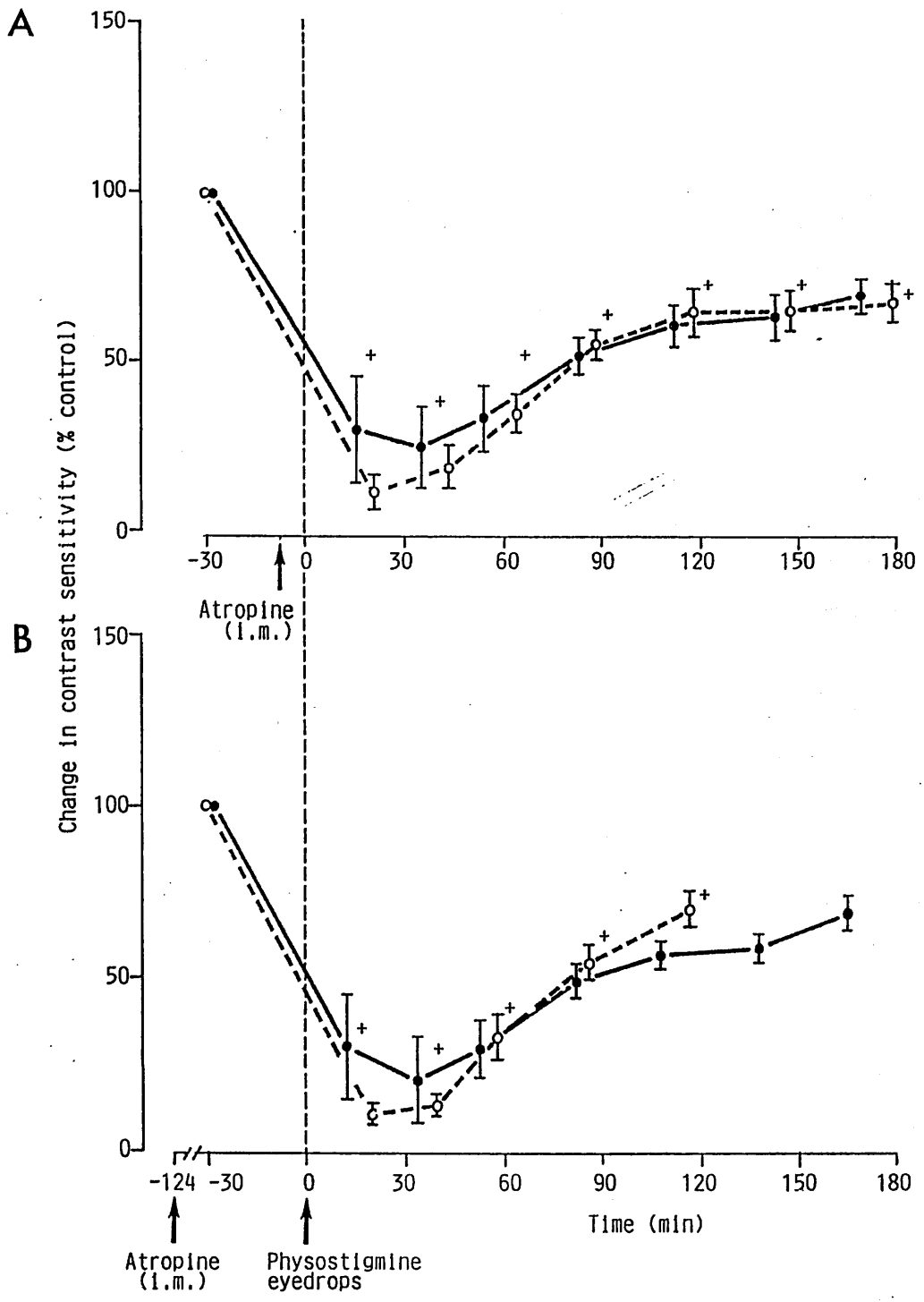


Fig. 48. 3 c/deg grating pattern phase-reversed at 5.5 Hz: effects of injection of atropine sulphate (2 mg i.m.) at 8 min (A) and 124 min (B) prior to 0.25% physostigmine eyedrops (O, broken line) compared with physostigmine alone (●, continuous line) on contrast sensitivity. None of the values was significantly different; + $p > 0.1$. Mean ± S.E. for 6 subjects.

(b) Atropine 124 min prior to physostigmine

The mean contrast sensitivity data for atropine at 124 min + physostigmine compared with that for physostigmine alone are shown in Fig. 49. Paired t-tests between the two control measurements prior to atropine showed no significant differences at 20 and 30 c/deg nor for the 3 c/deg phase-reversed grating pattern ($P>0.30$) but at 10 c/deg the second control measurement was significantly greater than the first ($P<0.01$). Contrast sensitivities were also measured at approximately 60 and 90 min after atropine administration and were compared with the control measurement just prior to atropine administration. Following atropine, contrast sensitivity to a 3 c/deg phase-reversed grating pattern was significantly less at 60 and 90 min ($P<0.05$) which confirms the results in part 4 of this study on another group of subjects. Contrast sensitivity to grating patterns of 10 and 20 c/deg were also significantly reduced but only at 60 min post-atropine ($P<0.01$). Contrast sensitivity at 30 c/deg was not significantly different from control at either 60 or 90 min post-atropine ($P>0.35$). Following the administration of physostigmine, there was a large and transient fall in contrast sensitivity at each spatial frequency measured. Contrast sensitivity to a 10 c/deg grating pattern did not recover completely to within control levels until 109 min, whereas at 20 and 30 c/deg recovery was complete at about 80 min after physostigmine. Contrast sensitivity to the 3 c/deg phase-reversed grating pattern remained significantly depressed compared with control levels at the end of the experimental period (2 hours in this case, $P<0.01$). The significance levels for the paired t-tests are shown in Table 14.

To compare the fall in contrast sensitivity after atropine 124 min before + physostigmine, with that seen after physostigmine alone, the data were normalised by expressing contrast thresholds as a percentage

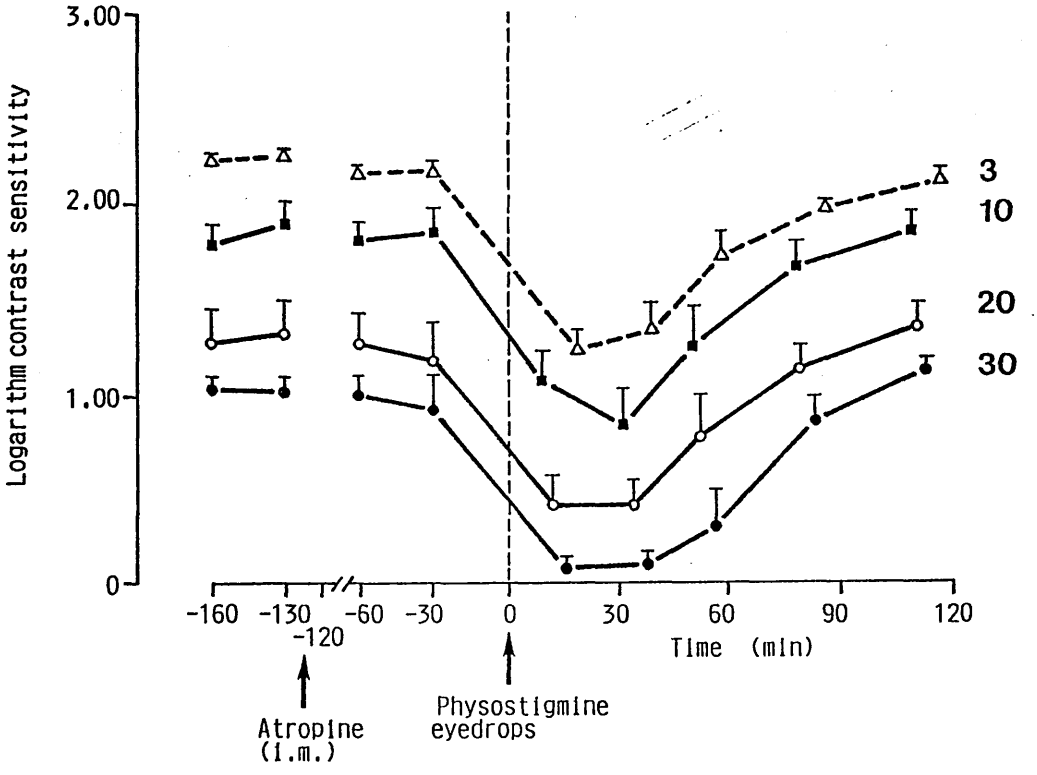


Fig. 49. Effects of injection of atropine (2 mg i.m.) at 124 min prior to 0.25% physostigmine eyedrops on contrast sensitivity (logarithmic scale) to stationary sinusoidal gratings of spatial frequencies 10, 20 and 30 c/deg and a 3 c/deg grating phase-reversed at 5.5 Hz. Mean data ± S.E. for 6 subjects.

Table 14. Atropine 124 min prior to physostigmine. Significance levels (paired t-tests) of the difference in logarithm contrast sensitivity between the test period and the control period C2, at the spatial frequencies indicated.

Period	C1	A1	A2	P1	P2	P3	P4	P5
Time (min)	-	(60)	(90)	(14)	(36)	(54)	(81)	(112)
10 c/deg	0.004	<0.001	0.29	0.005	0.002	0.008	0.05	0.27
20 c/deg	0.31	0.006	0.24	0.006	0.003	0.05	0.21	0.27
30 c/deg	0.51	0.67	0.36	<0.001	<0.001	0.01	0.33	0.18
3c/deg *	0.58	0.01	0.05	<0.001	0.001	0.005	0.01	0.008

C: control measurements A: measurements post-atropine and P: measurements post-physostigmine. * grating pattern phase-reversed at 5.5 Hz.

of C2. The reduction in contrast sensitivity for a 10 c/deg grating pattern is shown in Fig. 45B. Pre-treatment with atropine 124 min prior to physostigmine resulted in a fall to 12% of control by 31 min with a recovery to 89% of control by 109min after physostigmine. In comparison, physostigmine alone had caused a reduction in contrast sensitivity to 8% of control by 39 min, gradually recovering to 102% of control by 167 min. Again there were no significant differences between the physostigmine + atropine and physostigmine alone data at any time period ($P>0.07$; Fig. 45B).

The reduction in contrast sensitivity at 20 and 30 c/deg after physostigmine is shown in Figs. 46B and 47B respectively. In general, there was no significant difference in the reduction of contrast sensitivity seen for the physostigmine + atropine data compared with those for physostigmine alone, nor in the recovery of contrast sensitivity ($P>0.10$). The only exception was for 30 c/deg at 110 min ($P<0.05$).

For the 3 c/deg phase-reversed grating pattern, the loss of contrast sensitivity was slightly greater after the 124 min atropine pre-treatment which resulted in a maximal reduction to 11% of control compared with 21% of control for physostigmine alone (Fig. 48B). However these differences were not significant ($P>0.10$). As with the physostigmine alone experiment, contrast sensitivity to the 3 c/deg moving pattern returned to only 70% of the control level by the end of the experimental period ($P<0.01$).

In summary, neither of the pre-treatments with atropine significantly affected the amplitude or time course of the effects of physostigmine eyedrops, followed over 3 hours, on pupil diameter, accommodation and contrast sensitivity, as shown by the peak effects of physostigmine in Table 15.

Table 15. Comparison of effects of 0.25% physostigmine eyedrops 30min after instillation, when given alone or preceded by atropine at 8 min and 124 min. Mean data for 6 subjects.

