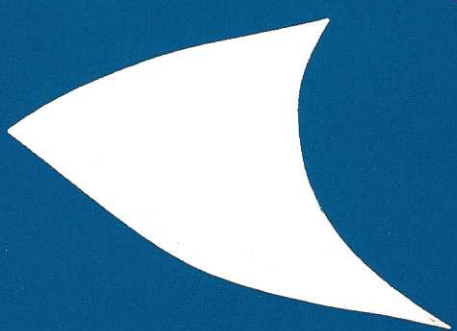
# *DYNAMICS* OF THE HUMAN PUPILLARY SYSTEM

An investigation into temporal aspects of light-induced constriction-related pupil behaviour, including contrast sensitivity and oscillations.

J.E. Bos



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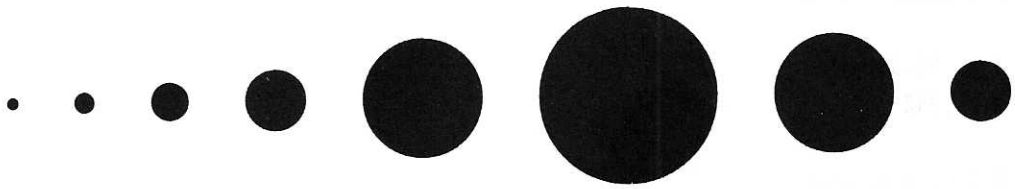

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# DYNAMICS OF THE HUMAN PUPILLARY SYSTEM

An investigation into temporal aspects of light-induced constriction-related pupil behaviour, including contrast sensitivity and oscillations

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor aan
de Vrije Universiteit te Amsterdam,
op gezag van de rector magnificus
dr. C. Datema,
hoogleraar aan de faculteit der letteren,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der geneeskunde
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door

JELTE EGBERT BOS

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Copromotor: dr. G.L. van der Heijde

Referent: dr. G.J. van der Wildt

To my closest relatives

## PREFACE

This thesis reports upon part of the research I have been doing from 1985 up till now at the Department of Medical Physics of the 'Vrije Universiteit' (VU), Amsterdam. For the first two years the work was subsidized by the 'Rotterdamse Vereniging Blindenbelangen'. Part of the patient investigations were performed at the 'Oogziekenhuis Rotterdam'. Subject is the pupil light reflex, that is the narrowing (constriction) and widening (dilatation) of the pupil when the amount of light, incident on the eye, is changed. The theme that runs through the thesis regards temporal aspects of pupil constriction. Basically, these temporal aspects concern the period of time that the pupil system needs to adjust the pupil size when the light condition is being changed. This delay is caused partly by neural conduction of electrical activity within the brains. When the work started, a newly developed pupillometer had just become available at the VU, with which the temporal aspects could be investigated accurately.

To study the pupil light reflex, it is important to know whether stimuli with a spatial structure, or just homogeneous stimuli should be presented. To sort this out, the contrast sensitivity of the pupil light reflex must first be characterized. Though it may seem that this does not concern temporal aspects, it is of importance because it lays the foundation for the stimulus to be used in investigations regarding temporal pupil behaviour.

An intriguing aspect of pupil behaviour is the occurrence of oscillation that can be induced artificially. This requires increasing the system's gain, or extending the delay that is inherent to the pupil system. High-gain-induced pupil oscillations have been obtained experimentally since 1957. Extended-delay-induced oscillations have, however, never been subject to experimentation. With a minor adaptation of the available pupillometer, this could also be achieved, and the results fit well within the scope of this thesis.

The constriction plays a major role in pupil reflexes, both when elicited by spatially structured stimuli and in induced oscillations. In addition to the above-mentioned scientific interest, the constriction may also be of interest in clinical diagnosis of diseases. For this, constrictions can simply be induced by sudden increases in stimulus-light intensity. The last part of this thesis describes the use of temporal parameters related to constrictions so induced in health and disease. Ways are indicated to discriminate between normal and abnormal pupil behaviour, and to locate the impairment within the pupil system underlying a disease. The iris encircling

the pupil consists of muscular tissue for changing the pupil size. Abnormal pupil behaviour may therefore reflect neural as well as muscular disfunctioning, and the possibility to differentiate between these two will also be dealt with.

TABLE OF CONTENTS

## ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| A | Pupil area |
| A/D | Analogue to digital |
| ANS | Autonomic nervous system |
| $\alpha$ | Rate constant determining constriction and dilatation velocity |
| BAEP | Brainstem auditory evoked potential |
| $\beta$ | External gain |
| CL | Constriction latency |
| CLTF | Closed-loop transfer function |
| CNS | Central nervous system |
| CRT | Cathode-ray tube |
| D/A | Digital to analogue |
| dCL | Difference between the constriction latencies of both eyes, either afferent or efferent |
| DDE | Delay differential equation |
| DM | Diabetes mellitus |
| dVEPL | Difference between visual evoked potential latencies of both eyes |
| E | Illuminance |
| EEG | Electro-encephalogram |
| Er | Retinal illuminance |
| G | Gain |
| L | Used as an index, refers to stimulation of the left eye |
| LED | Light-emitting diode |
| MS | Multiple sclerosis |
| MyD | Myotonic dystrophy |
| NCV | Nerve-conduction velocity |
| OD | Oculus dexter (=right eye) |
| OLTF | Open-loop transfer function |
| ON | Optic neuritis |
| OS | Oculus sinister (=left eye) |
| P | Oscillation period |
| PCMF | Piecewise-constant mixed feedback |
| PCNF | Piecewise-constant negative feedback |
| PCT | Pupil cycle time (=P) |
| PCVL | Peak constriction-velocity latency |
| PNS | Parasympathetic nervous system |
| R | Used as an index, refers to stimulation of the right eye |

| | |
|---|---|
| RHS | Right-hand side of an equation |
| RT | Recovery time (since disease onset) |
| SD | Standard deviation |
| SE | Standard error of mean |
| SEP | Somatosensory evoked potential |
| SNF | Smooth negative feedback |
| SNS | Sympathetic nervous system |
| Td | Troland |
| $\theta$ | Parameter determining midrange pupil area |
| $\tau$ | Time delay |
| VEP | Visual evoked potential |
| VEPL | Visual evoked potential latency |

# 1. GENERAL INTRODUCTION

## 1.1 AIM OF THE STUDY

In neuro-ophthalmologic diagnosis of diseases, there is a general need for demonstrating and quantifying abnormalities and locating eventual lesions. The latter involves the differentiation between receptor, central nervous system (CNS), and effector impairments. For diagnosing receptor and CNS impairments, the electro-encephalogram (EEG), and, more specifically, visual evoked potentials (VEP) are applied in clinical practice. VEPs, however, have their specific disadvantages, such as the small signal-to-noise ratio for which averaging techniques are required. Moreover, any technique is related to a specific subsystem. The VEP only reflects afferent visual functioning.

Pupil examination can also contribute to the neuro-ophthalmologic diagnosis, both for detecting and quantifying abnormalities and for locating impairments. The pupil system involves three major components, as outlined in Fig. 1. In the eye, where the pupil and the retina play a major role, incident light is 'translated' into electrical activity. Neurons and higher-order parts of the brain transport, transform, and delay this electrical activity, which is subsequently fed back to the eye, particularly the effector of the pupil system, i.e. the iris muscles.

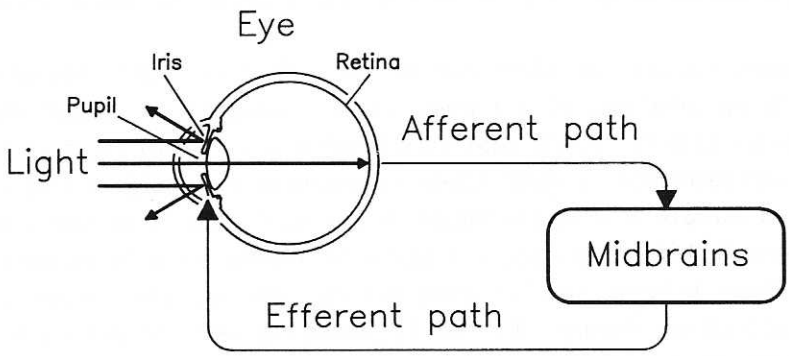


*Fig. 1. Outline of the pupil system regulating the amount of light incident on the retina.*

Pupillometry as a diagnostic tool offers a number of advantages, which can be briefly summarized:

- The neurologic part of the pupil system is regulated entirely by the autonomic nervous system (ANS). Pupil variations are thus independent of the human will, and investigation of the pupil reflex may lead to objective information about the ANS.
- In the ANS, both the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS) are involved, as well as afferent and efferent pathways. This dual-structure system may seem confounding, but its examination may also lead to better and more extensive insight.
- Because some parts of the pupil system are shared by other visual subsystems, pupillometry may also yield insight in these visual functions.
- The most prominent pupillary function is the regulation of the amount of light falling upon the retina. Changes in pupil size can be simply evoked by light.
- The pupil system's feedback loop can be opened by applying Maxwellian viewing conditions. Both the closed-loop and the open-loop transfer function can thus be subject to investigation.
- Pupil size can be recorded without physical contact with the subject's eye, i.e. non-invasively. Consequently, pupillometry is less of a burden for a subject than e.g. EEG techniques where electrodes have to be glued onto the skull.
- Manipulation of the pupil system's feedback can be realized by environmental clamping. This refers to a technique in which the system's loop is opened artificially and reclosed by an external device. Therefore the pupil system is apt to serve as a general paradigm for neural feedback.

Systems analysis has shown that an appropriate and simple way of gaining insight in and knowledge of a system, is by looking at the output evoked by step or pulse stimuli. In the pupil-light-reflex system, the input is a sudden increase (or decrease) in light intensity presented to a subject's eye. Fig. 2 provides an example of what can happen to the pupil size. A certain time after the increase in light intensity, the pupils of healthy subjects become smaller. The time lapse between stimulus onset and the start of pupil constriction is called constriction latency (CL, Fig. 2), which may last for 0.2 - 0.5 s. When the process of transforming light into electrical activity, transport or effectuation is affected, e.g. by an inflammation of the optic nerve as in optic neuritis (ON), by a degeneration of the optic nerve as in multiple sclerosis (MS), or by neuropathy in diabetes mellitus (DM), the CL may be altered. When the iris muscles are not functioning optimally due to a pathological stiffness, as possibly in myotonic dystrophy (MyD), the course of the

constriction may be changed, resulting in a change of velocity and duration. Related to this velocity and duration is the moment at which the velocity is at its maximum. This is represented by the peak constriction-velocity latency (PCVL, Fig. 2), a parameter that has not been applied so far. However, like the CL, it is a temporal parameter and both could be reliable parameters of pupil functioning if they can be demonstrated to be independent of signal amplitude.

Whenever a decrease in light intensity is presented to the subject's eye, the pupil dilates. Where pupil constriction is regulated mainly by the PNS, the far more complicated and less understood SNS is part and parcel of the regulation of the much slower dilatation.

