3 MATERIALS & METHODS

3.1 Viewing conditions during tests

All of the investigations in the present study were carried out under scotopic conditions. A dark adaptation of 30 min was required prior to the examination.

To avoid possible light adaptation due to flash exposure and presentation of a grating, different intervals were used between flashes and between gratings. In measurement of RAT, an interval of 3 min was adopted between two flash exposures (I, II, VI, VII). This was selected on the basis of preliminary studies indicating that the visual adaptation before the consecutive stimulation was not influenced by a prior stimulation.

In studies of OKN, an interval of 30 sec between two presentations of the grating was adopted (III, IV, V). The interval between grating presentations in measurements of the perceptual threshold was 20 sec due to the short presentation of the grating (III). These intervals were selected on the basis of pilot studies showing that the visual adaptation after the interval is equivalent to that before the grating presentation.

The interval between ERG recordings was 30 sec to avoid depression of the subsequent responses (II).

3.2 Flash stimulus

The flash stimulus was a xenon flash tube with a Metz 60 CT Photoflash reflector mounted in a light-tight box and fixed at the right upper corner of a Goldmann perimeter hemisphere. The light was directed to the roof of the hemisphere, which scattered light over the whole field (Lambertian view).

3.2.1 Temporal distribution of flash power

![Graph of flash power distribution](image)

Fig. 1
A typical tracing of the stimulus for flash blindness.
Half width = 0.75 ms.
The temporal distribution of the flash for visual adaptation (I, II, VI, VII) is shown in Fig. 1. The duration of the flash at 50% peak exposure was about 0.75 ms. The duration of the flash for the ERG recording was 0.01 ms (II).

### 3.2.2 Wavelength distribution of flash

Narrow band filters (Balzers, Liechtenstein) with half band widths ranging from 8 to 12 nm were used to obtain coloured flashes (I, II, VI, VII).

A total of 11 filters (449 nm, 456 nm, 477 nm, 498 nm, 502 nm, 520 nm, 565 nm, 580 nm, 591 nm, 666 nm, and 703 nm) were used to investigate wavelength dependence of RAT (I). A flash with a wavelength centred around 502 nm was adopted in part of the investigations of RAT (VI, VII) since this is the most optimal wavelength for RAT (I). The two wavelengths, 622 nm and 536 nm, were adopted for the flash (II) to elucidate the contribution of the receptor component in RAT. These wavelengths were selected on the basis of the concept that green is the most sensitive colour and red is the least sensitive colour for RAT.

### 3.2.3 Flash dose

The dose of the flash (J/m²) was measured in the plane of the subject's cornea towards the center of the hemisphere with a radiometer (United Detector Technology, USA, Opto-meter, model 40x). The radiometer was calibrated to US NIST traceable standard.

The flash dose was selected on the basis of the exposure that resulted in an identifiable RAT and was kept as low as possible to eliminate any damage to the eyes. All doses used in the present study were much lower than the permissible exposure limits (Sliney & Wolbarsht, 1981).

The radiant exposure on the retina (photons/m²) was calibrated according to the following model (I).

\[
N = \frac{E \times \lambda \times T}{hc}
\]  

Eq. 1

Here, \(N\) is the calculated radiant exposure hitting the retina (photons/m²), \(E\) is the flash dose (J/m²) measured in the plane of the subject's cornea with the detector directed toward the centre of the hemisphere, \(\lambda\) is the wavelength of the flash (10⁻⁹ cm), \(T\) is the transmittance of the ocular media for the corresponding wavelength (Geeraets et al., 1960; Geeraets & Berry, 1968), \(h\) is the Planck constant, 6.626 x10⁻³⁴J·s, and \(c\) is the velocity of light, 2.998 x10⁸ m/s in vacuum, (Hodgman, 1956).
The decrease in the transparency of the ocular media in human eyes as age increases (Lerman et al., 1976; Lerman & Borkman, 1976), which particularly influences the light transmission at short wavelengths, was not taken into consideration in the calculation of the flash dose. This may cause an overestimation of the flash dose hitting the retina.