	Pupil diameter	Defocus	Mean reduction in contrast sensitivity (%)			
	(mm)	(dioptres)	3c/deg	10c/deg	20c/deg	30c/deg
Physostigmine	2.0	7.0	77	92	96	98
+ 8min atropine	2.0	6.5	87	86	95	100
+ 124min atropine	2.1	6.0	86	88	91	95

Pre-treatment with homatropine hydrobromide eyedrops

Five of the six subjects who had taken part in the previous two experiments also completed this third test with physostigmine eyedrops. Each subject had shown a large increase in accommodation following physostigmine eyedrops on five previous occasions.

Pupil diameter

Following homatropine hydrobromide, all 5 subjects showed a dilatation of the pupil to ~8mm. This was followed by pupillary constriction after physostigmine to only ~6mm in diameter instead of the value of 2mm to physostigmine alone. Table 16 shows the individual responses, expressed as the difference in pupil diameter between the test eye and the control eye. Constriction of the pupil following physostigmine was sufficient to bring the diameter of the test eye to below that of the control eye for only two of the five subjects tested.

The mean data for the homatropine pre-treatment on the pupil diameter response to physostigmine eyedrops are shown in Fig. 50.

Table 16. Difference in pupil diameter between the test eye which received homatropine and physostigmine and the control eye at the time periods indicated for each subject.

	Difference in pupil diameter (mm)												
Period	C1	C2	C3	H1	H2	H3	P1	P2	P3	P4	P5	P6	P7
Time (min)	-	-	-	(30)	(52)	(94)	(11)	(34)	(54)	(82)	(112)	(143)	(169)
KO	0	+0.1	0	+2.3	+3.1	+2.3	+1.9	-0.6	-1.0	-0.6	-0.1	-0.2	0
DO	0	+0.1	-0.1	+2.1	+2.5	+2.0	+1.2	-0.1	-1.2	-0.3	+0.2	-0.2	+0.2
CK	0	+0.2	-0.1	+2.5	+3.2	+2.5	+3.5	+1.6	+1.5	+1.2	+1.8	+1.1	+1.1
GM	0	0	+0.1	+3.4	+4.4	+3.1	+2.6	+2.8	+2.7	+1.7	+2.3	+1.8	+2.3
SO	-0.2	-0.1	-0.1	+4.3	+4.7	+4.5	+4.3	+2.1	+1.7	+0.9	+0.4	+0.4	+0.6

C: control measurements H: measurements after homatropine at mean time (min) shown and P: measurements after physostigmine at mean time shown. Physostigmine was given 112 min after homatropine.

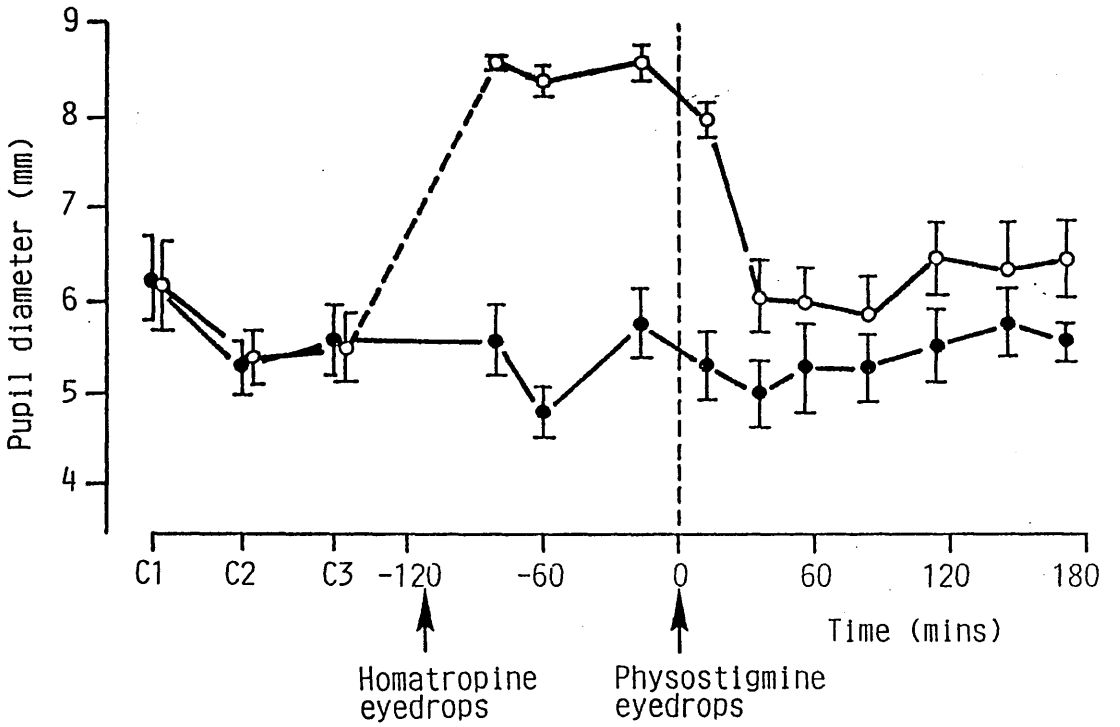


Fig. 50. Effects of 2% homatropine eyedrops prior to 0.25% physostigmine eyedrops on pupil diameter of test eye, O (which received both drugs) and companion eye, ●. Mean ± S.E. for 5 subjects.

Homatropine initially caused a pupil dilatation from about 5.5mm to about 8.6mm. To ensure that the homatropine effects were fully developed, physostigmine eyedrops were not given until, on average, 112 min after homatropine. Physostigmine then caused a constriction of the pupil to about 6mm, at 60-80 min when the pupil diameter was not significantly different from the pre-homatropine control for that eye (C3, $P>0.35$). Thereafter, the pupil diameter was significantly greater than the pre-homatropine control ($P<0.05$), as if the physostigmine-induced miosis was declining. After physostigmine, paired t-tests between the control and test eyes, however, showed no significant differences ($P>0.10$) except at 10 min when that of the test eye was still significantly larger than control ($P<0.001$).

Accommodation

Pre-treatment with homatropine resulted in a slight loss of accommodation which required a correcting lens (of the order of +0.25D) to be worn for optimal Snellen acuity at 5m. Administration of physostigmine then caused a small increase in involuntary accommodation with the result that the subject now had optimal Snellen acuity without additional refraction or needed a negative spherical lens to compensate for the increased accommodation. The individual results are given in Table 17 and the grouped data are shown in Fig. 51. Thus, administration of physostigmine causes a slight increase in accommodation. However, when compared with the effect of physostigmine alone, the changes are very small (e.g. -0.4 D change at 30 min as compared with -7.5 D).

Following application of homatropine eyedrops, all 5 subjects experienced a marked loss of near point accommodation (to less than 2 D, the limit of our measurements; Table 18). Physostigmine then caused an almost complete recovery of near point accommodation, but the effect was

Table 17. Additional refraction needed to bring bottom line of Snellen chart at 5m into sharp focus after homatropine eyedrops and physostigmine eyedrops for each subject.

	Strength of spherical lens (D)									
Period	H1	H2	H3	P1	P2	P3	P4	P5	P6	P7
Time (min)	(25)	(49)	(90)	(6)	(28)	(49)	(77)	(109)	(139)	(165)
KO	0	0	0	-0.75	-0.50	-0.25	-0.25	0	0	0
DO	+0.25	+0.50	+0.50	-0.50	-0.25	0	0	0	0	0
CK	0	0	0	0	0	0	0	0	0	0
GM	-0.25	0	0	0	0	0	0	-0.25	0	0
SO	+0.25	+0.25	+0.50	+0.25	-0.25	-0.25	-0.25	0	0	0

H: measurements after homatropine P: measurements after physostigmine, at mean time shown. Physostigmine given 112 min after homatropine.

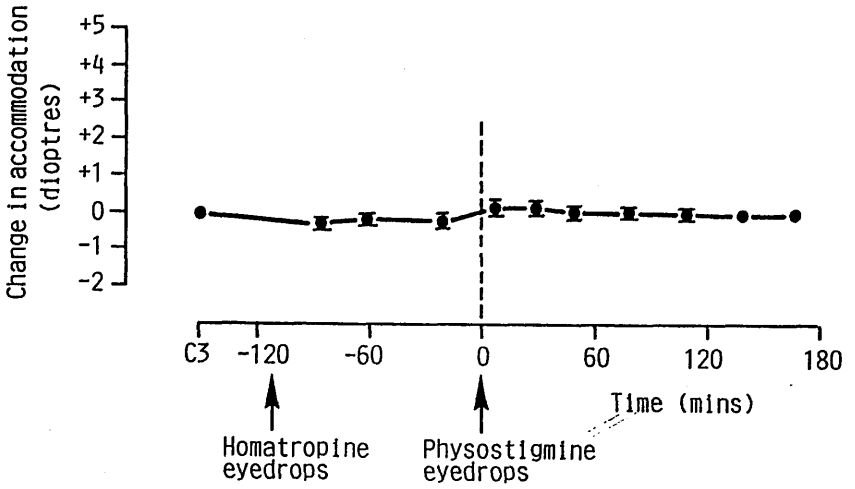


Fig. 51. Instillation of 2% homatropine eyedrops prior to 0.25% physostigmine eyedrops and amplitude of accommodation. Mean ± S.E. for 5 subjects.

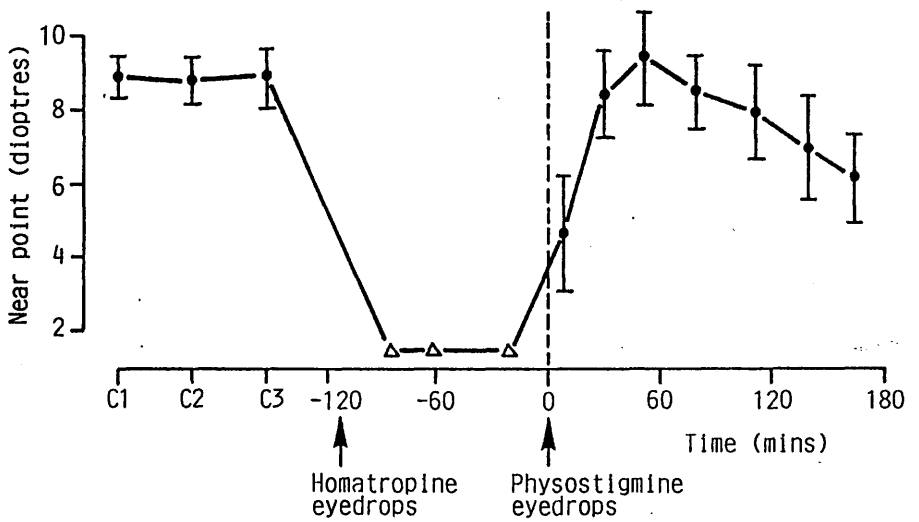


Fig. 52. Instillation of 2% homatropine eyedrops prior to 0.25% physostigmine eyedrops and near point accommodation. Mean ± S.E. for 5 subjects.

Table 18. Effect of homatropine eyedrops followed by physostigmine eyedrops on near point accommodation for each subject.

	Near point accommodation (D)												
Period	C1	C2	C3	H1	H2	H3	P1	P2	P3	P4	P5	P6	P7
Time (min)	-	-	-	(27)	(49)	(91)	(7)	(29)	(50)	(78)	(110)	(139)	(165)
KO	11.1	11.1	11.6	<2	<2	<2	8.2	11.4	13.2	11.1	11.3	10.5	8.9
DO	9.7	9.3	9.9	<2	<2	<2	9.1	11.1	11.6	10.7	9.9	8.6	8.2
CK	8.0	7.5	7.2	<2	<2	<2	<2	6.6	7.2	7.0	7.1	6.6	5.3
GM	7.9	8.0	8.0	<2	<2	<2	<2	6.0	7.8	6.2	3.8	<2	<2
SO	7.9	8.2	7.9	<2	<2	<2	2.0	6.7	7.0	7.4	7.5	7.1	6.8

C: control measurements, H: measurements after homatropine and P: measurements after physostigmine, at mean time (min) shown. Physostigmine given 112 min after homatropine.

transient as near point accommodation started to diminish by the end of the experiment. Indeed, in one subject this had again resulted in a complete loss of near point accommodation. The mean changes in near point accommodation are illustrated in Fig. 52. Physostigmine caused near point accommodation to return to pre-homatropine control levels ($P>0.13$). However, later there was an apparent loss of near point accommodation which attained significance by 3 hours post-physostigmine ($P=0.04$).