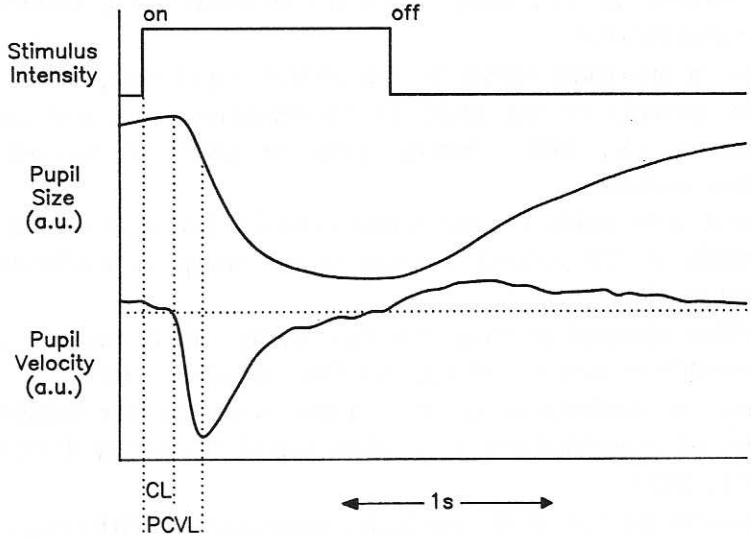


*Fig. 2. Example of pupil constriction and dilatation, induced by an instant increase and decrease, respectively, in light intensity. Indicated are the parameters of interest: the constriction latency (CL) and the peak constriction-velocity latency (PCVL).*

Systems analysis has shown also, that by increasing the gain or delay of a system, the system may become unstable. Then oscillations occur, as is true for the pupil system. Here the gain is related to the ratio between a change in light flux striking the retina due to a change in pupil size, and the reflex-originating change in stimulus flux.

Changes in pupil size have already proved their informative value to the physician (depth of anaesthesia, Marcus Gunn, Argyl-Robertson, life/shock/coma/death). This value is mainly restricted to qualitative observations. Moreover

not all abnormalities can be observed with the naked eye. Apparatus is then needed to manifest and quantify the differences. Pupil parameters thus measured, concern spatial aspects (changes in pupil size) as well as temporal aspects (CL, PCVL). Literature references to the spatial aspects outnumber the references to temporal aspects. This is mainly due to the limited time resolution of the current pupillometers. Incorporation of temporal aspects in the assessment of the pupil reflex has advantages though. Besides of complementary value along with amplitude information, the CL can also be of supplementary value, because:

- Changes in amplitude (and the derived velocity) may point to diminished PNS functioning as well as to increased SNS (stress) activity, or vice versa (Pfeifer et al., 1982). Amplitude information may therefore give confounding results.
- Changes in amplitude depend on the initial pupil size, which in turn depends strongly on the state of accommodation, age, and adaptation (Pfeifer et al., 1982). Results based on amplitude information are therefore ambiguous.
- The above also holds for the pupil velocity (as a derivative of the amplitude), as the velocity depends on the change in amplitude itself (Pfeifer et al., 1982).
- For evident abnormal or normal SNS functioning, the CL has been shown to be a reliable measure of PNS activity (Pfeifer et al., 1982).
- Analogous to knowledge on the VEP, latencies may reflect accurately the presence of demyelinations in a given neural trajectory (Halliday and McDonald, 1977).
- Analogous to the VEP in ON, amplitudes correlate well with visual acuity, whereas latencies do not (Halliday and McDonald, 1977). Therefore CLs are expected to yield additional information.
- Under well-controlled experimental conditions, light-induced amplitude changes lead to greater relative variations than CLs do (Lowenstein et al., 1964). Therefore the discriminating value of the latency is greater.
- In excitable subjects (i.e. individuals with strong supranuclear inhibition), pupil reflexes show latencies consistent with the presented stimulus, as opposed to the reflex amplitude (Lowenstein et al., 1964). In other words, the reflex amplitude shows a greater variability in the case of supranuclear inhibition than the CL does.
- Cases have been recorded where amplitude changes did not differ from those of controls, while the corresponding CLs did differ (Alexandridis et al., 1981). In ON the CL is abnormal more often than the matching

changes in amplitude (Ellis, 1979), which makes the CL more reliable as a diagnostic parameter.

- The period and amplitude of pupil oscillations depend not only on gain, but also on latency.

However:

- With respect to prolonged pupil latency, literature reveals limited information, and records a great diversity of values. This even holds for the literature on normative data.
- A great variety of often poorly documented stimulus conditions has been used so far. Thus it is hard to deduct standard values. Moreover, for the VEP, normative data have to be gathered under conditions identical to those under which the patient data are gathered. This also holds for pupillometric parameters opposed to the brainstem auditory evoked potentials (BAEP) and somatosensory evoked potentials (SEP). The pupil reflex may well depend more on stimulus conditions than the VEP does. This further reduces the value of normative data from the literature.
- Most examinations so far concerned monocular data, with respect to both the direct or consensual reflex and the eye that was stimulated.
- Most pupil examinations have been performed under 'closed-loop' conditions, where the retinal illuminance depends on pupil size. Therefore, the actual pupil stimulus is not given in the cases concerned. Moreover the pupil controls itself. Furthermore, when 'open-loop' stimuli have been used, often small retinal areas have been stimulated. This is why geographical variations in retinal functioning may have led to results ascribed to neuropathy.
- It remains unknown whether either spatial or temporal contrast can best be used for examining the pupil light reflex.
- CL data in the literature are based mainly on subjective parameter estimation, i.e. constriction-onset determination.
- A considerable amount of abnormal CL data presented in the literature are based on case reports, where statistical analysis based on larger sample sizes would be more cogent.
- In the literature only impairments of the afferent limbs of the pupil reflex arc have been demonstrated, while, in principle, demonstration of efferent defects is likewise possible.
- Differences between afferent pupil fibers, up to the optic tract, and fibers contributing to the VEP are unknown. Because a delay in the afferent limb may significantly prolong the VEP latency by less than 25

ms, such relative small differences in pupil latency have to be reckoned as well.

- So far, no quantitative pupil parameters are available for the differentiation between neurologic and muscular iris disfunction.
- Commercially available pupillometers have a limited spatial, and especially a limited temporal resolution, and this imposes a low precision on latency data.
- Whether pupil oscillations are of clinical value, has been tested phenomenologically only. There is a lack of theoretical knowledge for explaining oscillatory pupil behaviour, especially concerning delay dependency. Moreover, experiments, in which the delay is changed under well-controlled conditions, have never been performed. Therefore, the relationship between the pupil cycle time (PCT) and delay is not yet known.

Given these shortcomings, this thesis will describe a further examination of the latency of the human pupil constriction and its effect on pupil oscillations. In short, the aim of this thesis is:

> *The assessment and utility analysis, both for clinical practice and basic science, of temporal pupil-constriction parameters in healthy human subjects and in subjects with impaired afferent, efferent, or effector functioning.*

A general description of the pupil system is given following this section. Included is a review of the literature related to the pupil system's delay.

Prerequisite for further experimentation is proper equipment for stimulating and measuring the dynamic properties of the pupil reflex, which is described in Chapter 2. To present well-defined stimuli, Maxwellian view conditions will be applied where appropriate. The calibration of this device is also discussed. A pupillometer, with a high temporal resolution (1 ms) and the possibility to record pupil movements of both eyes simultaneously, was developed in our laboratory. This pupillometer enables a more detailed study of various aspects of pupil latency, and of possible relationships between the CL and the latency related to the VEP. Data acquisition is performed with computer aid.

Chapter 3 concerns results of investigations on the contrast sensitivity of the pupil system. It will be studied whether stimuli with some sort of

spatial structure or merely homogeneous stimuli should be used for further experimentation. Part of these investigations are also presented by van Dongen (1991) in his thesis.

Chapter 4 deals with pupil oscillations induced by high-gain and extended-delay. Interest will focus on the dependency of the oscillation period on delay.

Chapters 3 and 4 are more fundamental, while Chapter 5 will be more of clinical value. Chapter 5 concerns the latency of the pupil constriction, induced by steps in light intensity, in health and disease. A description will be given of the determination of the CL, facilitated by computer-aided pupillometry. Included are the establishment of the sample rate for digitizing the pupil records, and the presentation of both a simple and a more sophisticated algorithm for determining the moment of constriction onset. A general remark will be made on the averaging of raw pupil data. Part of this section has already been published (Bos, 1991). In back of an analysis concerning basic properties of the pupil light reflex in healthy subjects, the CL and PCVL are studied in three types of diseases, i.e. optic neuritis and multiple sclerosis, diabetes mellitus, and myotonic dystrophy. In these diseases, possible afferent, efferent, and/or effector pupil disfuctioning is investigated. Part of these studies have been published (Bos, 1988; Bos et al., 1990; Lanting et al., 1991; Den Heijer et al., 1991).

## 1.2 ANATOMY AND NEUROPHYSIOLOGY OF THE PUPIL SYSTEM

The basic anatomy and nervous innervation of the pupil are well described in elementary text books like those of Davson (Lowenstein & Loewenfeld, 1962), Voorhoeve (Veringa et al., 1978), Alexandridis (1985), Walsh and Hoyt (1985) or Moses (Thompson, 1987). For the sake of clarity, however, essentials relevant to my purposes are cited here.

When light enters the human eye (Fig. 3), it first passes a fixed focus refracting surface, the cornea. Secondly, part of the light will be shaded by a diaphragm, the iris. The aperture confined by the iris, the 'black nothing' of which the dynamics is a matter of supreme interest according to this thesis, is called the pupil.

The iris consists of layers of pigmented connective tissue, blood vessels and nerves. In between the layers of connective tissue two distinct muscle tissues are confined. These muscles are separated in an annular band, the sphincter pupillae, and a radial part, the dilator pupillae. The sphincter

consists of bundles of smooth muscle fibers (about 0.7 mm wide and 60 µm thick), closely encircling the pupil margin. The dilator consists of clear myo-epithelial tissue (2 µm thick), extended radially toward the iris exterior. The outer edge has a diameter of about 12 mm in man, the inner or pupil diameter can vary between about 2 and 8 mm. The sphincter and dilator are physically connected, as to directly act upon each other, in that the sphincter stretches the dilator, while the dilator unfolds the sphincter. The result is that each muscle, on contracting, places its counterpart in an optimum position for initiating its contraction (Alexandridis, 1985). At maximum constriction the fibers of the sphincter are shortened by almost 90% relative to their length in resting state (Moses, 1975), an extraordinary property possessed by no other smooth muscle in the human body.

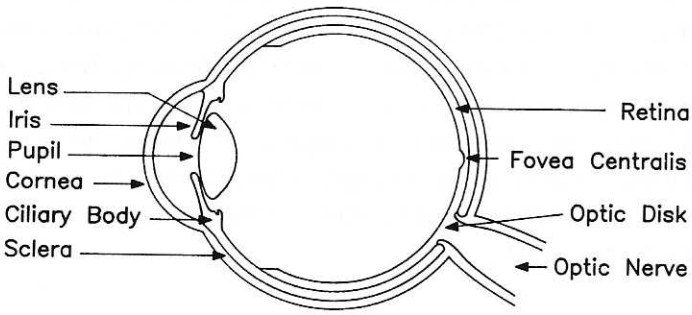


*Fig. 3. Schematic drawing of the human eye.*

The iris is positioned directly against, and concentrically pushed forward by a lens, which is able to change its thickness during accommodation. After the light has passed this lens, it travels through the vitreous body, and strikes the retina. The retinal photoreceptors, rods and cones, next elicit neural pulses, which travel along the pupillary neural pathways.