The distribution of the flash in the hemisphere was examined. The exposure dose was measured at different visual angles in the horizontal meridian (Fig. 2) on separate days. On each occasion, the measurement started from an angle of 30° and ended at an angle of 150° in increments of 15°. Three readings were obtained for each angle.

![Fig. 2](image_url)

Measurement of the light distribution of the flash in the hemisphere. a, b, c, d, e, f, g, h and i represent the area measured. The two neighboring areas had a visual angle of 15°. D = detector of radiometer.

The flash doses measured were analysed for variance according to a mixed effects model.

\[ X_{ijk} = \mu + \alpha_i + B_j + (\alpha B)_{ij} + \varepsilon_{k(ij)} \]  

Eq. 2

Here, the estimated radiant exposure of the flash, \( X_{ijk} \), equals the sum of the expected population mean, \( \mu \), a term for the fixed variation among measurement days, \( B_j \) (\( j = 1, \ldots, 3 \)), a term for the interaction between measurement angles, \( \alpha_i \) (\( i = 1, \ldots, 9 \)), a term for the random variation among measurement angles and days, \( (\alpha B)_{ij} \), and a term for the measurement error, \( \varepsilon_{k(ij)} \) (\( k = 1, \ldots, 3 \)).

The analysis of variance for doses of the flash (Table 1) showed that there is a statistically significant difference of flash dose among measurement angles. There is no statistically significant difference among measurement days. The distribution of the flash in the hemisphere is shown in Fig. 3 as the confidence interval for the mean of the measurements for three days.

The precision of the flash at a given measurement angle was estimated. The dose of the flash was measured at the center of the hemisphere 30 times in 30 min. The variation of the flash dose was about 1%.
Table 1
Analysis of variance for the flash dose in the hemisphere

| Source of variation | Degrees of freedom | Estimated mean square (10^-2 J/m^2)^2 | Expected mean square | Test statistic | Significance limit (0.95) |
|---------------------|--------------------|---------------------------------------|----------------------|---------------|--------------------------|
| Angles              | 8                  | 3.1842                                | \(\sigma_e^2 + b\kappa\alpha^2\) | 5876.00*      | 39.37                    |
| Days                | 2                  | 0.0156                                | \(\sigma_e^2 + an\sigma_B^2 + nk\alpha_B^2\) | 2.61          | 4.77                     |
| Angles vs days      | 16                 | 0.0060                                | \(\sigma_e^2 + nk\alpha_B^2\) | 10.99         | 39.43                    |
| Error               | 54                 | 0.0005                                | \(\sigma_e^2\)       |               |                          |

\(\sigma_e^2\) = expected variance for the indexed random source; \(\kappa^2\) = factor corresponding to the variation due to the indexed fixed source; \(\alpha\) = angles; \(B\) = days; \(n\) = number of days; \(\varepsilon\) = measurement error; * = significance (P < 0.05).

Fig. 3
The distribution of the flash in the hemisphere. A vertical bar represents the 95% confidence interval for the mean (df = 8).

3.3 Optokinetic stimulus

The OKN stimulus was a moving grating projected by a filament lamp (I) or a LED (Stedtner, Germany, CERLED, CR 10; II, III, IV, V, VI, VII) through a
striped film drum. The film comprised 38 pairs of alternating transparent and opaque vertical stripes.

3.3.1 Target luminance
The luminance of the grating was measured as the luminance of the light stripes when the grating was still. A Spectra Prichard Photometer (Photo Research, USA, model 1980) calibrated to a standard traceable to US NBS was used for the measurement. The measurement was done in the dark room used for the experiment. The measuring field was limited to the centre of the light strip (Fig. 4). The full scale of sensitivity of the instrument was used. A photopic filter, which matched the spectral response to the standard CIE V(\lambda) function, was used because of the lack of a scotopic filter. Two measurements were made on separate days for each light current. The mean of the two was used as the luminance for the corresponding current.

Fig. 4
Measurement of grating luminance. The circles illustrate the area measured for the luminance in the light and dark bars, respectively.

The current source of the LED was a function generator (Wavetek, USA, model 275) and the current was monitored by a current meter (Fluke, USA, Digital Multimeter 8000A). If the current drifted, the function generator was adjusted manually to obtain the proper current.