Contrast sensitivity

Contrast sensitivity was measured at 10, 20 and 30 c/deg for stationary grating patterns and at 3 c/deg for a grating phase-reversed at 5.5 Hz. Three control series of measurements were made prior to administration of any drugs. Following homatropine eyedrops, three further series of measurements were made. After administration of physostigmine eyedrops, measurements were made for a further 3 hours.

Individually, two out of five subjects showed little or no change in contrast sensitivity, at each spatial frequency measured, after homatropine and physostigmine and their results are shown in Fig. 53. These two subject's accommodation was also least affected (see Table 17). In another two subjects contrast sensitivity remained constant after homatropine eyedrops but a transient fall in contrast sensitivity was present mainly at 10, 20 and 30 c/deg after physostigmine (Fig. 54). The fifth subject showed a marked fall in contrast sensitivity after homatropine at 10, 20 and 30 c/deg, with a smaller reduction at 3 c/deg. Physostigmine administration then increased this subject's contrast sensitivity to about control levels (Fig. 55). The reason for the fall in contrast sensitivity following homatropine is not readily apparent, though homatropine nevertheless annulled the effect of physostigmine.

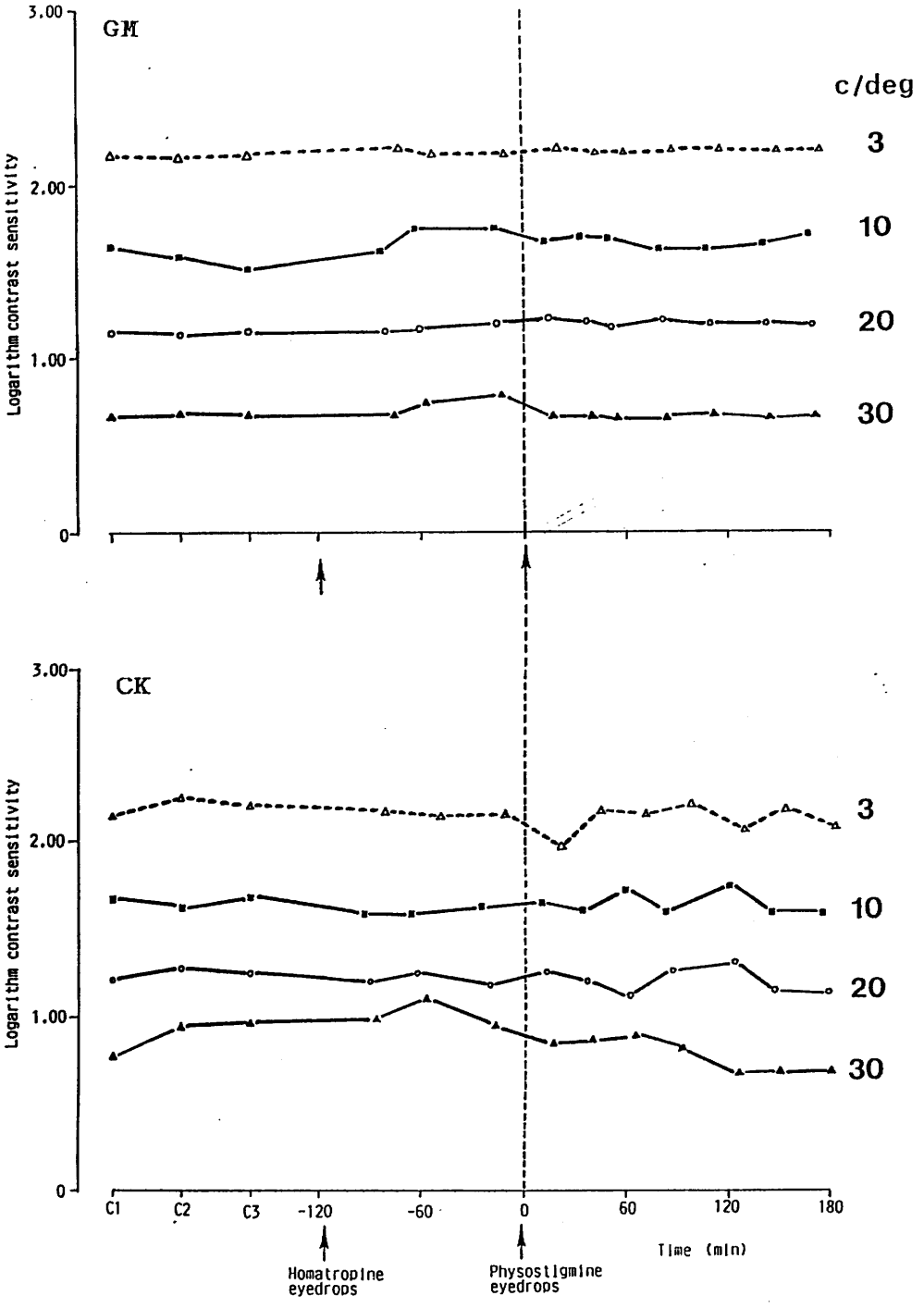


Fig. 53. Individual results of two subjects who showed little change in contrast sensitivity at the spatial frequencies indicated after 2% homatropine and 0.25% physostigmine eyedrops.

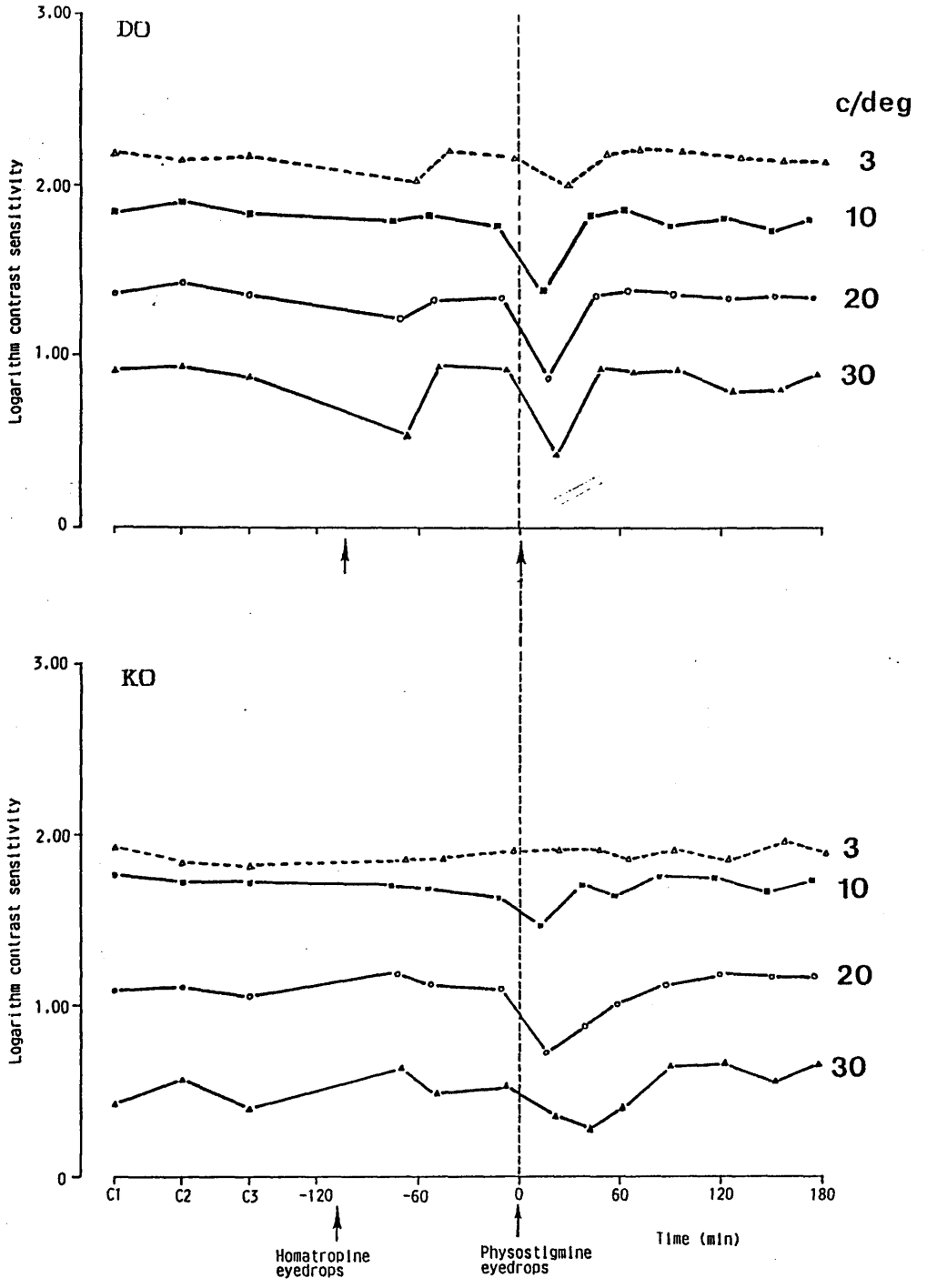


Fig. 54. Individual results of two subjects who showed a transient fall in contrast sensitivity at the spatial frequencies indicated following physostigmine eyedrops.

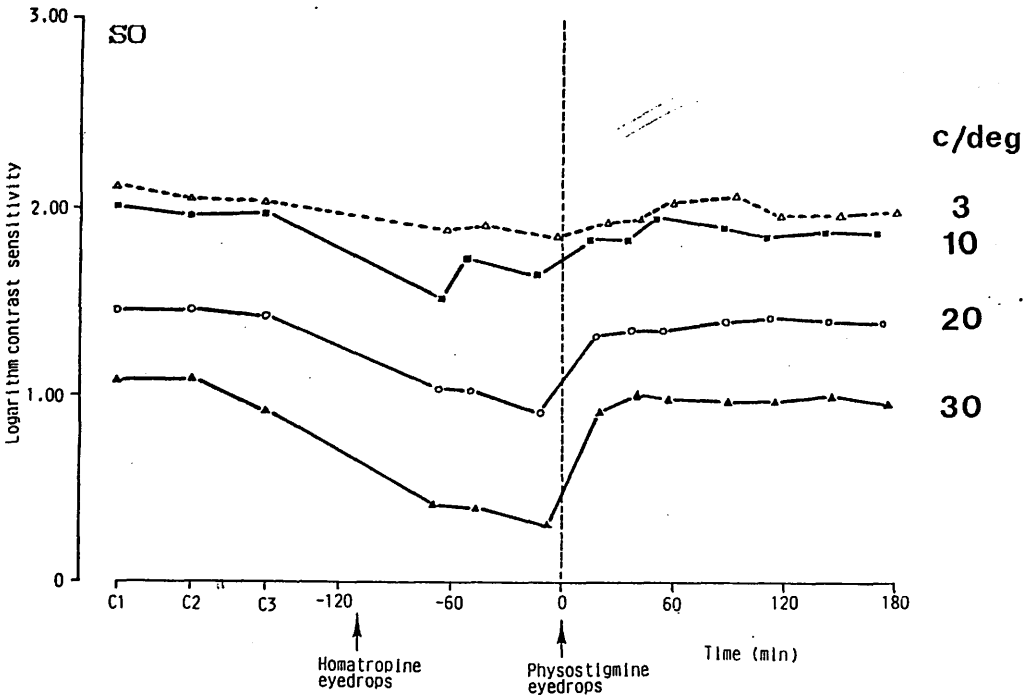


Fig. 55. Individual response of one subject who showed a reduction in contrast sensitivity at the spatial frequencies indicated following homatropine eyedrops which was then annulled by physostigmine eyedrops.