**Neural pathways**

The neural pulses, which originate in the retina and finally incite the pupil light reflex, are exclusively processed by the autonomic nervous system (ANS). Therefore, the pupil light reflex is independent of the human will, and this is a major advantage with respect to objective quantification of the reflex. The pulses travel along the afferent fibers to the midbrain, and the efferent fibers lead back again to the iris muscles (Fig. 4). A closed-loop system is thus constituted. It enables the iris to regulate the amount of light

falling onto the retina, and the amount of light falling on the retina to regulate iris size. This reflects the major role of pupil functioning.

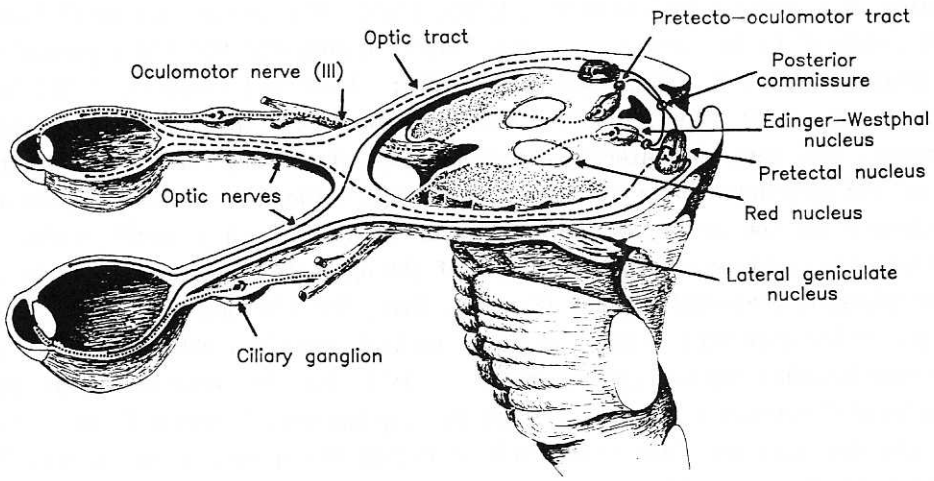


*Fig. 4. Anatomy of the parasympathetic neural pathways involved in the pupil light reflex (redrawn from Walsh and Hoyt, 1985).*

Once an electric pulse has been generated by the retinal receptors, it is transported via the nerves. Nerve conduction itself is an intriguing, but complex research subject. Nerves may be enveloped by myelin, interrupted by the so called nodes of Ranvier. Myelin is known to function as an isolator increasing nerve conduction velocity (NVC). Theoretical analysis of the passive core conduction and the descriptions of Hodgkin and Huxley (a short relevant review is given by Riemslag, 1986) give some insight in nerve functioning, but the models presented are an oversimplification. They do not take into account the function of the internodes and the mutual interaction of parallel fibers. Moreover, analytic solution of the mathematical equations involved is impossible and not all characteristics of NCV can be explained assuming passive processes. Yet undescribed active processes play their part as well.

The pupil latency is too long (> 100 ms) to be explained solely on the basis of NCVs, even if synapses are taken into account. The part of the reflex leading to the extra delay is presently controversial. Some authors favour an origin in the midbrain (Smith et al., 1970). Others suggest that it arises at the level of the iris, its musculature, and/or the neuro-muscular junction (Loewenfeld, 1966; Ellis, 1981).

**Afferent pathways**

The afferent pathway originates at the retinas and continues to the optic nerves (NII) and the optic chiasm, where fibers of the nasal retinal half fields, i.e. roughly 50 % (McCrary, 1977), cross over. So far, the pupil fibers are presumed to be identical to the fibers responsible for light perception (Alexandridis, 1967, 1968, 1970, 1971, 1973; Alpern and Campbell, 1962; Ohba and Alpern, 1972). Pupil fibers branch from the optic tract without making a synapse. They pass the lateral geniculate body, where they reach the pretectal area, and terminate in the ipsilateral pretectal nucleus. From there, axons are connected to the cells of the oculomotor nucleus, with a small number of fibers being distributed to the nucleus of the opposite side, as well as to the contralateral pretectal nucleus (Fig. 4). Thus, the afferent limb of the pupil light reflex undergoes a total of three partial crossings before reaching the Edinger-Westphal nuclei (Crosby et al., 1962). Due to these crossings both pupils will respond to light presented to only one eye. A change in pupil size of the eye that has been stimulated is called the direct light reflex. The change in the contralateral pupil is called the consensual light reflex. In healthy humans no differences have been found between the direct and consensual reflexes (Alexandridis, 1985).

**Efferent pathways**

The efferent pathway is innervated by both the sympathetic and parasympathetic nervous systems. The sphincter is predominantly innervated by the parasympathetic system, while the dilator is so by the sympathetic system. Parasympathetic fibers lead from the Edinger-Westphal nuclei to the sphincter pupillae via the oculomotor nerve (NIII), only interrupted by a synapse at the ciliary ganglion cells. The sympathetic innervation of the dilator arises from the hypothalamus. Because the parasympathically controlled sphincter system is of major interest in this thesis and dominates the total reflex, the much more complicated sympathetic innervation will not be discussed.

## 1.3 PUPIL REFLEXES

The pupil serves three main purposes (Lowenstein and Loewenfeld, 1962). It

- regulates the amount of light falling on the retina,
- increases the depth of focus by decreasing the aperture of the optical system, and

- reduces chromatic and spherical aberrations, especially in bright light conditions when the pupil is small.

**Light reflex**

Propensities of the pupil, with respect to the light presented to the eye, can be classified into static and dynamic pupil phenomena. Static properties refer to the steady-state pupil size, while dynamic characteristics refer to all other properties. The effects described mainly hold for the amplitude of pupil variations, in which most authors have been interested so far. As mentioned in the introduction, the major part of this thesis, however, will dwell upon dynamic properties.

**Static pupil phenomena**

The static pupil size can be described analogous to the *Weber-Fechner law*, stating that, when the eye is illuminated with a constant amount of light above threshold and below saturation, the finally perceived brightness increases logarithmically with stimulus intensity. The Weber-Fechner law thus also holds for the pupil size (Reeves, 1920; Bouma, 1962).

**Dynamic pupil phenomena**

A change in pupil size in reaction to a change of stimulus-light intensity is called the pupil light reflex. The change in pupil size of this reflex depends on the change in stimulus intensity, relative to background intensity (Webster et al., 1968; Webster, 1971), and this is in agreement with the *Weber law*.

For light pulses with a duration of less than 50 ms, the change in pupil area is linearly related to the product of their intensity and duration (Alpern et al., 1963; Webster, 1969, 1971). As in psychophysics, this relationship is sometimes called *Bloch's law*.

The above-mentioned relations hold for light-pulses, i.e. sudden increments succeeded by equal decrements in light intensity, superimposed on a certain background. The pupil then responds with a constriction. However, when a 'dark-pulse' is presented, i.e. a sudden decrease followed by an equal increase in light intensity, the pupil reacts with a constriction as well. But, for the dark-pulse the response amplitude is smaller and the latency lasts longer (Fig. 5). This effect is called *unidirectional rate sensitivity* (Clynes, 1961; Webster, 1971; Sun et al., 1981; Kollarits et al., 1982).

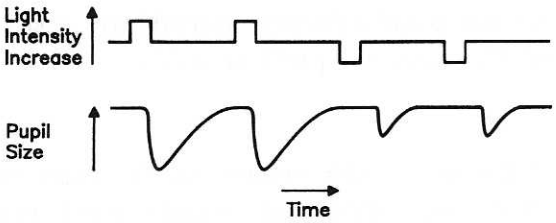


*Fig. 5. Example of the light- and dark-reflex: pupillary response to an incremental and decremental light-flash. The upper line represents the stimulus intensity presented to the human eye. By the lower trace the pupil size is sketched. (After Clynes, 1961)*

When the light intensity is modulated in time at low frequencies (2-20 Hz), the brightness is higher than the average presented intensity, and this is known as the *Brücke effect*. At higher frequencies, brightness is proportional to the average stimulus intensity, known as *Talbot's law*. Similar effects hold for pupil dynamics (Clynes, 1961; Varju, 1964; Troelstra, 1968; Webster, 1971; van der Wildt and Bouman, 1974).

In general two types of dynamics are encountered when the pupil reacts to steps in light intensity. When after the initial constriction the pupil dilates again to its pre-constriction size, the behaviour is called *escape* (Lowenstein and Loewenfeld, 1969). This only occurs when the initial pupil size is large and the step intensity is relatively small. In all other circumstances the pupil constriction is not followed by a dilatation, but the pupil remains constricted. This behaviour is called *capture* (Usui, 1974). The *pupil size effect* refers to the phenomenon that, depending on the initial pupil size and the step intensity, pupil behaviour is capture-like or escape-like (Fig. 6; Levatin, 1959; Semmlow and Stark, 1973; Usui, 1974; Shimizu and Stark, 1977; Sun and Stark, 1983; Sun et al. 1983a,c).

It has recently been found that the pupil responds to changes in *spatially structured* stimuli as well (Slooter and van Norren, 1980; Ukai, 1985; Barbur and Forsyth, 1986). The responses concerned are elicited by checkerboard reversals or appearance/disappearance stimuli. The origin of this response, however, remains uncertain, and will be subject to investigations described in Chapter 3.

**Reflexes of other origin**

Though light is the main originator of pupil reflexes, there are other sources as well. Because in practice it is hard to eliminate all but one of them, I will list them briefly here.

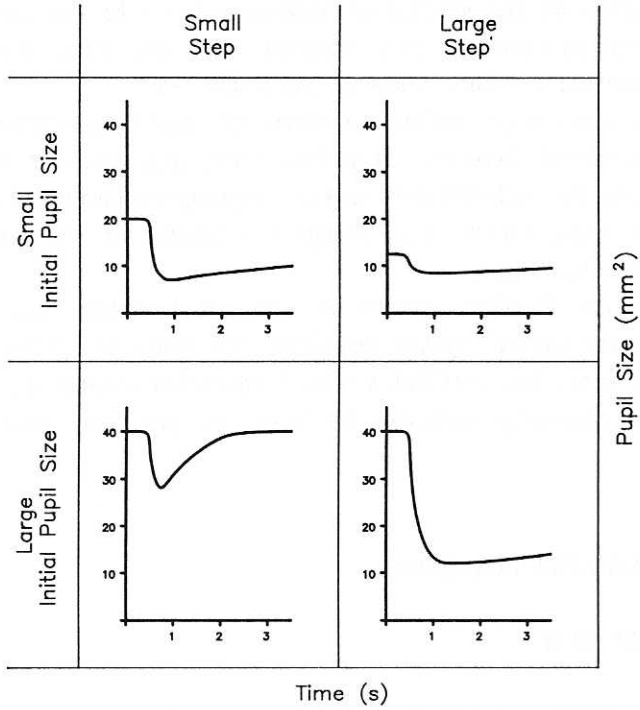


*Fig. 6. The pupil size effect. Depending on the initial pupil size and the intensity of a stimulus step, the pupil remains reduced in size (capture), or redilates immediately (escape).*

When the eye accommodates, a simultaneous pupil constriction, the *near reflex*, will occur. Cortical impulses for accommodation are carried to the oculomotor nerve outside the pretectal area (McCrary, 1977). Besides this synkinesis, vergence is involved as well. This results in what is called the *near triad*, a simultaneous appearance of pupil constriction, lens accommodation and vergent eye movements. The occurrence of pupillary constriction during accommodation has, however, recently been been questioned (Stakenburg, 1990). Reflexes of this kind may then be originated from differences in retinal illumination.