3.3.2 Appearance of colour of grating
The tungsten lamp as a light source
The luminance of the grating was adjusted by changing the lamp current (I). This may induce a shift in the dominant wavelength of the grating. The significance of the shift in the dominant wavelength was estimated by means of the CIE standard colorimetric system, XYZ (CIE, 1932).

The relative spectral irradiance of the white strip of the grating, E(\lambda), was measured with a spectroradiometer (Optronic Laboratories, USA, model 742) at intervals of 5 nm over the range 300-780 nm. At each interval, the average of four readings was taken as a single measurement. This was done at lamp currents producing a luminance of $1.1 \times 10^{-5}$ cd/m$^2$ and $2.6 \times 10^{-5}$ cd/m$^2$, respectively.

The obtained spectral radiance measurements, E(\lambda), were inserted in Eq. 3, 4 and 5 to calculate the tristimulus values, "X", "Y" and "Z", in the XYZ system (Le Grand, 1968).
Here, $X(h)$, $Y(h)$ and $Z(h)$ are the respective distribution coefficients in the XYZ system for the standardised primary colours, red, green and blue (Le Grand, 1968).

The trichromatic coordinates, "$x$" and "$y$", were calculated as

$$X = \frac{\int E(\lambda)X(\lambda)d\lambda}{\int E(\lambda)d\lambda} \quad \text{Eq. 3}$$

$$Y = \frac{\int E(\lambda)Y(\lambda)d\lambda}{\int E(\lambda)d\lambda} \quad \text{Eq. 4}$$

$$Z = \frac{\int E(\lambda)Z(\lambda)d\lambda}{\int E(\lambda)d\lambda} \quad \text{Eq. 5}$$

For the target luminance, $1.1 \times 10^{-5}$cd/m$^2$, the trichromatic co-ordinates were estimated to be $x = 0.56$ and $y = 0.43$. For the target luminance, $2.6 \times 10^{-5}$cd/m$^2$, they were $x = 0.55$ and $y = 0.43$. Thus, there was no difference in the colour appearance of the white strip of the grating between the two luminances tested for.

The LED as a light source
The wavelength of the LED was 567 nm ($T_{50} = 50$ nm). This colour was close to the waveband where the eye has its highest sensitivity for scotopic vision (CIE, 1983).

3.3.3 Speed of target
The speed of the film drum was monitored by an external frequency counter (Fluke, USA, Digital Counter-Timer 1952A). A motor powered by a D/A converter rotated the drum. A photo interrupter (Sharp, Japan, GP 1A06) fixed at the bottom of the drum. When the drum was moving, the striped film passed through the gap of the photo interrupter. The transparent strip of the film was detected. The pulses from the photo interrupter gated pulses of 4.0 kHz generated by a crystal clock to an interval counter. The D/A converter, the
crystal clock and the interval counter were provided by a PC data acquisition board (Scientific Solutions, USA, Lab Master). For a given drum speed, an ideal value (pulses/bar) was found and set as a parameter in the computer. If the detected value differed from the ideal value, the computer program automatically sent an adjusted value to the D/A converter to force the motor to maintain the expected speed. With this system, the variation of the drum speed was limited to ±1 deg/s.

3.3.4 Field of target
The viewing conditions in the present studies were scotopic. Rods are known to be distributed most densely around the fovea with a visual angle of 20° (Adler, 1959). A grating having a horizontal visual angle of 60° was used. Vertical OKN was not examined in the present studies. A visual angle of 30° in the vertical direction was adopted for the grating.

3.3.5 Contrast of target
The contrast of the grating was defined as \((L_{\text{max}} - L_{\text{min}})/(L_{\text{max}} + L_{\text{min}})\) in the present studies. Here, \(L_{\text{max}}\) was the luminance of the light stripes and \(L_{\text{min}}\) was the luminance of the dark stripes. The luminance of the light stripes was very low. The measurement of the luminance of the dark stripes was at the limit of the instrument used. Thus, the grating contrast was estimated only roughly (I, II, VI, VII).