The grouped data for all 5 subjects were calculated and the mean contrast sensitivity measurements are shown in Fig. 56A. For comparison, Fig. 57 shows the mean contrast sensitivity measurements after physostigmine alone for the same 5 subjects. Pre-treatment with homatropine eyedrops prevented the large decrease in contrast sensitivity normally seen after physostigmine eyedrops. Although there was a small transient fall in contrast sensitivity, particularly for the 10, 20 and 30 c/deg grating patterns with less effect at the 3 c/deg phase-reversed grating pattern, contrast sensitivity was not significantly different from the control measurement prior to homatropine administration (C3) at any of the spatial frequencies measured (paired t-tests, $P>0.12$). Homatropine *per se* did not significantly affect contrast sensitivity at any spatial frequency measured, including the phase-reversed grating pattern ($P>0.10$).

The changes in contrast sensitivity have also been shown as a percentage of the pre-homatropine control value in Fig. 56B. Immediately after physostigmine, a 20% fall in contrast sensitivity was seen at 10, 20 and 30 c/deg and a 12% fall was seen for the 3 c/deg phase-reversed grating pattern. By 30 min after physostigmine, however, contrast sensitivity had returned to, or exceeded the control level. This is compared with a peak fall in contrast sensitivity of 92% at 10c/deg; 95% at 20 c/deg; 97% at 30 c/deg and 78% at 3 c/deg (phase-reversed) after physostigmine alone. Overall, the deleterious effect of physostigmine eyedrops on contrast sensitivity was very effectively prevented by homatropine.

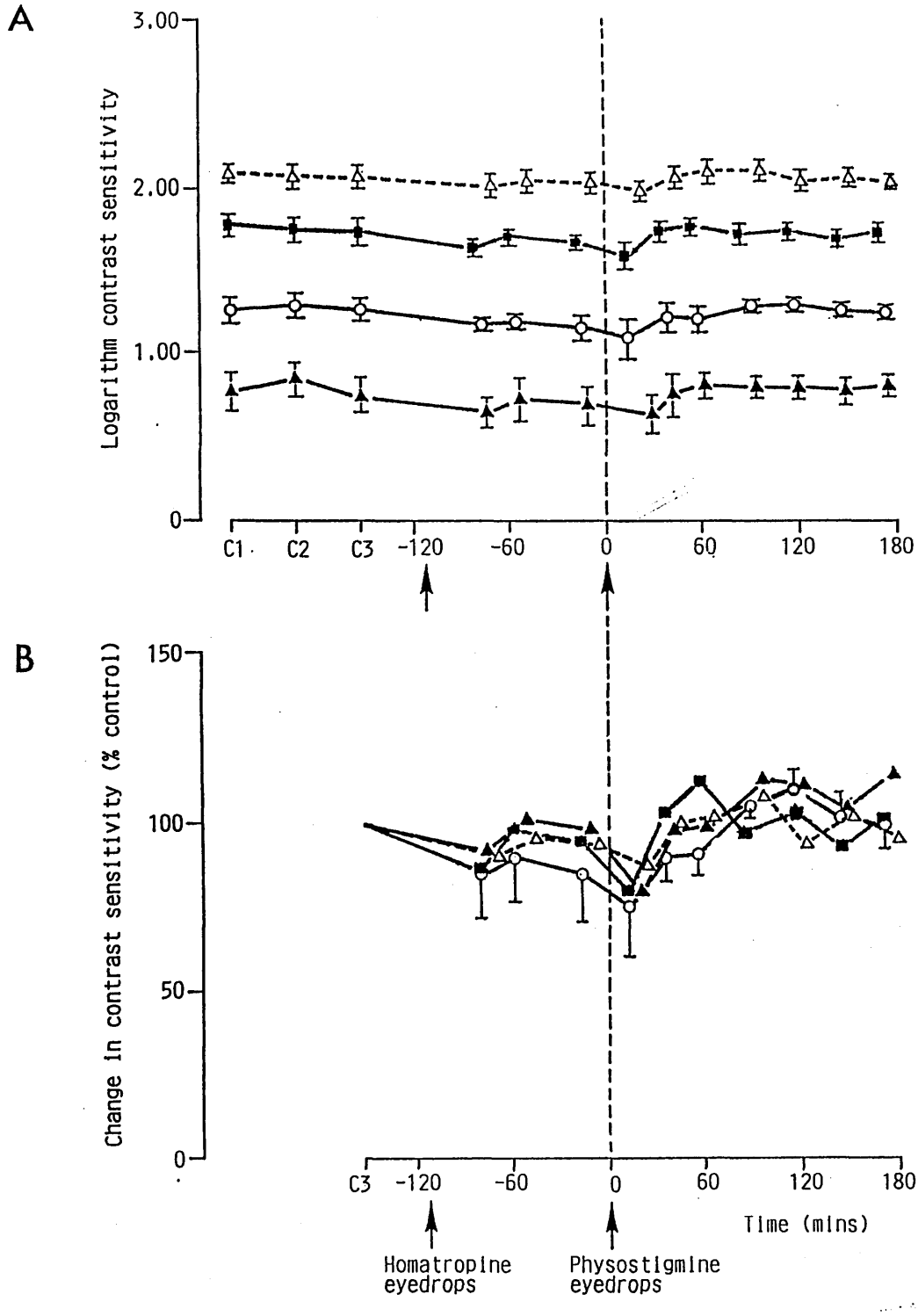


Fig. 56. Instillation of 2% homatropine eyedrops prior to 0.25% physostigmine eyedrops and contrast sensitivity to stationary sinusoidal grating patterns of 10 ■, 20 O and 30 ▲ c/deg and a 3 Δ c/deg grating phase-reversed at 5.5 Hz (A). The change in contrast sensitivity (linear units) from control value C3 for each spatial frequency (B). Mean results ± S.E. for 5 subjects.

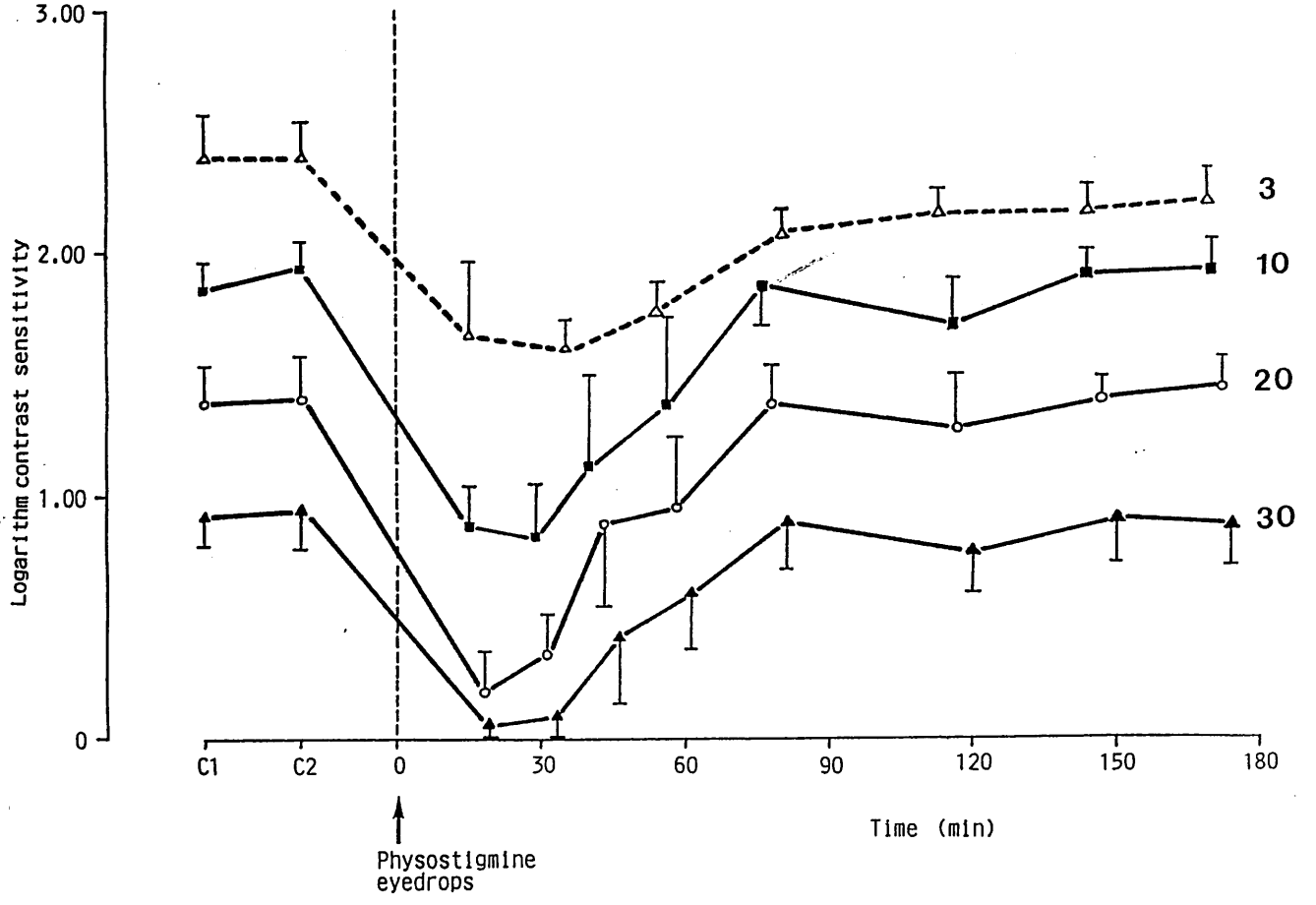


Fig. 57. Instillation of 0.25% physostigmine eyedrops alone on contrast sensitivity to stationary sinusoidal gratings of 10, 20 and 30 c/deg and a 3 c/deg grating phase-reversed at 5.5 Hz. Mean results ± S.E. for the same 5 subjects as in Fig. 56.

DISCUSSION

Assessment of visual performance mainly by measurement of contrast sensitivity has provided several new findings with respect to the action of anticholinesterases and cholinergic antagonists. By far the most important finding was the profound deleterious effect caused by defocus in contrast to the minimal effects caused by the changes in pupil diameter. In addition, evidence has been presented for neural actions of atropine and physostigmine. Intramuscular injection of atropine had a deleterious effect on motion sensitivity at low spatial frequencies. Physostigmine eyedrops caused a transient reduction in contrast sensitivity at higher spatial frequencies with an additional sustained action on low spatial frequency channels. Thus, as far as the visual system is concerned it is most undesirable for there to be a premature injection of atropine in the face of anticipated anticholinesterase poisoning. Even in the presence of the anticholinesterase, atropine has no beneficial effects in protection of visual function and may actually exacerbate perception difficulties by impairing motion sensitivity.

Contrast sensitivity to the CRT display

In the present study, the mean CSF for 12 subjects measured for the CRT display (Fig. 7), viewing with the natural pupil of ~6 mm diameter, had a peak of 81 units (S.D., 0.20) at 3 - 5 c/deg. This mean CSF is very similar to the individual data of Campbell & Green (1965) and Charman (1979). It can also be directly compared to the measurements of Morrison & McGrath (1985) (which were made using the same apparatus), for a group of young subjects, the range of contrast sensitivity they found was similar to that in the present study.