The *blink reflex* refers to a pupil constriction when the eyelids are being closed or attempted to close. It is not a real reflex, but merely a synkinesis due to connections between facial nerve nuclei and the oculomotor nucleus (Moses, 1975).

The *irritation reflex* consists of a short constriction followed by dilatation when the eye surface or the lids are touched.

Stimulation of the vestibular apparatus leads to the *vestibular reflex*, i.e. pupillary dilatation. This results from the fact that some of the pupillary sympathetic fibers traverse the middle ear.

The *psychosensory reflex* consists of pupil dilatation elicited by emotional excitement (anxiety, surprise, fear, joy, pain or mental activity, such as memory or mathematical tasks). Psychopathological factors may also influence the light reflex, e.g. change the latency of the pupil constriction (Grünberger et al., 1985).

Due to the feedback nature of the pupil and/or neurological noise, injected into the control system somewhere, the pupil in living humans is never completely at rest, but oscillates with frequencies between 0.1 and 3 Hz. This phenomenon is generally indicated by *hippus* or *pupillary unrest* (Thompson et al. 1971).

## 1.4 LATENCY-RELATED LITERATURE

### 1.4.1 *NORMATIVE DATA*

Donders (1866) was the first author to publish data concerning the delay of the pupil system. At that time, measuring techniques consisted of subjective entoptic methods (see below). Later on, photographic methods using visible light were applied, such as photography, cinematography, and kymography. From about 1940 onwards invisible infrared light was used, once even ultraviolet light. Nowadays high-tech infrared light reflection methods are in use. Despite all these measuring techniques, in nearly all publications referring to the latency of the pupil constriction, research on the latency itself has been limited.

To evoke pupil constrictions, homogeneous square wave modulated light stimuli i.e. intensity pulses, are mostly used. The constriction latency (CL) is generally determined by looking for the time interval between stimulus and constriction onset. Stark and Shermann (1957) employed small amplitude sinusoidal-modulated light with varying frequency, applying a linearized systems approach. In such a case, the relation between the non-minimum phase-shift and the CL, as measured by using intensity pulses, is known. Since the pupil system is now known to be highly nonlinear, and phase-shift measurements require elaborate experiments, literature concerning this way of CL determination is limited to this sole presentation.

In step-induced constrictions the period of time the pupil system needs to respond is generally referred to as latency. When dealing with oscillations, the system's lag, an essential for oscillatory behaviour in the pupil system, is generally referred to as delay. There is, however, no difference between these two. Nevertheless, I will use both terms, latency and delay, in their usual context.

**Step-induced constriction latency**

Literature lists a great variety of stimulus conditions with respect to square-wave modulated light stimuli (background- and pulse-intensities as well as in interval- and pulse-durations, open- or closed-loop, field angle and colour). As a result, CLs are found within the range from 60 ms (Lowenstein and Westphal, 1933) to about 550 ms (von Vintschgau, 1881; Lowenstein and Loewenfeld, 1959a).

One of Stark's many merits is his proposal to present the stimulus under Maxwellian view, i.e. open-loop, conditions for dosing the amount of light falling upon the retina. Despite the great advantage of independence of retinal illuminance from pupil size, only about one third of the subsequently published investigations were performed under open-loop conditions. There are two ways of realizing open-loop stimulation. One can either present a small parallel beam of light with a diameter smaller than the smallest pupil diameter (3% of the investigations), or use Maxwellian view conditions in which a greater retinal area can be stimulated (the remaining 27% of the investigations). In the latter, a beam of light converges in the centre of the pupil. The diverging beam thereafter stimulates a large retinal area. Utsumi et al. (1978) investigated the difference between open- and closed-loop stimulation, especially with respect to possible diurnal effects. They found a diurnal variation in the CL determined under closed-loop conditions and not when determined under open-loop conditions.

The CL also depends on stimulus colour. Lowenstein et al. (1964) have shown that the CL increases with increasing wavelength. If the CL-versus-wavelength curves are compensated for the thresholds per colour, they coincide however. In about 38% of the literature the stimulus colour is mentioned. Mostly white light is used (34%), sometimes yellow (5%), red (3%), or green (3%).

It is known since long (Piltz, 1904) that the CL in healthy subjects decreases with increasing stimulus intensities. This has been confirmed by Lowenstein and Loewenfeld (1951, 1959b), Feinberg and Podolak (1965), Lee et al. (1969), Müller-Jensen and Hagenah (1976), Müller-Jensen, (1978), Cibis et

al. (1977), Ellis (1981), Pfeifer et al. (1982) and Link and Stark (1988). However, up to the late fifties some authors explicitly denied this dependency (Machemer, 1941; van der Tweel and Denier van der Gon, 1959). Feinberg and Podolak (1965) suggested an inverse relationship between the CL and $Log(I_{on}-I_{off})$, in which I represents the light intensity. Lee et al. (1969) hypothesized the latency to be independent of the level of adaptation due to adaptation processes. The latency would then be affected solely by the size of the step from the adaptation level to which the eye has fully adapted. They confirmed this hypothesis up to a certain level by demonstrating that $CL = 312 - 18.6 \cdot Log(I_{on}) - 5 \cdot Log(I_{on}-I_{off})$, in which I is given in Trolands. Müller-Jensen and Hagenah (1976) and Müller-Jensen (1978) assumed an exponential decay of the CL versus Log(I). Ellis (1981), using a zero background intensity, found a dependency according to $CL = 445.7 - 22.9 \cdot Log(I_{on}) + 76.2 \cdot Log^2(I_{on})$, in which I is given in $cd/m^2$. Pfeifer et al. (1982) performed experiments under closed-loop conditions and found a dependency according to $CL = 466 - 3.25 \cdot I_{on}$, in which I is given in foot candela. Link and Stark (1988) included the repetition rate (R) of the light pulses. Finally they found a dependency according to $CL = 253 - 14 \cdot Log(I_{on}) + 70 \cdot R + 29 \cdot R \cdot Log(I_{on})$, in which I is given in foot Lamberts. This relationship implies some kind of short-term adaptation requiring only fractions of seconds, while the classical view tends to consider it as a process requiring minutes. All above-mentioned descriptions only have an inverse logarithmic dependency in common. They vary considerably in specific stimulus conditions, such as temporal parameters and background dependency.

The latency also depends on the retinal area that has been stimulated. This reflects both the total stimulus angle and the retinal position (Cibis and Campos, 1977).

Because of the difference in wielded stimulus conditions (stimulus angle, colour, background intensities, stimulus durations), a comparison between cited CLs is hazardous. An extrapolation towards experiments to be performed is therefore inappropriate.

Partial crossings of fibers to the opposite sides of the brains, as mentioned before, lead to a direct and consensual light reflex. Kristek (1966), Cibis et al. (1977), Ellis (1981), and Pfeifer et al. (1982) showed that in healthy subjects the CL of the direct and consensual reflexes do not differ. Likewise, there is no difference in healthy subjects between the CL when stimulated by either eye (Müller-Jensen and Hagenah, 1976). These findings are conform those concerning the VEP, SEP and BAEP (Allison et al., 1983).

There is a great variation in intra- (within subjects) and inter-individual (between subjects) CLs described. But for Drischel (1957), Künkel

(1961, 1963), Müller-Jensen and Hagenah (1976) and Müller-Jensen (1978), authors made no explicit distinction between these differences in general. Drischel (1957) reported an average intraindividual difference in latency of 15 ms, and 29.5 ms interindividually. Künkel (1961, 1963) found an average interindividual difference of 26 ms. Müller-Jensen and Hagenah (1976) and Müller-Jensen (1978) mentioned 5 ms for the intraindividual difference, and 18 ms interindividually. The average overall variability amounts to about 30 ms. As compared to other reports, a conspicuously small variation of 4 ms has been reported by Friedman et al. (1967), of 5 ms (intraindividual) by Müller-Jensen and Hagenah (1976), and Müller-Jensen (1978), of 8 ms by Utsumi et al. (1978) and of 4 ms by Pfeifer et al. (1982). It has been reported that CL variations diminish under high-stimulus-intensity conditions (Müller-Jensen and Hagenah, 1976; Cibis and Campos, 1977).

**Age and gender**

Whether the CL does depend on age or not has been discussed by some authors, however, without referring to a possible origin. Kumnick (1956), Borgmann (1972a), Namba et al. (1980), Beaumont et al. (1987) and Grünberger (1987) found no significant age dependency. Petersen (1956), Feinberg and Podolak (1965), Müller-Jensen and Hagenah (1976), and Pfeifer et al. (1983), however, did, and found the CL to increase with increasing age. Feinberg and Podolak (1965) represented the dependency as a regression line: CL = 222.3 + 0.77•age. Pfeifer et al. (1983) showed that CL = 314 + 0.89•age. The latter also showed that, based on dark-adapted pupil sizes, SNS activity diminishes during adulthood. So, if the pupil latency decreases with increasing age, PNS activity certainly decreases. Because the visual acuity decreases with increasing age, Müller-Jensen and Hagenah (1976) related the age dependency to the visual acuity as well. However, they showed that the increase in latency is only caused by age, i.e. by reduced NCV due to age, and not to a reduced acuity.

With respect to similar neurophysiological control systems, distinct age dependencies have been described for the BAEP (Stockkard et al., 1978; Allison et al., 1983; Thivierge and Cote, 1987; Regan, 1989) and the SEP (Allison et al., 1983), and possible origins have been discussed. Spaans (1981) contributed reduction of NCV with increasing age in general to a demyelination of the nerve fibres (see also Kimura, 1987). Dorfman and Bosley (1979) mentioned axonal degeneration, diminution in number and density of nerve fibres, increase of connective tissue, and myelin loss as possible causes.

So far only Drischel (1957) and Grünberger (1987) distinguished between male and female CLs. Both found the female CLs to be shorter than male CLs. Müller-Jensen and Hagenah (1976) and Namba et al. (1980), however, explicitly found no such a difference. The fact that Müller-Jensen and Hagenah (1976) did not find a sex difference with respect to the CL is surprising in view of the small intra- and interindividual differences found by them.

In other neurophysiological control systems, similar latency differences due to gender have been reported in the VEP (Stockard et al., 1979; Allison et al., 1983), the BAEP (Beagley and Sheldrake, 1978; Stockard et al., 1978; Thivierge and Cote, 1987; Regan, 1989), and the SEP (Allison et al., 1983). A possible explanation for the sex differences is sought in the obvious differences in brain weights and sizes (Dekaban and Sadowski, 1978), probably associated with differences in nerve lengths between males and females. For the VEP and BAEP, latency differences are then within the predicted range (Allison et al., 1983).