It is believed that the contrast of the grating differs as the luminance of the grating changes. The measurements showed that the contrast of the grating varied between 0.78 and 0.82 (III, IV, V). Considering that the contrast shifted within a small region and its influence on OKN was not investigated in the present study, the average is presented.

3.4 Recording and calibration of eye movement

Binocular eye movement was measured as the DC potential change detected between the two inputs into a balanced amplifier. The two electrodes (Medicotest, Denmark, A-10-VS) were fixed laterally to the outer canthi. The earth electrode was taped to the forehead.

The potential change was recorded as a function of time with a Siemens Mingograf 34 (I, II, III). In the later investigations, the potential change was amplified by a DC differential amplifier (Tektronix, USA, 7A22), A/D converted and then stored digitally at 50 samples/s (IV, V) or 20 samples/s (VI, VII) on a hard disk.

The ratio (volt/angle) for eye movement calibration was noted in each test session after dark adaptation. The subject was asked to shift the fixation between two fixed point lights inside the hemisphere. At least 6-8 EOG
oscillations were required. Overshoot (positive or negative) was ignored during the measurement. The mean of the recorded ratio was calculated as the calibration factor (Fig. 2, IV).

3.5 Methods for recognition of optokinetic nystagmus

Two methods, manual and semiautomatic, were used for the recognition of OKN after flash exposure. The manual method required the operator to detect the first reappearance of the OKN and measure the RAT on a paper print out of the EOG recording with a ruler. The semiautomatic method was a computer program designed for the recognition of OKN. The strategy of this program was to distinguish the electrical equivalent of an eye beat from noise based on four characteristics (VI). They were the voltage recorded at a maximum and a minimum in the trace of an eye beat, the duration between the maximum and the minimum, the slope of the slow phase and the residual variance for a regression between the maximum and the minimum according to a first degree polynomial.

The boundaries of the characteristics were set by the operator. It was impossible to set general tolerances for the boundaries. A further development of the algorithm resulting in fully automatic recognition of RAT would provide a tool for clinical practice.

3.6 Electroretinography

The ERG is an objective measure of retinal function. After Holmgren (1865-1866) first recorded the bioelectrical potentials in a fish eye, Cooper et al. (1933) succeeded in obtaining the first electronically amplified human ERG. The technique of recording ERG has been developed considerably.

The technique described by Wanger & Persson (1984) was adopted in the present study. The a-wave mirroring the activity in the photoreceptors (Dowling, 1987a) was used to follow the recovery of the receptor activity after flash exposure (II).

A Grass PS 22 photic stimulator was used as the stimulus for the ERG recording. The stimulator was fixed at the upper left corner of the Goldmann perimeter hemisphere (Fig. 1, II). The light from the stimulator was reflected to the whole hemisphere. The duration of the light was 0.01 ms. The intensity was controlled by an adjustable diaphragm.

A gold-foil electrode was inserted under the lower eye lid with a reference fixed at the skin close to the outer canthus to record ERG. The ERG was amplified with a high and low frequency filter set at 1 and 128 Hz,
respectively. The recorded single ERGs were averaged in an 8-channel Medelec Sensor Unit with a resolution time constant of 300 ms.

### 3.7 Filter goggles for visual adaptation

A pair of diving spectacles was used to simulate dark adaptation. The glasses were covered by wratten gelatine filters (Kodak, USA, No. 70). The cut-off wavelength of the filter, together with the glasses was 650 nm with 5% transmittance. The wavelength of the filter was selected on the basis of the action spectrum of photopic and scotopic vision (CIE, 1983). It was expected that the stimulation of rods would be eliminated by wearing the goggles in the light.

### 3.8 Experimental design and data analysis

#### 3.8.1 Selection of subjects

A total of 79 subjects participated in the present study. They were all members of the hospital staff with normal or corrected-to-normal visual acuity. Subjects with histories of oculomotor, colour vision or night vision abnormalities were not included.

The distribution of subjects by age and sex is presented in Table 2. Owing to the limitations on the selection of subjects, the subjects were selected over a rather wide age range. This may increase the variability among subjects and reduce the precision in population estimates. Increasing the number of subjects might further augment the precision in population estimates.