Contrast sensitivity at low spatial frequencies was measured from 1.0 m in the first part of the present study, where the display subtended 4.5 deg. McCann et al., (1974) showed that the viewing of low

spatial frequencies is unaffected by distance, but one factor which may limit contrast sensitivity at low spatial frequencies is the number of cycles of the grating visible. At least three cycles should be present for our screen luminance (Hoekstra et al., 1974). Thus some additional attenuation of contrast sensitivity may have been present at 0.5 c/deg as just under three cycles of the grating were visible. Howell & Hess (1978) found that the angular length of the bars of a grating was also important at low spatial frequencies. In absolute terms the contrast sensitivity measurements at the lowest spatial frequencies may not be entirely normal, but any effect would be present for all subjects under each of the test conditions; therefore, the measurements are acceptable for making comparisons. The presence of a dark surround also reduces contrast sensitivity, especially at low spatial frequencies (Estevez & Cavonius, 1976). The reduction would, however, not be constant throughout the present work since the action of the defocusing lenses would be to reduce the gradient of luminance between display and surround. Again, this factor should be constant between subjects, allowing comparisons to be made.

Contrast sensitivity to the laser display

Contrast sensitivity measurements to laser interference fringes observed in the Maxwellian view provide a measure of the transfer characteristics of the retina/brain portion of the visual system. The contrast sensitivity measurements to laser interference fringes made in the present study (Fig. 11) are, in general, comparable to those of Campbell & Green (1965), in that peak contrast sensitivity of similar magnitude occurred at 5-10 c/deg. High spatial frequency resolution was also comparable to Campbell & Green since spatial frequencies as high as 50 c/deg could be detected without difficulty, as was also found by

Morrison & McGrath (1985) for a group of young subjects. These results are higher than those from earlier studies before gas lasers were available, using partially coherent light (Arnulf & Dupuy, 1960; Westheimer, 1960). Higher contrast sensitivities, particularly at high spatial frequencies were reported by Williams (1985a), in a study which employed a constant fraction of coherent light. In the present study and that of Campbell & Green (1965), the fraction of coherent light varied since a polariser was used to modulate the contrast of the interference fringes. Williams (1985a) has suggested that the presence of coherent spatial noise both from the apparatus and from irregularities in the eye itself, such as vitreal floaters, can have a masking effect. This reduces contrast sensitivity to interference fringes and is especially marked when a greater proportion of coherent light is present i.e. when the contrast threshold is high, as for high spatial frequency fringes in the present study. At spatial frequencies above 60-70 c/deg, the individual bars of the interference fringes are no longer visible as aliasing due to the spacing of the foveal cones distorts the image and a low spatial frequency moire pattern is seen (Williams, 1985b). Aliasing, however, should not have been a problem in the present study.

The method of presentation of the stimulus can also influence the contrast threshold to high spatial frequency interference fringes. These fringes can only be seen at the macula and disappear with steady viewing due to adaptation. To prevent this, subjects were instructed to make rapid determinations of threshold and to look away from the display then return to it if necessary. The range for normal subjects was considerable and generally greater than that for the CRT display but was similar to that found for the six subjects tested by Williams and in the study with subjects of different ages by Morrison & McGrath (1985).

This emphasises the need for relatively large samples of subjects wherever possible.

The ocular modulation transfer function

Comparison of contrast sensitivity measurements to laser interference fringes with those made for conventional CRT generated grating patterns allows the ocular MTF to be calculated. This method of comparing contrast sensitivity for the two displays has the advantage that light must pass through the optics of the eye only once. Methods of determining the ocular MTF from measurements of the linespread function require the light to pass through the eye twice (Westheimer & Campbell, 1962; Campbell & Gubisch, 1966; Charman & Jennings, 1976) and may be confounded by backscattered illumination (Kerker, 1969). However, one disadvantage of the method used in the present study is that it does not allow for intraocular scatter of light. This will degrade the modulation of the image of both the laser interference fringes and the CRT grating patterns and may, therefore, lead to an overestimation of the ocular MTF (Charman, 1983). The fact that the two displays used in the present study (and also by Campbell & Green) were of different wavelength emissions should not be a problem, for contrast sensitivity to monochromatic gratings does not change with wavelength, providing the mean luminance is constant (van Nes & Bouman, 1967).

The mean ocular MTF (shown as the continuous line uppermost in Fig. 12) shows that image contrast was reduced to 50% at 3 c/deg with a further fall to 30% at 20 c/deg. Over 10-38 c/deg, it remained fairly constant whereas the retinal transfer characteristics (i.e. contrast sensitivity to the laser interference fringes) declined at a much faster rate, indicating that the high frequency cut-off (i.e. the resolution limit) would be set by the retina/brain rather than by the ocular media.

This is in agreement with the results of Campbell & Green (1965) and also those from a study with subjects of different ages (Morrison & McGrath, 1985). While at first it may seem that the present results which measured down to 1 c/deg extend the data of Campbell & Green (1965) which measured down to 4 c/deg, this was not the case. Due to the failure to anticipate certain methodological problems, and the fact that it was thought more objective to analyse the data after all the experiments had been completed, this was discovered only later. For spatial frequencies less than 3 c/deg the MTF, for all 12 subjects measured, fell due to a greater difference between the CRT and laser contrast sensitivities. This was due to three factors: first, the luminances of the two displays were not exactly matched (difference of 1.3 log units) although this should have least effect at low spatial frequencies (van Meeteren & Vos, 1972). Second, there was a difference in field size which would have had greatest effect for low spatial frequency grating patterns generated on the CRT display (Howell & Hess, 1978). Third, the shape of the two displays was different (CRT, rectangular; laser, circular) and edge effects due to the truncation of the grating pattern would have influenced contrast sensitivity only for the CRT display since they average out for circular apertures (Kelly, 1970). When field size, shape and luminance of the two displays was carefully matched, the ocular MTF was found to increase towards unity at low spatial frequencies (Fig. 13; Fraser & Morrison, 1987), in agreement with conventional physical optics.

Reproducibility of the measurements

In order to be able to use contrast sensitivity as a monitor of visual performance throughout an experimental session consisting of repeated sets of measurements, the measurements themselves must be

reproducible. This is especially important if one wishes to detect any small changes in performance which may occur, for example, following administration of a drug. The reproducibility of the contrast sensitivity measurements made in the present study was assessed in Part 4 when a full "dummy" run of experiment 2 of the atropine study was performed. This consisted of four consecutive hourly series of measurements to both CRT and laser displays (shown on left of Figs. 37 & 39 respectively). There was a gradual trend for contrast sensitivity to increase on each repeat session over the four hour experimental period. However, these changes were relatively rather small as seen in Fig. 38 which shows the consistency of contrast sensitivity for the CRT display, for all spatial frequencies measured. Contrast sensitivity was generally not significantly different from that determined on the first session for either stimulus display (Figs. 37 & 39). Contrast sensitivity on the second day (prior to atropine administration) was, however, significantly greater than the last series of control measurements on the first day. Thus, a significant practice effect or change in criterion for judgement of threshold had occurred between days. If the effect of some drug was to be examined by contrast sensitivity measurements, it would be necessary to at least randomise the sessions or preferably to establish a series of controls on the same day prior to administration of the drug. De Valois (1977) reported that two highly practised subjects showed a large increase in sensitivity as a result of repeated testing, but this was for almost daily testing over a period of 1.5 years. In contrast to the present work, Kelly & Tomlinson (1987) for a group of 20 subjects, showed that no significant practice effects occurred over a five day training period. They concluded that the technique was suitable for longitudinal studies of visual performance. Throughout the present study, an initial set of

practice measurements was made for each display used at a low, medium and high spatial frequency. These results though analysed were not used. After practice at a high spatial frequency the subject usually became attuned to the method of determining threshold and then a full series of measurements could be taken with confidence.

Part 1: A quantitative investigation of the effects of defocus, pupil diameter and spatial frequency on contrast sensitivity.

Pupil Diameter

In general, the change in pupil diameter from 2mm to 8mm was without significant effect on contrast sensitivity as compared with the natural pupil of ~6mm (Fig. 8). There was a tendency for contrast sensitivity over 3-38 c/deg to be reduced at 2mm compared with 3mm pupil diameters, but the reduction was not significant (Fig. 8A). The only significant reduction in contrast sensitivity observed was at 0.5 and 1.0 c/deg for a 2mm pupil.

It has been established that, when retinal illumination is arranged to be constant, optical quality increases as pupil diameter decreases until a critical pupil diameter is reached below which diffraction degrades image quality. The optimal pupil diameter has been demonstrated by psychophysical measurements of the MTF (Arnulf & Dupuy, 1960; Campbell & Green, 1965; Charman, 1979; Bour, 1980), objective measurements of the line spread function (Campbell & Gubisch, 1966; Charman & Jennings, 1976) and measurements of visual resolution (Campbell & Gregory, 1960; Jenkins, 1963; Tucker & Charman, 1975) to be around 3mm. Thus, in the present study, the theoretical improvement to be gained by reducing the pupil diameter appears to be annulled by the reduction in retinal illumination. Indeed, it has been previously shown

that at low light levels, a small fixed pupil leads to a large loss in visual resolution giving rise to the proposal that the pupil diameter resulting naturally from a given level of illumination is optimal for visual acuity at that illumination (Woodhouse, 1975). The results of the present study are in agreement with the findings of Morrison & McGrath (1985) who showed no difference in contrast sensitivity for young subjects viewing with either their natural pupil (8mm) or through a 3mm artificial pupil and have since been confirmed by Tobimatsu, Celesia & Cone (1988) for natural, constricted and dilated pupils.

In the present work, a significant reduction in contrast sensitivity was found for 0.5 and 1 c/deg gratings when viewed through a 2mm pupil but not a 3mm pupil. This result is unlikely to be due to the reduction in retinal illumination alone for this should have least effect on low spatial frequency gratings (van Meeteren & Vos, 1972). A possible explanation is that increased diffraction effects from a 2mm pupil, in combination with lower retinal illumination, significantly degrades contrast sensitivity at low spatial frequencies. This also leads to the tendency for contrast sensitivity to be reduced for the 2mm pupil compared with the 3mm pupil over the 3-38 c/deg range. The effect becomes significant at low spatial frequencies due, perhaps, to the involvement of other factors. It is known that at low spatial frequencies, subjects scan the grating in order to aid detection of the pattern (Kulikowski & Tolhurst, 1973) as eye-movements increase contrast sensitivity at low spatial frequencies (Kelly, 1977). With the introduction of a small artificial pupil in a fixed position relative to the orbit, this scanning could result in compromising of central fixation. Perhaps more likely, the significant reduction seen at 0.5 and 1 c/deg is related to the number of cycles being close to the minimum limit for normal contrast sensitivity.

Defocus

Defocus had a profound effect on contrast sensitivity, each increment in defocus causing a further decrement in contrast sensitivity. All spatial frequencies were affected by defocus but those below the peak of the CSF less so than those above. Spatial frequencies above the peak were all affected to the same extent, as a downwards parallel shift of the CSF was observed with increasing defocus, i.e. there was no significant change in the slope of the regression of the CSF above the peak. If increasing defocus were having a more detrimental effect on the higher spatial frequencies, the slope of the regression would be expected to increase with increasing defocus.

Campbell & Green (1965) showed an increase in slope with +1.0D defocus, as also shown in a brief study by Regan et al., (1977). Although no significant changes in slope were found in the present study, a fairly consistent finding was that between 0D (optimum focus) and +1.0D, a slight (but insignificant) increase in slope occurred, thereafter the slope remained the same or decreased (Table 3). At optimum focus for high spatial frequencies, the image is situated at the hyperopic point of the depth-of-focus (Kasai et al., 1971). The fact that at large pupil diameters, there is a difference of -0.8 to -0.9D between optimal focus for high and low spatial frequencies (Green & Campbell, 1965), offers a possible explanation for the change of slope caused by +1.0D of defocus. Assuming a -0.8D difference in optimal focus between high and low spatial frequencies, this would mean that the image of a high spatial frequency grating would be situated at the hyperopic point of the depth-of-focus whereas the image of a low spatial frequency grating would be 0.8D in front of this point. By adding a +1.0D lens, the image of the high spatial frequency grating would be shifted by 1D behind the hyperopic point (effectively defocused by 1.0D)

whilst the image of a low spatial frequency grating would be shifted by 1D from its original position (i.e. through the depth of focus range to a point +0.2D behind the hyperopic point) and therefore only effectively defocused by 0.2D. For further increases in defocus (i.e. for +2.0D and +4.0D) both low and high spatial frequencies would then be defocused by the same amount and hence the slope of the CSF would remain constant. Although a difference in optimal focus was not seen when viewing through a 2mm pupil (Green & Campbell, 1965), which was attributed to a reduction in spherical aberration, some effect may possibly be present at the 3mm pupil used in this study. Indeed, pupils of 5.4 and 3.8mm diameters also show a spatial frequency dependence of optimal focus (Charman & Heron, 1979). Perhaps if the effects of defocus on contrast sensitivity had been investigated for larger pupil diameters in the present study, +1.0D may have caused a significant increase in slope.