**Peak constriction-velocity latency**

I am not aware of any normative data ever presented on the latency of the peak constriction-velocity (PCVL) and on its relation to the constriction latency.

**Delay and oscillations**

The pupil system can be made unstable under certain conditions, producing oscillations. This is described in detail in Chapter 4. A prerequisite for these oscillations is delay. An extended delay will, in general, produce oscillations with a larger period. The oscillation period for the pupil system, usually referred to as the pupil cycle time (PCT), has been determined by many authors, both in health and disease (Stern, 1944; Campbell and Whiteside, 1950; Wybar, 1952; Miller and Thompson, 1978a,b; Ukai et al., 1980; Weinstein et al., 1980; Manor et al., 1981; Safran et al., 1981a,b; Manor et al, 1982a,b; Gadoth et al., 1983; Hamilton and Drewry, 1983; Martyn and Ewing, 1984; Sood et al., 1985; Blumen et al., 1986; Martyn and Ewing, 1986; Alio et al., 1987). However, the relationship between the PCT and the delay is still unknown. Consequently, data on the delays determining these oscillations cannot be given.

### 1.4.2 *CONSTRICTION LATENCY IN DISORDERS*

**Optic neuritis and multiple sclerosis**

Most patients suffering from optic neuritis (ON) in the acute phase, characterized by a decrease in visual acuity without abnormalities in media or fundus, recover within a few weeks (Bradley and Whitty, 1967). Follow-up of these patients may also reveal signs of demyelinating lesions outside the optic pathway. In 1884 Parinaud had already described a relation between ON and multiple sclerosis (MS). About half of the patients suffering from MS have a clinical episode of ON at some time during the course of the disease (van der Poel, 1985). Furthermore, in several investigations progression to MS has been observed in 8% to 85% of the patients who have suffered from ON (Cohen et al., 1979). Why and how this happens is not yet understood. It is known, however, that an impairment of the optic nerve may cause an abnormal latency prolongation of both the pupil system and of the major peak in visual evoked potentials (VEP). The afferent pathways leading to both responses are thought to be identical in mediating both evoked responses up to the posterior third of the optic tract (Ohba and Alpern, 1972; Alexandridis, 1985). It is therefore reasonable to assume that at least some of the characteristics of VEP latency prolongation do also hold for the pupil. Moreover, VEP abnormalities in ON have been described extensively, such as by Halliday et al. (1972, 1973a,b) and McDonald (1977).

It was argued that in ON and MS with abnormal VEP latencies, a severe demyelination leads to a total conduction block of nerve fibres. Less severe degrees of demyelination were argued to result in impaired transmission due to impaired NCVs and to the increased refractory period by which faster pulse trains are not transmitted properly (McDonald and Sears, 1970; Rasminsky and Sears, 1972; Halliday and McDonald, 1977; McDonald, 1977; Ellis, 1979). Vice versa, if a pupil or VEP abnormality is found, it is contributed to a disfunctioning (demyelination) of the afferent visual pathway.

Abnormal CL prolongation due to inflammations, as in ON, has been described by Lowenstein (1954), Thompson (1966), Müller-Jensen (1978), Müller-Jensen and Zschocke (1979), Argyropoulos et al. (1980) and Alexandridis et al. (1981, 1982). Ellis (1979) and Müller-Jensen and Zschocke (1979) described VEP and pupillometric results in ON. Ellis (1979) encountered difficulties when dealing with latencies because of a limited time resolution and, consequently, inaccurate detection of the moment of constriction onset. That paper therefore described spatial rather than temporal aspects, though the results have shown that pupillary abnormalities are a constant feature of acute ON.

Besides a neural cause, pupillary abnormalities may be due to retinal disfunction as well. But latency prolongations found by Alexandridis et al. (1982) cannot be explained solely by the reduction in light perception. The authors therefore stated that, especially by using high (photopic) stimulus intensities, "pupil latency prolongation is, at least in part, due to slow-down of neural transmission velocity".

According to Müller-Jensen and Zschocke (1979) quantification of the CL can be used for obtaining additional information on the location of the optic nerve and/or tract impairment as revealed by VEP measurements. On the other hand, they state that "based only on pupillography, disturbances between the efferent and afferent arcs of the pupillary reflex cannot be differentiated". An unequivocal relation between pupil latencies and VEP latencies, however, has not been found so far.

Compared to the amount of literature describing VEP abnormalities, the literature describing pupil abnormalities is sparse. This holds especially for quantitative measurements of the aforementioned temporal parameters.

**Diabetes mellitus**

The involvement of the autonomic nervous system (ANS) in diabetic neuropathy makes the pupil reflex also of interest in research on diabetes mellitus (DM). In addition to possible impairment of the sympathetic nervous system (Hayashi and Ishikawa, 1979) or iris disfunction, CL prolongation up to about 60 ms has been described in connection with DM by Friedman et al. (1967), Namba et al. (1980), Pfeifer et al. (1982, 1984) and Hreidarsson and Gundersen (1985). There is evidence that in diabetic patients prolonged CL is caused by reduced parasympathetic activity (Pfeifer et al. 1982).

Increased VEP latencies (VEPL) in diabetic patients have also been reported (Puvenendran et al., 1983; Cirillo et al., 1984; Pozzessere et al., 1988; Algan et al., 1989). These VEPL prolongations indicate that the afferent arc can be impaired in diabetic patients, but Hreidarson and Gundersen (1985) indicated that possible damage to the efferent pathway may not be excluded in DM. There are, however, no available data presenting pupil and VEP data from the same diabetic subjects.

**Myotonic dystrophy**

The pupil has also been studied in some investigations concerning myotonic dystrophy (MyD). MyD is an autosomal, dominantly inherited, multi-system disorder. Weakness, wasting, and myotonia of skeletal muscle are the most prominent symptoms. A still unanswered question is whether the ANS is

involved or not. Pupillometry was thought to be useful in answering this question because the pupil depends both on the ANS and on muscular function. So far, however, mainly qualitative results have been published (Saenger, 1902; Bramwell and Addis, 1913; Maas and Zondek, 1920; Scharnke and Full, 1920; Kehrer, 1923; Guttman and Stokes, 1939; Maas and Paterson, 1947; Morone, 1948; Kyrieleis, 1958). More recently, Thompson et al. (1964) and Bird et al. (1984) presented quantitative data including the latency of the pupil constriction. Thompson et al. (1964) compared pupil data of 15 MyD patients with normative data. A, what they called "slightly", prolonged CL of 325 ms was found in MyD versus 250 ms in healthy subjects. The PCVL was prolonged significantly, but quantitative data were not given. Bird et al. (1984) found three of five patients showing a prolonged CL, but the population mean did not differ significantly from normal (372 ±54 ms versus 340 ±30 ms). They also could not detect a prolongation of the pattern reversal VEPL. The authors of the two last references were not able to conclude the involvement of the ANS in abnormal pupil behaviour in MyD. Furthermore, a sluggishness of the near reflex in MyD, as demonstrated by Thompson et al. (1964), suggests at least an efferent defect. This efferent defect may include a defective iris-muscle performance. Thompson et al. (1964) also stated that the iris may get rigid after a long lasting spastic miosis, even if the origin of the impaired pupil reflex is a neural one.

In Appendix A a tabular summary is included reviewing and extending the above information in chronological order.

# 2. MEASUREMENT AND STIMULATION OF THE PUPIL LIGHT REFLEX

## 2.1 INTRODUCTION

Although Donders (1866) was able to give an estimation of the CL based on subjective observations, the accuracy obtained was far too low to detect possible differences. It is this interest in possible CL differences in this thesis that makes precise recording indispensable. Besides exact registration of the pupil size, stimulation of the eye with well-defined stimuli is also essential.

Assessment of the pupil size can be performed in various ways. Measuring techniques formerly consisted of subjective *entoptic methods* (Broca, 1924; West, 1988). These involved the projection of the edge of the pupil on the retina by means of a small (point) light source (Fig. 7). The subject is then able to observe his own pupil.

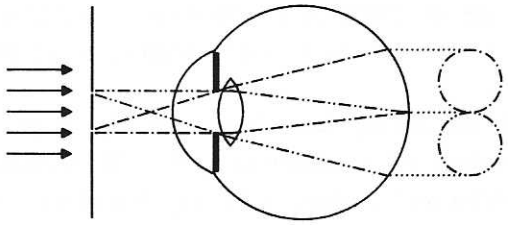

*Fig. 7. Principle of the entoptical pupillometer. When two pinholes, illuminated from behind and serving as light point sources, are held in front of the eye, two images of the pupil are projected onto the retina. When the distance of the two pinholes equals the apparent pupil diameter, and the eye is in the unaccommodated state, extra-ocular parallel rays will focus on the retina and the retinal images will touch.*

The earliest published data concerning the CL (Donders, 1866) were based on this method. Later on *photographic methods* using visible light were applied. These methods consisted of ordinary photography of the pupil, sometimes with motor-driven cameras taking up to 20 pictures per second. *Cinematography* was actually only a small improvement, although higher speeds could be achieved (high-speed cinematography). *Kymography* consisted of the exposure of a film, moving at constant speed through a narrow slit positioned perpendicular to the direction of film transport, continuously giving the pupil size as a black amplitude-modulated band on the developed film. Because of the interference

between the light, necessary for observing the pupil, and the pupil light reflex itself, Garten (1897) introduced the use of invisible light. He has been the only one using ultraviolet light for this purpose. Since about 1940 infrared light was used. Matthes (1941) introduced the detection of the overall reflected infrared light by light-sensitive transducers. This yielded a continuous relative measure of the pupil size. Such techniques are generally called *infrared reflecting (IR) techniques*. The term may not seem well-chosen, because all techniques using infrared light are based on the reflection of the light by the eye. Most pupillometers used infrared light only to replace visible light for photographic methods. Lowenstein and Loewenfeld (1958) developed a *scanning technique*, using a small beam of infrared light which scanned the eye. The reflected beam was analysed for periods of low intensities corresponding with absorption of the light by the pupil. Taking the velocity of the scanning beam into account, this yielded the size of the pupil. From 1950 onwards the image of the eye could also be recorded by means of a television camera. The *TV pupillometers* that are available nowadays have a processing unit incorporated, running software. More or less sophisticated algorithms can provide the pupil size, and eventually some other parameters of the measured pupil light reflex may be calculated. The name *PC pupillometer* would be more appropriate for the latter type of TV pupillometer, based on personal computers with a frame grabber.

Both infrared reflecting techniques and TV or PC pupillometers have their specific advantages and disadvantages. With IR techniques, the pupil size can be recorded continuously, whereas TV pupillometers yield the pupil size only 60 times per second at its most. As opposed to IR techniques, a major advantage of TV pupillometers is their possibility to provide a calibrated signal, i.e. the pupil diameter in mm or the pupil area in $mm^2$. Applying TV techniques, however, is expensive. TV equipment for recording one pupil costs over 30,000 US$, whereas IR techniques for recording both pupils simultaneously cost less than 10,000 US$.

Because small latency differences may also have to be taken into consideration (< 25 ms, conform pathological VEPL differences), the time resolution of the pupillometer has to meet the highest requirements. For this reason an infrared-reflecting technique was chosen for the study described in this thesis. As such devices were not commercially available, the IRIS system was developed in cooperation between the 'Vakgroep Medische Fysica' and the 'Instrumentele Dienst' of the 'Academisch Ziekenhuis Vrije Universiteit', Amsterdam (Reulen et al., 1988).