#### Table 2

Age and sex distribution of subjects

| Investigation | Age range (years) | No. of females | No. of males |
|---------------|-------------------|----------------|--------------|
| I             | 28 - 46           | 2              | 16           |
| II            | 32 - 45           | 5              | 4            |
| III           | 32 - 34           | 1              | 1            |
| IV            | 28 - 47           | 5              | 4            |
| V             | 28 - 47           | 5              | 4            |
| VI            | 29 - 47           | 6              | 6            |
| VII           | 23 - 52           | 10             | 10           |

Sex was not considered a factor in most of the experimental designs, based on the assumption that the difference in RAT or OKN between females and males
is not significant. This was supported by the finding of insignificant difference of RAT between females and males (VII).

Corrected myopes were included in the present study. In order to minimise the influence of spectacles on the transmission of the light flash, these subjects were examined with their spectacles off in the studies on RAT (I, II, VI, VII). Good visual acuity is essential for a normal occurrence of OKN (Duke-Elder, 1968d). Subjects with myopic vision therefore wore spectacles during the experiment in OKN studies (III, IV, V).

3.8.2 Sample sizes and experimental variables
The sample sizes and experimental variables in RAT and OKN studies are given in Tables 3 and 4.

| Investigation | No. of subjects | Flash Wavelength (nm) | Target dose at the cornea | Target luminance |
|---------------|-----------------|-----------------------|--------------------------|-----------------|
| I 1)          | 15              | 449, 456, 477, 498, 520, 565, 591, 622, 703 | 80 mJ/m² at the cornea | 2.6(10⁻⁵cd/m²) |
|               |                 |                       |                          |                 |
| II 1)         | 4               | 622                    | 20 mJ/m² at the cornea   | 5.1-6.7(10⁻⁶cd/m²) |
|               |                 |                       |                          |                 |
| II 2)         | 5               | 536                    | 20 mJ/m² at the cornea   | 5.2-6.7(10⁻⁶cd/m²) |
| VI            | 12              | 502                    | 38 mJ/m² at the cornea   | 5.9(10⁻⁵cd/m²)  |
| VII           | 20              | 502                    | 38 mJ/m² at the cornea   | 5.9(10⁻⁵cd/m²)  |

The sample sizes in the present studies were limited. The sample size was considered in the confidence intervals estimated. In one of the investigations (III), only two subjects participated in the experiment. Therefore, general conclusions for the population were not possible.
Table 4
Sample sizes and experimental variables in OKN studies

| Investigation | No. of subjects | Viewing situation | Target velocity (deg/s) |
|---------------|-----------------|-------------------|------------------------|
| III           | 2               | binocular         | 16-56                  |
| IV            | 9               | monocular         | 20-120                 |
| V             | 9               | monocular         | 20-120                 |

3.8.3 Statistical tests used

Analysis of variance
The mixed effects models (I, II, V, VI, VII) and the random effects models (VI) were applied to examine the sources of variation in measurements. The estimated sources of variation (VI) were used in the analysis of the precision of the measuring procedure (Friedman et al., 1981).

Non-linear regression analysis
The plot of OKN gain against target luminance at a given target velocity suggested an exponential function (IV, V). Non-linear regression was used to estimate the parameters in the adopted model.

Owing to the imbalance of the individual data obtained, analysis of variance was not feasible for an examination of the directional preference in OKN. A linear model (Eq. 1, IV) was used to examine the difference in OKN gain as a function of target luminance for each target velocity and each subject. Using this model, the systematic difference in OKN gain at varying target luminance in the population was indicated by the estimation of the confidence interval for the mean constant for a given target velocity.

This examination is reconsidered. It is found that the confidence interval for the mean constant in the linear model (Eq. 1, IV) is not a proper estimation for the directional difference in OKN gain. The confidence interval for the mean estimated difference in OKN gain, \( \bar{y}_1 \), in Eq. 1 (IV), for a given target velocity is a better expression of the systematic difference in OKN gain between different target directions at different target luminances.

3.8.4 Statistical parameters
The confidence intervals for means were given as estimations of experimentally found parameters in the population throughout the present studies. Significance levels and confidence coefficients were set at 0.05 and 0.95, respectively, in consideration of the small sample size.