The conclusion of Campbell & Green (1965) that higher spatial frequencies were disproportionately affected compared with lower spatial frequencies was based largely on contrast thresholds of one subject, viewing through a 2mm artificial pupil, at spatial frequencies of 1.5, 9, 22 and 30 c/deg (Fig. 6 in their original paper). However, when they examined contrast sensitivity over a wider range of spatial frequencies for 0, +0.5, +1 and +2.0 dioptres of defocus (Fig. 7 in their paper) contrast sensitivity was seen to decrease at all spatial frequencies from peak upwards, with some apparent tailing-off at the very highest spatial frequencies. However, calculation of the linear regression of the CSF greater than peak for each amount of defocus, revealed no increase in slope with increasing defocus ($P>0.25$). With a 7mm pupil, a parallel downward shift in contrast sensitivity occurred (Green & Campbell, 1965) in agreement with the present work. Charman (1979) also showed a basically parallel fall in contrast sensitivity over 5-30 c/deg

for +0.25, +0.5 and +1.0D of defocus measured for a range of pupil diameters from 1 to 6mm. Marmor & Gawande (1988) also found a parallel loss of contrast sensitivity over 3 to 18 c/deg for +0.5, +1.0 and +1.7D of defocus.

From theoretical predictions of the effect of defocus on the MTF of the human eye, considered as a diffraction limited optical system (Hopkins, 1955), one would expect a steep increase in the effect of defocus with increasing spatial frequency and with increasing defocus (as shown in Fig. 2); this was not seen in this study. Also "zero minima" would have been expected where the grating would appear to reverse in contrast, but were not found in this study, nor in the work of Campbell & Green (1965). Charman (1979) did find some modulation but not always at the predicted spatial frequency (i.e. for +1.0D of defocus minima occur at 6.6 and 10 c/deg in the diffraction-limited eye, Smith, 1982). Thus, the refractive media of the eye perform in a way that cannot be predicted by the characteristics of a simple diffraction-limited optical system.

In the present study, contrast sensitivity to low spatial frequencies below the peak of the CSF was also degraded by defocus though to a lesser amount than spatial frequencies above the peak. The decrease in contrast sensitivity for these low spatial frequencies was, however, only statistically significant when viewing through a 3mm pupil, not a 2mm pupil. The difference between the two conditions is most probably due to the greater depth-of-focus available with a 2mm pupil. Also a reduction in retinal illumination itself is also known to increase the depth-of-focus (Campbell, 1957).

Campbell & Green (1965) had concluded that low spatial frequencies were not degraded by defocus, since the results for one subject, viewing a 1.5 c/deg grating through a 2mm pupil were not affected by increasing

positive or negative lenses (Fig. 6 in their original paper). However, if one examines the more complete CSF (their Fig.7) there does seem to be some decline in contrast sensitivity, with defocus, for spatial frequencies less than peak. With a 7mm artificial pupil, low spatial frequencies were markedly reduced (Green & Campbell, 1965). Also, in Charman's work (1979), contrast sensitivity at 1, 2 and 5 c/deg was reduced for a range of pupil diameters between 1 and 6mm.

In the repeat experiments of this study, which entailed viewing with the natural eye, several subjects noticed that their accommodation was fluctuating, presumably as their accommodative mechanism sought to bring the blurred image into focus. Regardless of this, viewing with the natural eye produced very similar results to the original study for the homatropinised eye. One exception was that +1.0D of defocus did not significantly reduce contrast sensitivity as it had done previously for the homatropinised eye. For 6 of the 8 subjects, a reduction in contrast sensitivity was found (though this was not significant) with +1.0D compared to optimal focus, but for 2 subjects contrast sensitivity was indistinguishable on the two occasions. Differences between individuals of this nature had been described by Reese & Fry (1941) who showed that not all individuals respond to defocusing lenses in the same manner. Some observers relax their accommodation when viewing through a defocusing lens (as for the 2 subjects above) whereas many observers' accommodation remains unchanged. Some others will, in fact, increase their level of accommodation in response to the external lens. Only a small relaxation of accommodation would be necessary to compensate for the external lens (0.25D for viewing from 3m and 0.75D for viewing from 1m) because there is already a considerable amount of depth-of-focus (0.6D for a 3 mm pupil, Campbell, 1957) in addition to a tolerance of defocus blur of some 0.2-0.25D (Whiteside, 1957; Campbell & Westheimer,

1958). This emphasises the fact that even a very small amount of defocus degrades the CSF, for the same depth-of-focus and tolerance of blur would also be present for the homatropinised eye with a 3 mm pupil, where +1.0D did significantly degrade the CSF. Indeed, this is as predicted from optical theory, for Hopkins (1955) calculated that the optical transfer function would be appreciably reduced for only 0.125D of defocus (which corresponds to n=1 in Fig. 2) for a 3mm pupil viewed in green light.

The reproducibility of the contrast sensitivity measurements made in these long experiments was very good; repeat measurements at optimum focus made at the end of the session were not significantly different from those made at the start.

Quantitative analysis

To predict contrast sensitivity at the CRT luminance used in the present study, the best-fitting multiple regression equations were derived to incorporate terms for pupil diameter, defocus and spatial frequency. Pupil diameter was found to have only a small effect on contrast sensitivity, whereas defocus had a profound effect particularly over 3 - 38 c/deg where it reduced contrast sensitivity by 51% and 45% per dioptre in the homatropinised and natural eyes, respectively. At 0.5 - 3 c/deg, the reduction was only 19% and 11% per dioptre respectively. The relative insensitivity of low spatial frequencies will mean that the visual system will at least be able to rely on this information when defocus has reduced fine detail recognition. Thus, object detection should be relatively unimpaired as this has been correlated with the peak of the CSF (Ginsburg et al. 1982). Stereopsis should also be spared, for fusion of the two images can occur in the presence of low spatial frequency information only (Julesz, 1971).

Stereoacuity, however, requires the full range of spatial frequencies to be present and has been shown to be degraded by defocus to a greater extent than visual acuity itself (Westheimer & McKee, 1980). Also, in the absence of high spatial frequency information, the accommodative mechanism will not be able to attain a sharp focus (Charman & Heron, 1979).

To summarise the findings of this part of the work, pupil diameter had such a small effect on contrast sensitivity that it can effectively be ignored. Therefore, in the mesopic range of illumination, for defocus of up to 4 dioptres, contrast sensitivity will be halved for each dioptre of defocus, for all but the very lowest of spatial frequencies.

Part 2: The effects of a single oral dose of pyridostigmine bromide on contrast sensitivity.

The principal finding of this study was that a 60mg oral dose of pyridostigmine bromide was without significant effect on the CSF measured for either the CRT or laser display. Borland et al., (1985) stated without elaboration that a 30mg dose of pyridostigmine, repeated at 8 hourly intervals for 3 days, had no effect on contrast sensitivity at several spatial frequencies between 0.5 and 25 c/deg. In the present study, a small (7%) but significant improvement of the mean contrast sensitivity, for the CRT display was seen over 3 - 40 c/deg after pyridostigmine. The mean contrast sensitivity for the laser display, which gives an entirely neural assessment of visual function, over the same range showed no such improvement. Below the peak of the CSF, no significant difference in contrast sensitivity was seen for either display. Thus, the small improvement in visual performance for

externally viewed grating patterns in the absence of an effect on neural sensitivity may be attributed to an increase in ocular quality which was presumably due to the small reduction in pupil diameter (Fig.16 A). However, the actual change in pupil diameter was very small. It was interesting that a larger difference in pupil diameter was present between the first and second tests (Fig. 16 B). It may be that the effect of the sympathetic nervous system on the first visit, perhaps due to the stress of performing tests in a strange laboratory environment or of taking an unknown tablet (anticipating possible ill-effects), were having a greater effect on pupil size than the actions of the anticholinesterase. Borland et al., (1985) noted that pyridostigmine caused an increase in arousal. Therefore, it may even have been that any decrease in pupil diameter, expected from direct action of an anticholinesterase agent on the sphincter pupillae, was counteracted by an increase in pupil diameter associated with increased central arousal (Hess & Polt, 1964) due to a central action of the drug. However, pyridostigmine is a qua ternary compound and should not readily cross the blood brain barrier. This is supported by the constancy of contrast sensitivity to laser interference fringes in the present study.

There is evidence, from electrophysiological studies in the cat, that pyridostigmine alters visual cortical cell activity; approximately half of the cells studied were excited and half inhibited by pyridostigmine (DeBruyn & Bonds, 1987). Furthermore, physostigmine depressed visual cortical function, measured by the VER, to moving grating patterns of low spatial frequencies (0.1 - 1 c/deg), whereas high spatial frequencies were unaffected (Harding et al., 1983). Since contrast sensitivity in the cat is shifted down ten-fold with respect to human vision (Campbell, Maffei & Piccolino, 1973), these spatial frequencies may be analagous to spatial frequencies of 1 - 10 c/deg in

man. Interestingly, in part 4 of the present study, atropine sulphate (2mg, i.m.) was found to have a deleterious effect specifically on movement sensitivity at low spatial frequencies (1 - 5 c/deg). This being so, for completeness it would be desirable to undertake further experiments to assess the effect of pyridostigmine on visual performance to moving stimuli.

In any future experiments, the double blind protocol would not be used, for although one can depend on good reproducibility of contrast sensitivity measurements on any one day, this is not necessarily so between days. Therefore, both tests should be performed on the same day with control followed by test (in case of persistence if performed the other way round), though subjects must not know this.

To conclude, the present study demonstrates that a 60 mg dose of pyridostigmine does not significantly affect contrast sensitivity for stationary grating patterns. This suggests that this dose may be used as a pre-treatment against organophosphorus anticholinesterases without fear of impairment of stationary visual function, though a possible effect on movement function remains to be resolved.

Part 3: The effects of topical application of 0.25% physostigmine sulphate on visual performance.

In accordance with the established literature, physostigmine sulphate eyedrops caused a marked and long lasting pupillary constriction and an increase in the amplitude of accommodation. The extent of pupil constriction was greater than that caused by a single drop of 0.25% physostigmine salicylate (Ogle, Whisnant & Hazelrig, 1966), but of a similar time course. Systemic absorption by way of the conjunctival vessels or the nasal mucosa was unlikely in the present

study, as there was no effect on the contralateral eye. Aside from the predominant muscarinic effects on the smooth muscle of the sphincter pupillae and the ciliary body, some physostigmine must also have passed through the palpebral conjunctiva and prolonged the action of acetylcholine at nicotinic receptors of the orbicularis oculi palpebral muscle. This caused involuntary twitches of the eyelids.

An interesting finding of the present study was that while all subjects showed a similar degree of miosis there was wide variation in the amplitude of the increase in involuntary accommodation, ranging from practically zero to +10 dioptres (Table 6). Several factors were explored to try to account for this variation.