## 2.2 IRIS SYSTEM

The IRIS pupillometer is basically a modification of the Heidelberg pupillograph (Alexandridis, 1968; Alexandridis and Krastel, 1972). It is based on the principle of reflection and absorption of invisible infrared light by the iris and the pupil, respectively. The smaller the pupil, the larger the iris, and consequently more infrared light will be reflected (Fig. 8).

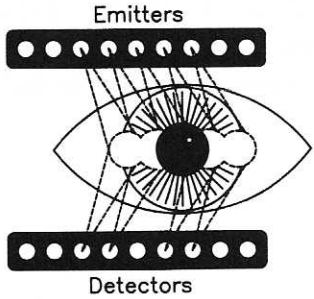


*Fig. 8. Principle of projection, reflection, absorption, and detection of infrared light by the IRIS-system/eye combination. Actual projections are not as distinct as sketched.*

This principle was first applied by Matthes in 1941. It has since been used by several others (Tschirren, 1947; Cüppers, 1951; Drischel, 1957; Stark and Shermann, 1957; Künkel, 1961; Alexandridis and Krastel, 1972; Müller-Jensen and

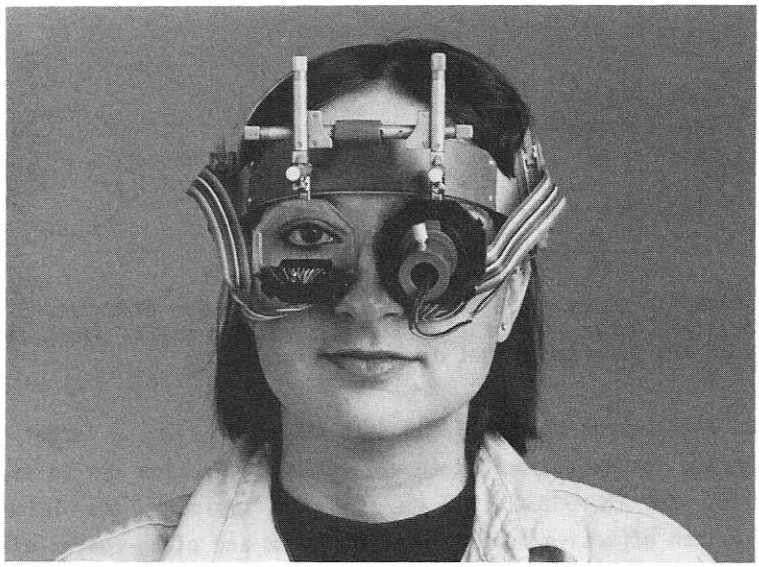

*Fig. 9. Subject wearing the IRIS head frame with the Maxwellian view stimulator mounted in front of one of the eyes.*

Hagenah, 1976; Argyropoulos et al., 1980). Our IRIS pupillometer (Reulen et al., 1988) illuminates the central part of the eye by five small photo-diodes. The infrared light is on for only 1/4th of every millisecond to minimize the radiated energy and to reduce noise. Five photo-transistors detect the reflected infrared light, only during the periods of infrared light emission. Emitter and detector arrays are mounted in units which can be positioned about 3 cm in front of both eyes, and can be adjusted in three dimensions. The whole set-up is rigid with respect to the head and eyes, and insensitive for head movements made by the subject (Fig. 9). With the IRIS pupillometer, signals of both eyes are recorded simultaneously. It has been shown that the signals are linearly related to pupil area. For more details reference is made to Reulen et al. (1988).

## 2.3 MAXWELLIAN VIEW STIMULATOR

For experimental control of the retinal illuminance independent of the pupil size, a Maxwellian view stimulator has been used. This stimulator (Fig. 10) images a small yellow LED in the centre of the pupil by means of two lenses in such a way that the iris can never mask incoming light. The retinal illuminance is then independent of the pupil size.

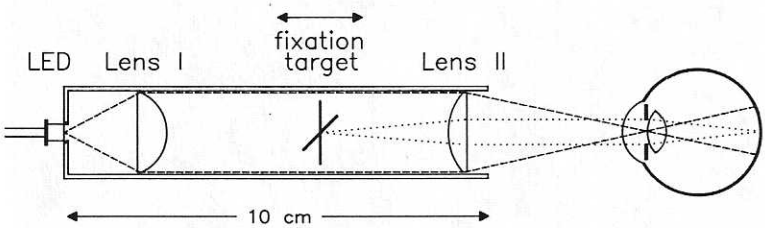


*Fig. 10. Schematic representation of the Maxwellian view stimulator. The dashed lines delimit the outer rays of the illumination. The dotted lines indicate the projection of the fixation target on the retina.*

For maximum elimination of the bias due to the near triad, a target is mounted between the two lenses to facilitate eye-movement fixation and to avoid accommodation. The LED (HLMP3850, 583 nm, 140 mcd typical), the lenses (plano-aspheric with an effective focal length of 17 mm and plano-convex with a focal distance of 35 mm), and the fixation target are placed in a tube (diameter 20 mm, length 100 mm) which can easily be mounted onto the IRIS system in front of one of the subject's eyes (Fig. 9). Accordingly, the retina is illuminated

centrally with a circular field enclosing a total angle of approximately 30 degrees. To set a standard for accommodation, the fixation target is conjugated with the subject's far point at the start of each experiment. This rigid fixation keeps Maxwellian view conditions adjusted properly, even during head movements, once they are established. Cross-talk between the visible light radiated by the stimulus LED, and infrared detectors of the IRIS system, can be avoided by keeping the stimulator LED current at zero when the infrared detection is on every 1/4th of a millisecond. A diagram showing this principle is given in Fig. 11.

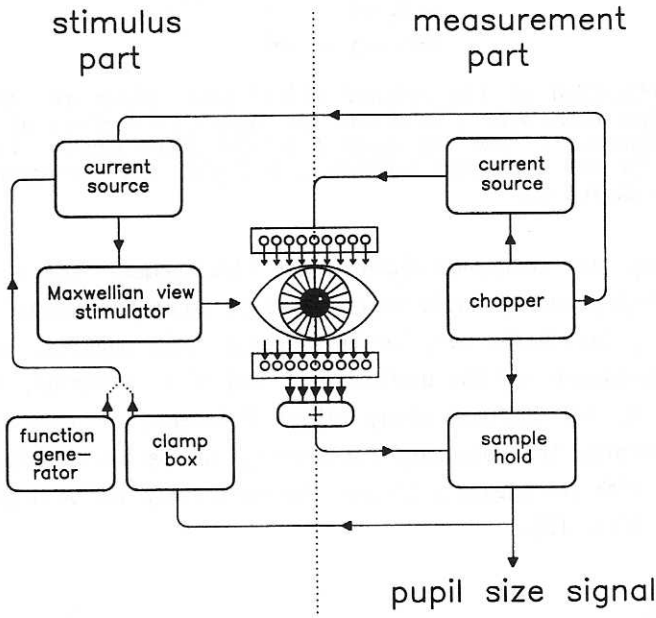


*Fig. 11. Principle of the IRIS pupillometer.*

**Calibration of retinal illuminance**

For quantitative measurements concerning the visual system, quantification of the amount of light incident on the retina will be of great importance. If the stimulus device is of a Maxwellian type, the appropriate measure of light is the retinal illuminance (Er) in Troland (Td). Nygaard and Frumkes (1982) presented an improved and easier technique for calibrating the retinal illuminance provided by a Maxwellian view device, originally given by Westheimer (1966). They showed that the retinal illuminance can be expressed as

$$Er = 10^6 \cdot E \cdot d^2.$$

Here E represents the illuminance (in lm/mm²) as measured by an illuminometer, and d (in mm) the distance between the reference plane (R) of that illuminometer and the source image (S) of the Maxwellian view device (Fig. 12).

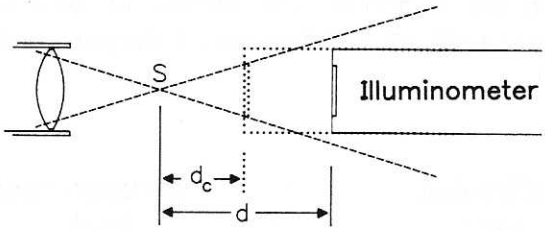


*Fig. 12. Calibration of the retinal illuminance. Shown are the end of the Maxwellian view stimulator (left) and the detection surface of the illuminometer at a distance d from the image S of the light source. If the emission cone just fills the detection surface with light, the corresponding critical distance d is called $d_c$.*

In their set-up, the reference plane of the illuminometer should be completely filled with light, and d should be at least 10 times the radius of the source image. However, this holds only for homogeneous light bundles, where according to the inverse-square law the product of E and $d^2$ is constant, independent of the distance d. For devices using ribbon filaments or carefully apertured tungsten filaments, the necessary homogeneity can be reasonable. But, with a LED, combined with two separate lenses, the result may not be a perfect uniform emission lobe (Fig. 13).

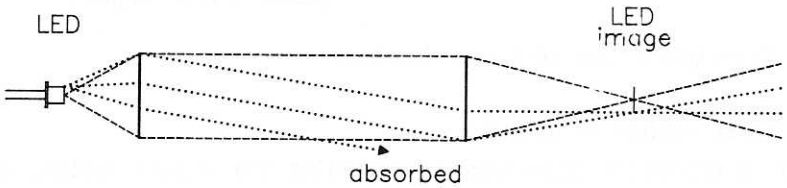


*Fig. 13. Inhomogeneous illumination by the Maxwellian view stimulator caused by the distance between the two lenses. Some rays coming from the periphery of the first lens are absorbed by the tube before reaching the second lens. This results in a decreasing paraxial intensity.*

In such a case the intensity of the central part of the illuminated zone will be greater than that of the peripheral part. The illuminometer will then detect

more of the central (=brighter) part at large distances d than at small distances. This results in a distance-dependent calculated retinal illumination. Fig. 14a shows the results of a measurement with the Maxwellian view device described above, which gave a subjectively homogeneous illumination.

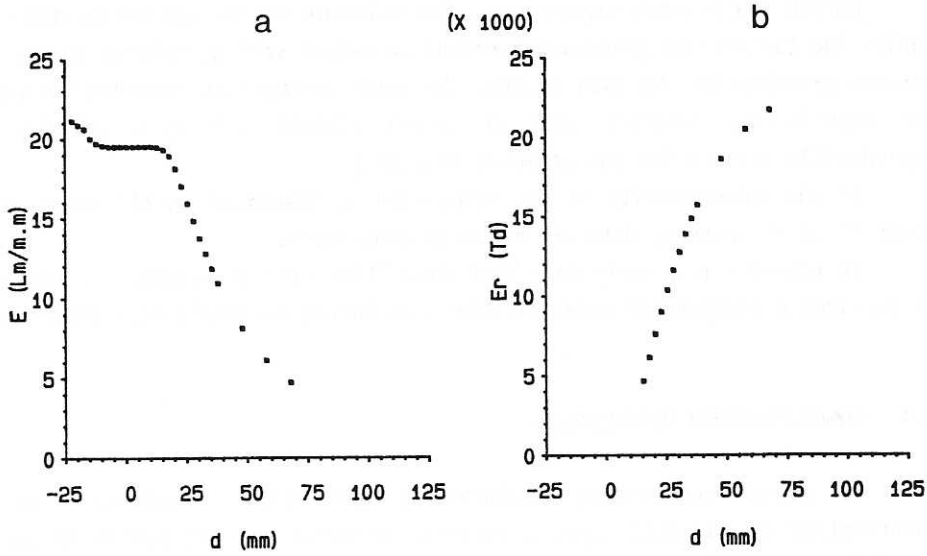


*Fig. 14. (a) Illuminance (E), and (b) retinal illuminance (Er) versus distance (d) between source image and detection surface according to the setup shown in Fig. 12.*

The distance d between the source image S and the detection surface R (Fig. 12) was changed also for $d<d_c$, i.e. beyond the range valid according to Nygaard and Frumkes (1982). As long as all light is kept within the limits of the detection surface, the luminance does not change, indicating the integrating properties of the illuminometer, as is shown by the plateau in Fig. 14a. Retinal illuminance was calculated only for $d>d_c$. A dramatic failure of the inverse-square law for inhomogeneous devices is shown in Fig. 14b. Here the calculated retinal illuminance is not constant, but varies by a factor of over 3.5.

For an unambiguous calibration, the average retinal illuminance can be determined. For this, an amendment will be given, by combining the methods presented by Nygaard and Frumkes (1982) and Buck and Makous (1982). The determination of the average retinal illuminance necessitates the taking into account of all the flux coming from the source image. This can be done by choosing $d=d_c$. In practice, $d<d_c$ can also be chosen (for example d=0) subject to the integrating properties of the detection surface of the illuminometer.

Although $d<d_c$ can be chosen, $d=d_c$ has to be substituted in the above equation to calculate the mean retinal illuminance. In practice the value $d_c$ to be substituted can be calculated based on the optics of the Maxwellian view device and the detection surface of the illuminometer, or be read from a curve such as shown in Fig. 14a.

During the present experiments, the stimulus device was calibrated regularly. The calibration procedure involved an output voltage related to the LED-current provided by the IRIS system. The same voltage was recorded throughout the experiments, together with the pupil signals, giving a reliable and reproducible measure for the stimulus intensity.

If the inhomogeneity of the stimulator is identical in all experiments, which it is at present, data are mutually comparable.

In Appendix B a newly developed Maxwellian view stimulator is described. It provides a homogeneous emission lobe, but became available only recently.

## 2.4 COMPUTER-AIDED PUPILLOMETRY

To avoid long-lasting elaborations and noise, resulting from the intervention of magnetic tape, a personal computer was programmed to perform the following options. At first, pupil data had to be acquired by the system and converted to binary data. Secondly, it had to be possible to display the converted data on the CRT of the computer, making the use of a separate oscilloscope redundant and improving portability. A third option for increasing the utility of the system was to provide the stimulus signal simultaneously with the acquisition. Fourthly, the recorded data had to be analysed, so that the parameters of interest could be extracted as objectively and accurately as possible.

A personal computer (PC) with additional analogue-to-digital (A/D) and digital-to-analogue (D/A) convertor was programmed to perform the options mentioned. The PC used was a portable Olivetti M21. The programme was mainly written in FORTRAN-77. The routines necessary for signal sampling and plotting on the CRT and those to output the stimulus signal were written in assembly language. In this way high sample rates could be assured (> 1000 Hz/chn). A one-to-one relation between the sampled data and real time was maintained by running the sample and display routines in background mode with the highest possible priority. Interactively, without interrupting the sampling, scaling parameters for the display of the signals on the screen could be changed. All parameters had preset values, that could be changed by means of the same user

interface. The whole set-up: spectacle frame, electronics, and PC (Fig. 15), were all together confined to the manageable size of about 50x50x50 cm. After trial recording, data were stored on disk for off-line analyses. Off-line analysis was chosen in order to achieve general applicability of the computer programme. Data analysis, i.e. searching for special events, was performed with dedicated algorithms. These allowed control by hand, if necessary, via the display of the data on the CRT. In addition, the programme allowed digital filtering, differentiating, X-Y plotting, auto- and cross-correlation, curve-fitting, etc. Data could be exchanged between the programme and additional software, such as the statistical and graphical software package STATGRAPHICS.

During the past few years the software has been developed into an easy-to-use package that can be run on several IBM compatibles. At present it supports CGA, AT&T, EGA, VGA, and Hercules graphics. Several A/D-D/A convertors are supported, such as Scientific Solutions Labmaster, Keithley 500, Metrabyte DASH-16, and Analog Devices RTI-800/850.

In performing the experiments, three signals were always recorded: one representing the stimulus intensity, and two for the pupil signals of both eyes. A sample rate of 200 Hz was chosen to acquire pupil responses. Hence, temporal resolution of parameters to be measured was at least 5 ms.

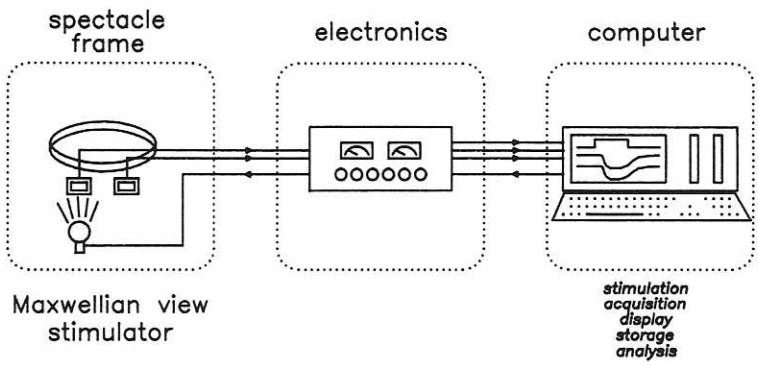


*Fig. 15. Computer-aided pupillometry. Diagram of the complete pupillometry unit: The IRIS pupillometer with the Maxwellian view stimulator, the electronics incorporated in the pupillometer, and a personal computer for generating the stimulus and recording, display, storage and analysis of the pupil signals.*

# 3. CONTRAST SENSITIVITY

## 3.1 INTRODUCTION

In most investigations concerning the pupil, some sort of homogeneous stimulus with varying overall luminance is applied to evoke a response. It has been reported that the pupil reacts on spatially structured stimuli as well, even if the total amount of light remains constant (van der Kraats et al. 1977; Slooter and van Norren, 1980; Slooter, 1981; Ukai, 1985; Barbur and Forsyth, 1986; Barbur and Thomson, 1987). This can be achieved by presenting a checkerboard reversal or by presenting an appearance-disappearance stimulus. In presenting a checkerboard reversal, two sets of squares, A and B of Fig. 16, are modulated in counterphase, and each reversal results in an equal amount of constriction.

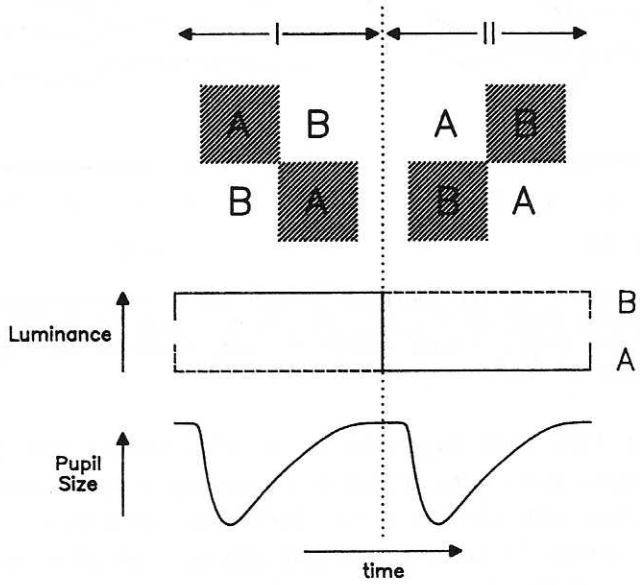


*Fig. 16. Sketch of a pupil response (bottom line) to a pattern reversal (top). If two sets of checks, A (solid luminance line) and B (dashed luminance line), of a checkerboard are modulated in counterphase, equal responses appear during the periods I and II.*

Furthermore it was found that in decreasing the check size, the response amplitude decreased also. Moreover, the response amplitude correlated well with

the psychophysically assessed visual acuity. The latter two phenomena were first reported by Slooter and van Norren (1980), and confirmed by Ukai (1985), Barbur and Forsyth (1986), and in our laboratory. Specimen pupil responses of two healthy subjects, with different visual acuities looking at a pattern reversal of different check sizes, are given in Fig. 17. The response amplitude of a subject with a visual acuity of 1.25 does not decrease significantly, while that of another one with an acuity of about two times less becomes smaller with decreasing check size.

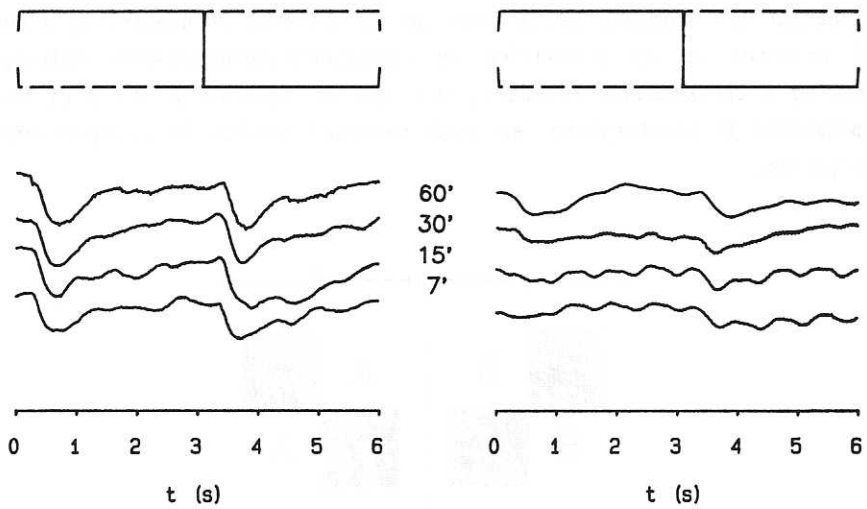


*Fig. 17. Pupil responses of two subjects evoked by a checkerboard reversal (upper traces) at check sizes of 7', 15', 30' and 1°. Results at the left were obtained from a subject with a visual acuity of 1.25, those at the right from a subject with an acuity of 0.65.*

The responses have been suggested to be elicited by the change in contrast, and the pupil system was suggested to include a neural pathway from the lateral geniculate body via the visual cortex to the pretectal nucleus, because the visual cortex is known to contain neurons sensitive to contrast modulation (Slooter, 1985). These considerations together opened promising avenues for an objective visual acuity assessment.