It is well known clinically that dark irises dilate very slowly with mydriatic agents. It is believed that darkly pigmented irises show a prolonged drug absorption phase (Shell, 1982), due to drug binding to melanins, the drug is then released slowly from this store prolonging the duration of the response (Nagataki & Mishima, 1980; Patil, 1984). However, the variation in the degree of defocus found in the present study was not related to any particular eye colour.

A significant amount of topically applied drugs is lost via drainage through the lachrymal apparatus, the rate of drainage increasing as the volume of instilled drug increases (Chrai et al., 1973). Similarly, if excess tears were formed on administration of the eyedrops, increased washout of the drug solution may have occurred. However, in the present study, there appeared to be no relation between the amount of tear formation and the extent of the defocus.

Wide variation in the extent of defocus after physostigmine, up to a maximum of +7.5D, was also found by Rengstorff (1970). He found the visual task which he set his subjects, immediately following instillation of the eyedrops, influenced the amount of defocus

experienced. Those subjects who performed near tasks produced significantly larger increases in accommodation than those subjects performing far visual tasks, presumably because more acetylcholine accumulates at the endplates of an active muscle. However, the visual tasks performed immediately after instillation of the eyedrops can not account for the variation in the amplitude of defocus in this present work. All subjects followed the same sequence of tests involving both near and far visual tasks.

The possibility that some genetic factor may be involved in the degree of defocus caused by physostigmine was also investigated in the present study. Two groups of siblings were tested. One family all showed large increases in accommodation and the other showed only moderate or small increases in accommodation. Both families showed the same miosis (Table 7). These results are consistent with a genetic trait affecting the response of the ciliary body, but further experiments would, of course, be necessary to establish this point.

In the present study, the effect of physostigmine on visual performance was initially assessed by a Snellen test which is the only assessment that has in the past been made (Mattila et al., 1967; Rengstorff, 1970). A more extensive assessment of visual function was then made by measuring contrast sensitivity.

Contrast sensitivity to oscilloscope-generated stationary grating patterns declined transiently in response to physostigmine with a time-course similar to that of the increase in accommodation. Since the miosis did not recover during the experimental period, it seems that only defocus of the image contributes substantially to the decline in contrast sensitivity, which is consistent with the findings of part 1 of this study. Indeed, at the peak of the response, the decline in contrast sensitivity was proportional to the degree of defocus

($P < 0.01$) as shown in Fig. 25. At this time, contrast sensitivity to each spatial frequency measured (3, 10, 20 and 30 c/deg) was similarly reduced. However, contrast sensitivity at 3 c/deg did not fully recover within the time period of the experiment (Fig. 23).

Contrast sensitivity to oscilloscope-generated moving patterns also declined, spatial frequencies of 2 and 3 c/deg were more severely affected than those of 1 and 0.5 c/deg, as would be predicted from the present study with defocusing lenses. The decline in contrast sensitivity to these moving gratings was also proportional to the degree of defocus ($P < 0.001$ at 1, 2 and 3 c/deg gratings) as shown in Fig. 28. A point of particular interest was that for each of these low spatial frequency moving gratings, as well as for the 3 c/deg stationary grating, full recovery of contrast sensitivity was not seen during the experiment. Contrast sensitivity for the higher spatial frequencies, however, recovered to control levels by 90 - 120 min post-administration. Since diffraction effects, arising from the small pupil would be present for all spatial frequencies and the reduced retinal illumination should, if anything, have less effect at low spatial frequencies (van Meeteren & Vos, 1972), this may represent some prolonged neural effect of physostigmine on the low spatial frequency channels. This may also be related to the present finding of a reduction in movement sensitivity following atropine administration.

Contrast sensitivity measurements made for laser interference fringes also showed a transient reduction following physostigmine. Again, since the miosis outlasted the fall in contrast sensitivity, the reduction in retinal illumination could not account for this change. Although the interference fringes themselves, which were formed by coherent light and observed in the Maxwellian view, would not be defocused by an increase in accommodation, the aperture surrounding the

fringes would be defocused. This would reduce the luminance gradient between the interference fringes and the surround and produce a blur circle around the edge of the display which may alter the phase of truncation of the waveform, both of which can influence the contrast threshold (Estevez & Cavonius, 1976; Kelly, 1970). However, a defocus of +7.0 D due to myopia, which would cause a similar defocus of the aperture, did not significantly change contrast sensitivity to the interference fringes (Fig. 31). Thus, defocus of the aperture seems not to be the cause of the decline in contrast sensitivity to the laser display. Since the control eye showed no miosis, systemic absorption of physostigmine which may have led to an effect on the central nervous system seems improbable. This suggests that there may be a direct effect from trans-corneal absorption of physostigmine on the retinal neurones. Further evidence to support a retinal/central effect of physostigmine was the transient reduction in mean critical fusion frequency measured with a constant entrance pupil, as also shown by Alpern & Jampel (1959). The transient time course of both these effects, while mirroring the time course of the defocus response, may, instead, represent the time course of the presence of physostigmine at the retina.

Large contractions of the ciliary muscle, however, are known to cause forward movement of the choroid and retina. Enoch (1973) has calculated that +10D accommodation results in a 2.5% increase in retinal area. This is not confined to the anterior margins of the retina as the central retina also stretches during marked accommodation (Hollins, 1974). Blank & Enoch (1973), found that large increases in accommodation induced significant distortions of horizontal spatial perception which may have altered the spatial frequency of the interference fringes in the present study. However, subject 12, who

showed very little increase in accommodation following physostigmine, also showed a small decline in laser contrast sensitivity, but only for the 25 c/deg pattern (Fig. 30). This again suggests that the decline in laser contrast sensitivity is not related to the ciliary muscle contraction but a direct effect of physostigmine on the retina.

Physostigmine may also have some general vascular effect on the retinal neurones which affects visual function, since contraction of the ciliary muscle also contracts the ciliary arteries, but, at the same time, outflow from the vorticose veins is facilitated. This leads to a reduced volume of blood in the eye and hence, a reduced intra-ocular pressure (O'Connor Davies, 1981).

The cholinergic cells in the retina have been identified as a population of amacrine cells which, in the monkey retina have been shown to contact other amacrine cells, bipolar cells and ganglion cells (Mariani & Hersch, 1988). Both nicotinic and muscarinic receptors have been demonstrated in the inner plexiform layer. The consensus of opinion seems to be that nicotinic receptors are found on ganglion cells in rabbit, chick and rat retinae (Masland & Ames, 1976; Morgan & Mundy, 1984; Redburn et al., 1984 respectively) and on "transient" ganglion cells in the cat (Ikeda & Sheardown, 1982), whereas muscarinic receptors are found on amacrine cells (Morgan & Mundy, 1984; Redburn et al., 1984). However, Schmidt et al., (1987) have suggested that both "sustained" and "transient" ganglion cells in the cat possess muscarinic receptors. The inhibitory effect of physostigmine on the retinal contrast sensitivity found in the present study is consistent with the enhancement of the effect of acetylcholine on muscarinic receptors of amacrine cells which are inhibitory to the retinal ganglion cells. This action must be stronger than any potentiation of cholinergic transmission to the nicotinic receptors on the "transient" ganglion

cells which subserve movement detection since the effect of physostigmine on the low spatial frequency grating patterns which will be detected by the movement-dependent channels (Tolhurst, 1973) showed a sustained loss of contrast sensitivity which was not apparent at higher spatial frequencies.

The main findings of this part of the work were: (1) that physostigmine caused a transient reduction in contrast sensitivity to externally viewed grating patterns which was correlated with the increase in the amplitude of accommodation, (2) physostigmine had a direct effect, of transient time course, on contrast sensitivity to laser interference fringes particularly at higher spatial frequencies which was not affected by defocus of the image, and (3) physostigmine caused a prolonged reduction in contrast sensitivity to low spatial frequency grating patterns indicating an additional action on the movement-dependent channels of the visual system.

Part 4: The effect of an intramuscular dose of 2mg atropine sulphate on visual performance.

In the present study, a 2 mg i.m. injection of atropine sulphate produced the well known effects of a general antagonism of muscarinic receptors throughout the body (Martindale, 1982). The overall degree of discomfort expressed by each subject was quite variable, although all three subjects who completed both parts of the study experienced less discomfort (in particular, dryness of the mouth and throat) on the second day, as was also noted by Cullumbine et al., (1955). Atropine caused a transient bradycardia during the first five minutes after the injection which has been previously reported following low doses (0.5-2.4 mg) of atropine i.m. (Cullumbine et al., 1955; Chamberlain, Turner &

Sneddon, 1967; Kalser & McLain, 1970; Holland, Parkes & White, 1975). It is believed to be due to either a slight central stimulant action of atropine on the nucleus ambiguus - the source of cardiac vagal fibres, or to an agonist action of atropine on muscarinic receptors of cardiac muscle, since low doses of antagonists can act as partial agonists, before the antagonist action develops (Bowman & Rand, 1980). As the bradycardia was not found at higher i.m. doses of atropine (Mirakhur, 1978) nor for a 1 mg i.v. dose (Cullumbine et al., 1955), the second explanation may be the more probable. The more pronounced effect on heart rate was a tachycardia which then rapidly developed, due to inhibition of the vagal inputs to the heart. Resting heart rate increased by approximately 50% at the peak of the response (30 min - 1 hour post-injection) and then declined gradually to normal by 4 - 5 hours post-injection.

Atropine's effects on the ocular smooth muscle developed more slowly and were more prolonged than its effect on heart rate. Differences in the initial time course of the action of atropine are due to differences in regional blood flow and the rate of diffusion of the drug into the various tissues (Mayer, Melmon & Gilman, 1980). The prolonged duration of the effect on the ocular smooth muscle is related to binding of the drug to proteins in the muscle (notably to pigments in the iris). In the present study, a maximum pupil dilation of 1.2 - 1.5mm was seen between 2 - 2.5 hours post-injection and the pupil remained significantly dilated for up to 4 hours, as also found by Herxheimer (1958) and Rozsival & Ciganek (1978). The reduction in the amplitude of accommodation was sustained in the present study for 6 hours post-injection, as previously found by Mirakhur (1978). A small amount of this reduction may be due to the decreased depth-of-focus available with a dilated pupil but as only 0.6 D is available with a 3mm pupil, its

contribution must be minimal (Campbell, 1957). The loss of ability to focus on near objects was large enough that most subjects noted difficulty in reading, even though they still had a reserve accommodation of around 8 D. Near visual acuity was not measured in this study but Haegerstrom-Portnoy et al., (1987), reported deficits of about one and a half lines on a Bailey near acuity chart.

The effect of atropine on the extraocular muscles which caused a change in horizontal heterophoria and cyclophoria was rather short lasting. It seemed to coincide with the change in heart rate, which reflects plasma concentration of atropine (Kalser & McLain, 1970), rather than with the changes in pupil diameter and accommodation. This may be due to a non-specific membrane effect at the motor end plate (Wray, 1980). However, there is evidence for muscarinic receptors which are blocked by atropine in the superior oblique muscle of cat (Sanghvi & Smith, 1969). The present change in muscle balance is, therefore, consistent with the presence of muscarinic receptors on human extraocular muscle fibres.

In general, atropine did not affect the performance of any stationary visual tasks in the present study. Atropine did not significantly affect reaction time or choice reaction time to a visual stimulus, followed for 6 hours post-injection, despite subjective complaints of lightheadedness and an inability to concentrate from many subjects. Red-green colour balance was unchanged by atropine as also reported by Baker et al., (1983). Stereoacuity, the ability to discriminate between features at different distances in space, was a particularly demanding task for the subject which, if anything, should reflect any cerebral effects of the drug. However, stereoacuity was unaffected by atropine in the present study. Since stereoacuity has been shown to be more markedly affected than visual acuity by a given amount

of defocus (Westheimer & McKee, 1980), it was not surprising therefore, that visual acuity was also unaffected by atropine. Similarly, Rozsival & Ciganek (1978) reported no change in distant vision, Baker et al., (1983), found no change in acuity to a Landolt C chart and Haegerstrom-Portnoy et al., (1987) found no change in distant acuity to either high or low contrast test charts.

In case, by measuring only visual acuity, we were perhaps missing some more subtle effects of atropine on spatial vision, a full examination of its effect on the CSF was performed. However, atropine had no significant effect on contrast sensitivity to stationary grating patterns of spatial frequencies 1 - 30 c/deg, generated on a CRT. This is in accord with the absence of an effect of pupil size on contrast sensitivity and with Baker et al., (1983) who also found contrast sensitivity to be mainly unaffected by atropine. Also, Haegerstrom-Portnoy et al., (1987) found no change in sensitivity to contrast differences on a "Border contrast sensitivity" test following 2 and 4 mg atropine. Contrast sensitivity to stationary laser interference fringes was similarly unaffected by atropine confirming an absence of any neural effect on stationary visual function.

An important finding of this study was that an i.m. injection of atropine sulphate had an adverse effect on contrast sensitivity to low spatial frequency moving grating patterns. Detection of moving gratings of 1, 2 and 3 c/deg was significantly impaired by some 25 %, with a smaller reduction at 5 c/deg. The time course of the adverse effect on contrast sensitivity to these grating patterns was sustained, spanning the whole of the experimental period (6 hours). Since contrast sensitivity to stationary grating patterns of the same spatial frequencies was unaffected by atropine, this must reflect a specific effect on movement sensitivity. An adverse effect on movement

sensitivity is consistent with the recently reported impairment of visual tracking of a moving stimulus (Penetar & Beatrice, 1986; Haegerstrom-Portnoy et al., 1987) after atropine. In both studies, the subject aligned the cross-hairs of an eyepiece on the centre of a moving target and tracking scores were computed. A 4 mg dose produced a significant increase in tracking errors under dim and bright lighting conditions, but a 2 mg dose only reduced performance under dim illumination. Topical application of hyoscine has also been shown to selectively depress contrast sensitivity, by 30 - 50%, to low spatial frequency grating patterns (Morrison & Reilly, 1989).

Cholinergic inputs are present throughout the visual system, in the LGN and visual cortex, but are often not involved in the transmission of visual information (Phillis et al., 1967). The cholinergic input to both the LGN and visual cortex seems to originate from neurones of the reticular formation (Singer, 1979). Ionophoresis of acetylcholine has a predominantly excitatory action on neurones of the dLGN (Sillito et al., 1983; Crunelli et al., 1988) but some cells may also be inhibited (McCormick & Prince, 1987). Little difference between "Y" and "X" dLGN cells' response to acetylcholine has been reported except for one study where a specific enhancement of a peripheral response of "Y" cells was seen (Eysel et al., 1987). Cortical cells are also influenced by ionophoresis of acetylcholine, an effect which can be antagonised by atropine (Sillito & Kemp, 1983). Firing of cells in layer 4 of the cortex was depressed by acetylcholine and their dominance of the VER would explain its depression at low spatial frequencies after physostigmine (Harding et al., 1983) mentioned earlier. Also, in man, i.m. injections of low doses of the muscarinic antagonist hyoscine (0.25 - 0.75 mg) caused some amplitude and latency changes to flash and pattern (2.4 c/deg reversing checkerboard) VERs, while simultaneously

recorded electroretinograms were unaffected, suggesting that the effect was central and not retinal (Sannita et al., 1988).

However, the specific effect of atropine on movement sensitivity found in the present study may reflect an effect on the cholinergic cells in the retina as they are known to be particularly responsive to flickering or moving stimuli (Masland & Livingstone, 1976; Ariel & Daw, 1982b). In the previous section, it was argued that physostigmine enhanced the efficacy of amacrine cells which were surmised to have cholinergic inputs and released inhibitory transmitters onto the ganglion cell. This effect outweighed any potentiation of the direct excitatory cholinergic inputs onto the ganglion cell. It is at the latter synapse which the action of atropine is now surmised to have its dominant effect in selectively depressing movement sensitive ganglion cells. The present results are partially in agreement with the results of Ikeda & Sheardown (1982) who described in cat retina, excitatory cholinergic inputs to transient or "Y" ganglion cells, but they found dH β E antagonised the effect while atropine was described as having a non-specific depressant effect, when applied in high concentration. Thus, they suggested that "Y" cells possessed nicotinic rather than muscarinic receptors. The present results are also in partial agreement with the work of Schmidt et al., (1987). These authors found that acetylcholine was excitatory to both "X" and "Y" cells and that the response was antagonised by hyoscine, whereas dH β E blocked the effect of acetylcholine only on OFF-cells, suggesting the presence of muscarinic receptors on the ganglion cells, but on both "X" and "Y" types. Given that the central actions of hyoscine are generally recognised to be more potent than those of atropine in man (Herxheimer, 1958; Mirakhur, 1978), it may be that atropine especially in low dosage may discriminate between the ganglion cells underlying the movement-

dependent ("Y"-like) and movement-independent ("X"-like) mechanisms which have also been described in man (Tolhurst, 1973), whereas hyoscine is more non-selective.

In conclusion, stationary pattern functions viz. contrast sensitivity to stationary grating patterns, visual acuity, stereoacuity and colour perception are unaffected by atropine administration. However, contrast sensitivity to low spatial frequency moving gratings is adversely affected by atropine indicating an impairment of movement detection.

Part 5: The effectiveness of prior administration of an intramuscular injection of atropine sulphate or instillation of homatropine eyedrops against physostigmine eyedrops.

Intramuscular injection of 2 mg atropine sulphate was ineffective in antagonising the effects of physostigmine eyedrops on vision whether given 8 min or 124 min previously. Whereas, prior instillation of 2% homatropine hydrobromide eyedrops effectively blocked the marked miosis, the increase in near point accommodation, the increase in amplitude of accommodation for distance and the accompanying decrease in contrast sensitivity, induced by physostigmine. Thus, neither peak plasma concentration of atropine which occurs at ~ 30 min after injection (Berghem et al., 1980) nor atropine bound to muscarinic receptors of the ocular smooth muscle, which was maximal at 124 min, was sufficient to antagonise the effects of physostigmine eyedrops.

Atropine, during the 124 min period prior to physostigmine administration, significantly reduced contrast sensitivity to the 3 c/deg phase-reversed grating pattern, but not to the higher spatial frequency stationary gratings (Table 14). Furthermore, the loss of

contrast sensitivity for the 3 c/deg phase-reversed grating was, if anything, greater after both atropine pre-treatments although the difference was not significant (Fig. 48). This therefore confirms the result of part 4 of the present study that a 2 mg i.m. injection of atropine sulphate *per se* has a deleterious effect on contrast sensitivity to low spatial frequency channels which are sensitive to movement. In addition, contrast sensitivity to a 3 c/deg phase-reversed grating pattern had returned to only 70 % of the control level by the end of the experiment, indicating a prolonged inhibition of the low spatial frequency channels by physostigmine. This confirms the result of part 3 of this study that physostigmine has an additional prolonged action, presumably at the retina, on the movement-dependent mechanism of the human visual system.

Atropine sulphate i.m. has been reported to prevent the proposed central elevation of the absolute scotopic threshold induced by exposure to Sarin vapour (Rubin & Goldberg, 1958). This is interesting because the only variable which atropine sulphate antagonised in the present study was the physostigmine-induced reduction in critical fusion frequency at 30 min post-administration (Fig 43), which was also thought to be a central effect.

Thus, it seems that the rate of delivery of atropine from an i.m. injection is insufficient to compete against the ocular effects of locally applied physostigmine. Although some of the muscarinic receptors of the ocular smooth muscle are occupied by atropine (shown by the dilatation of the control eye) this is overcome by the large quantities of acetylcholine which accumulate due to the inactivation of AChE by physostigmine. Ocular instillation of homatropine, however, occupies sufficient numbers of muscarinic receptors to render any build-up of acetylcholine ineffective.

Wood (1950) and Grob & Harvey (1953) briefly stated without elaboration that topical administration of 2% homatropine hydrobromide eyedrops was sufficient to relieve all the ocular symptoms in mild organophosphorus poisoning, but that the usual systemic dose of atropine was ineffective. In more severe cases, the administration of 0.5 or 1% atropine solution directly to the eye was necessary. Scholz & Wallen (1946) showed 5% homatropine was ineffective in reducing the miosis or the increase in accommodation for distance viewing, induced by DFP vapour. 0.25% atropine relieved the increased accommodation and returned distance vision to normal within 2.5 days, compared with an average of 5.3 days for the control eye. 1% atropine returned distant vision to normal in 3 hours. 5% homatropine only slightly relieved the intense pain experienced when focusing on close objects, while both 0.25% and 1.0% atropine gave complete relief in the treated eyes in 3 hours. Thus, the antagonism produced by the three solutions is clearly concentration dependent which is in agreement with the present work.

It is difficult to relate the results obtained in this present study with physostigmine to the effects of the generally more potent organophosphorus anticholinesterases. Although these agents are known to cause a more marked and much longer lasting pupil constriction, the amplitude of any increase in accommodation as a result of exposure has yet to be firmly established. Certainly, blurred vision was one of the first symptoms ever noted by workers involved in the initial synthesis of dimethyl and diethylphosphorofluoridate in the 1930s in Germany (Holmstedt, 1963). Increased accommodation of between 1 and 6 D was reported after exposure to DFP, which came on within 3 hours of exposure and lasted from 2 to 7 days (Scholz & Wallen, 1946) and an increase in accommodation was also reported after exposure to TEPP (Upholt et al., 1956). In contrast, other studies have reported little increase in

accommodation after exposure to DFP (Aldridge et al., 1947) or Sarin (Rengstorff, 1985) despite a marked miosis. The lack of effect of organophosphorus anticholinesterases on accommodation may be explained if their binding potency was such that considerable binding to the iris occurs without substantial amounts reaching the ciliary muscles. However, Rubin et al., (1957) noted that Sarin applied direct to the eye was ineffective in raising the threshold of dark adaptation which was caused by the vapour. This implies that the cornea may constitute a barrier to absorption of appreciable amounts of Sarin. Hence, those amounts which do penetrate are sufficient to cause miosis but not accommodative spasm. Finally, since relatively few subjects have been studied, the possible genetic disposition may be of relevance as suggested for physostigmine in the present study. Nevertheless, both Wood (1950) and Grob & Harvey (1953) state, without giving any detail, that exposure to traces of organophosphorus vapour causes constriction of the pupils within minutes, and slightly greater exposure induces ciliary muscle spasm. If the dose is sufficient to cause ciliary muscle spasm, it seems that a great deal of pain is felt on focusing and a headache and drawing sensation is felt behind and around the eyes. These symptoms were also experienced by subjects who were severely defocused by some 8 - 10 D following physostigmine eyedrops in the present study.

In conclusion, if visual function were the sole consideration, the standard i.m. injection of 2 mg atropine sulphate would seem ineffective as a pre-treatment against anticholinesterase poisoning. This is due to delivery of insufficient atropine to counter the anticholinesterase in the ocular tissues. Atropine may also actually worsen the deleterious effect of the anticholinesterase on movement perception. Also, if the atropine was injected prematurely in what turned out to be a false alarm

it would, in itself, have a deleterious effect on movement perception at low spatial frequencies and make close work requiring accommodation difficult. Homatropine eyedrops, given alone, have no deleterious effect on contrast sensitivity to stationary grating patterns, measured for a wide range of spatial frequencies (in part 1 of this study). Also, since contrast sensitivity to a 3 c/deg phase-reversed grating pattern was unaffected by homatropine eyedrops (Fig. 56), it would seem that, unlike i.m. atropine, homatropine eyedrops do not have a deleterious effect on movement perception at low spatial frequencies. Thus, a pre-treatment with homatropine eyedrops, prior to any exposure to anticholinesterase agents, would be far more effective, as far as the visual system is concerned.

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