The literature, as already mentioned, only reports about the contrast origin of these pupillary constrictions. The term contrast refers here to the (relative) difference between luminances of adjacent fields, i.e. to spatial contrast. This spatial contrast is generally changed experimentally by changing the local-luminance distribution, i.e. by changing adjacent luminance

differences. The latter is also called temporal contrast. At present I will adapt the nomenclature by calling contrast 'spatial contrast' and local-luminance changes 'temporal contrast'. This points directly to the difficulty in distinguishing between the fundamentally different contrast and local luminance. However, under these circumstances these local-luminance changes may still generate the pupil reflex instead of the contrast. This possibility has been overlooked so far. It is therefore sorted out here which of the two origins holds for the reflex under consideration: is it contrast or is it local luminance?

## 3.2 THEORETICAL CONSIDERATIONS

The problem was to find an appropriate spatially structured stimulus for distinguishing between contrast and local-luminance change, Fortunately, a solution had already been given by Spekreijse et al. (1972) and Riemslag et al. (1985) for the distinction between the possible origins of the pattern visual-evoked potential and the pattern electroretinogram. They proposed a number of trials. Within each trial one set of checks (A in Fig. 18) is modulated tempo-

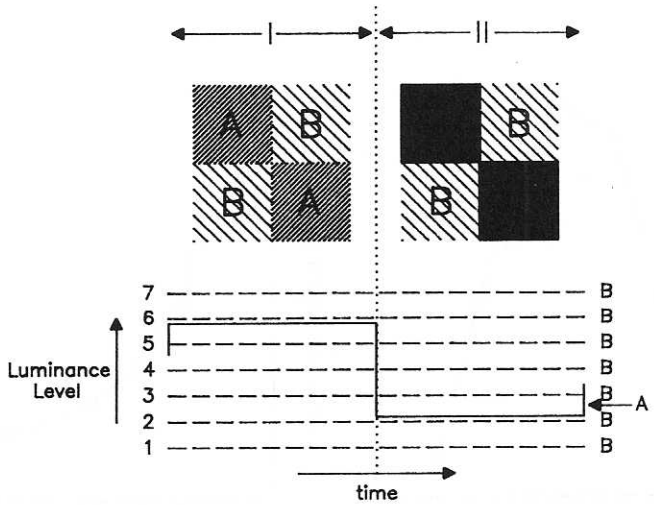


*Fig. 18. Stimulus to distinguish between contrast and local luminance. Only one set of checks, A, is modulated temporally according to the solid line at the bottom over fixed amplitudes (levels 2 and 6 in this example), while the luminance level of set B is kept constant (dashed lines) within every trial, but is adjusted at different levels over the different trials.*

rally with fixed amplitudes, while the other set (B in Fig. 18) is kept constant at different levels for the separate trials. If, for example, the luminance of set A changes between the levels 2 and 6, and set B is kept constant at level 2, an appearance-disappearance stimulus is generated, in which the contrast arises at luminance increase. If set B is kept constant at level 6, also an appearance-disappearance stimulus is generated, but contrast then arises at luminance decrease.

The changes in contrast (dC) as well as the relative local-luminance changes (dL) can be calculated based on the luminances of sets A and B during the periods I and II, applying the Michelson contrast definition, and taking into account the Weber law respectively , according to:

$$dC = \frac{A_I - B}{A_I + B} - \frac{A_{II} - B}{A_{II} + B} \qquad \text{and} \qquad dL = \frac{A_I - A_{II}}{B}.$$

Of course, this yields different values whenever the luminance of set B is varied from level 1 to level 7 over the different trials. Essential for this experiment is that, when the changes in contrast and luminance are both plotted versus the level of the unmodulated checks, theory predicts a peak in contrast change, while the relative local-luminance changes decrease monotonically. If the Weber-Fechner law is taken into account and the true illuminances of the

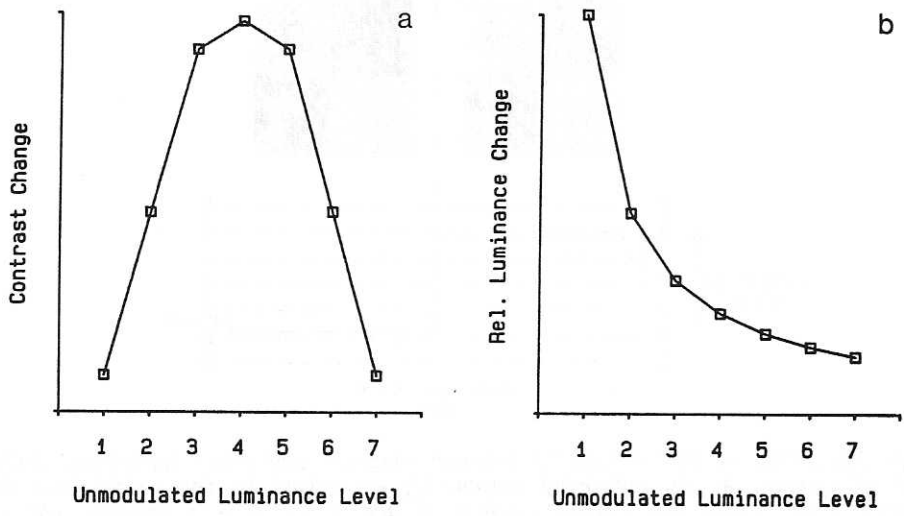


*Fig. 19. Theoretical changes in contrast (a) and relative luminance (b) versus the level of the unmodulated set of checks.*

sets A and B are chosen as a power function of the levels 1 to 7 (see below), the curves of dC and dL versus the unmodulated luminance resemble those drawn in Fig. 19.

The luminance levels were originally chosen according to Riemslag et al. (1985), where modulation depth varies to a minimum of 50% when B is maximal. In this case the contrast curve is not symmeterical (Fig. 19a). The peak location of dC versus B is given by ddC/dB = 0, and therefore

$$\frac{ddC}{dB} = \frac{2A_{II}}{(A_{II}+B)^2} - \frac{2A_I}{(A_I+B)^2} = 0 \quad \Rightarrow \quad B = \sqrt{(A_I A_{II})}.$$

This explains why the dC-versus-B curve is asymmetrical if the luminances of A and B are chosen according to a linear scale. If, hoewever, the luminances are chosen according to

$$A_I' = g^{A_I},\ A_{II}' = g^{A_{II}} \text{ and } B' = g^B$$

with g arbitrarily, then the peak is located at

$$B' = g^B = \sqrt{(g^{A_I} g^{A_{II}})} = g^{\frac{1}{2}(A_I+A_{II})} \quad \Rightarrow \quad B = \tfrac{1}{2}(A_I+A_{II}).$$

It can subsequently be shown by mathematical analysis or in a graphical manner, as depicted in Fig. 19a, that in the exponential case dC is symmetrical around $\frac{1}{2}(A_I+A_{II})$. Moreover, the latter case has the advantage over the linear case of giving a more pronounced peak in contrast dependency. Furthermore, at greater modulation depths, a luminance response may dominate the contrast response. An additional experiment was therefore performed with the true illuminances chosen exponentially increasing and minimum modulation depth as small as practically possible.

In the stimulus described, the over-all illuminance is not constant, as in a real pattern reversal. When presenting a true pattern reversal, the responses, due to the modulation of both sets of checks over both periods I and II, are added by the pupil system. If the periods I and II are equally long, this leaves only even harmonic responses, since all odd harmonics are added to zero (Fig. 20). For the stimulus as described by Riemslag et al. (1985), the responses may contain odd harmonics as well. Hence, a valid comparison between the responses to be measured requires a comparison between the even harmonics only. If the periods I and II are equally long, it does not matter whether the responses of A and B are summed simultaneously, or whether they are added over

the periods I and II. For obtaining only the even harmonics in the single reversal case, the responses will therefore have to be added artificially over the periods I and II.

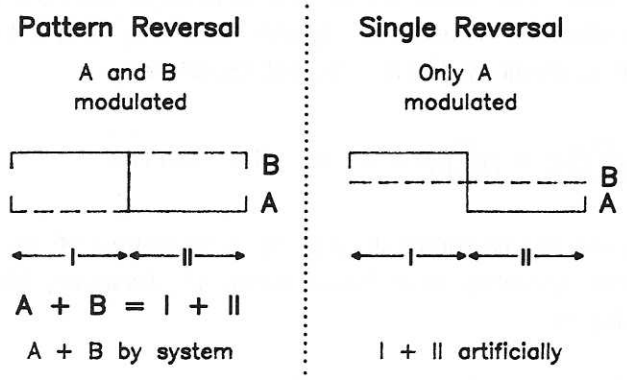


*Fig. 20. Spatial versus temporal addition of responses. In a true pattern reversal (left) where both sets A and B are modulated, the responses to A and B are added by the pupil system, cancelling all odd harmonics. When only one set of checks is modulated (right), the response consists of even as well as of odd harmonics. To cancel the odd harmonics in the single reversal case, the response over the intervals I and II can be added artificially.*

For the distinction between the contrast and luminance origin of the pupil reflex, the remaining question now is whether the amplitude changes of the even harmonics over the different trials follow the contrast curve or the luminance curve according to Fig. 19a and 19b, respectively.

## 3.3 EXPERIMENTAL CONDITIONS

### Stimulus

The stimulus as described was presented on a computer-controlled TV monitor enclosing a square field of 15 x 15 degrees, viewed at a distance of 1 m. Experiments were performed with four different check sizes (7', 15', 30' and 1°), using whole numbers of checks only. Subjects were asked to fixate at the central contrast transition, indicated by a little fixation target on that spot. The period of one complete stimulus cycle always amounted to 6 s (period I = period II = 3 s), in order to ensure total restoration (escape) of pupil size. Maximum illuminance amounted to 245 cd/m$^2$. Two different level settings were chosen, resulting in a minimum modulation depth of 50% and 8%. The

luminance levels at 8% minimum modulation depth are chosen exponentially increasing.

**Pupillometry**

Although the IRIS pupillometer yields a relative measure of the pupil size, it could be used for the present purpose, because it has been shown to yield a signal linearly related to pupil size. So, changes in pupil amplitude could be compared reliably within one experiment as long as the gain and mechanical adjustment of the emitters and detectors in front of the eyes were not changed. The ultimate response of every trial was calculated by averaging over at least 20 artefact-free periods, whereby responses of both eyes were recorded. To obtain even harmonic responses only, the ultimate response was separated in two equal periods, one from 0 to 3 s (period I) and one from 3 to 6 s (period II), which were finally added. For every subject the maximum amplitude within one experiment was set at 100%, to which all other amplitudes were related.

Before each experiment the subjects were adapted to the lowest luminance level of the stimulus for 10 minutes.

**Subjects**

The experiments were performed with seven young subjects (mean age 31 years) with good vision, normal acuity, normal pupillary behaviour and no ophthalmological defects evident at routine examination. All subjects were submitted to the 50% modulation-depth stimuli, and one in addition to the 8%-modulation depth.

## 3.4 RESULTS

As an example, Fig. 21 represents one set of trials for one subject. On the left the averages of the raw data are shown for one complete stimulus cycle, and on the right the sum of the second harmonic responses are shown. For all subjects, onset to peak amplitude of the even responses was thus determined and averaged separately for every trial. These averages were plotted against the luminance level of the unmodulated checks, which is shown in Fig. 22a. In Fig. 22b the even harmonic response amplitude is shown for the minimum luminance / maximum contrast modulation stimulus. All experiments applying different check sizes yielded similar results: a monotonically decreasing response amplitude without any significant peak around the centre.

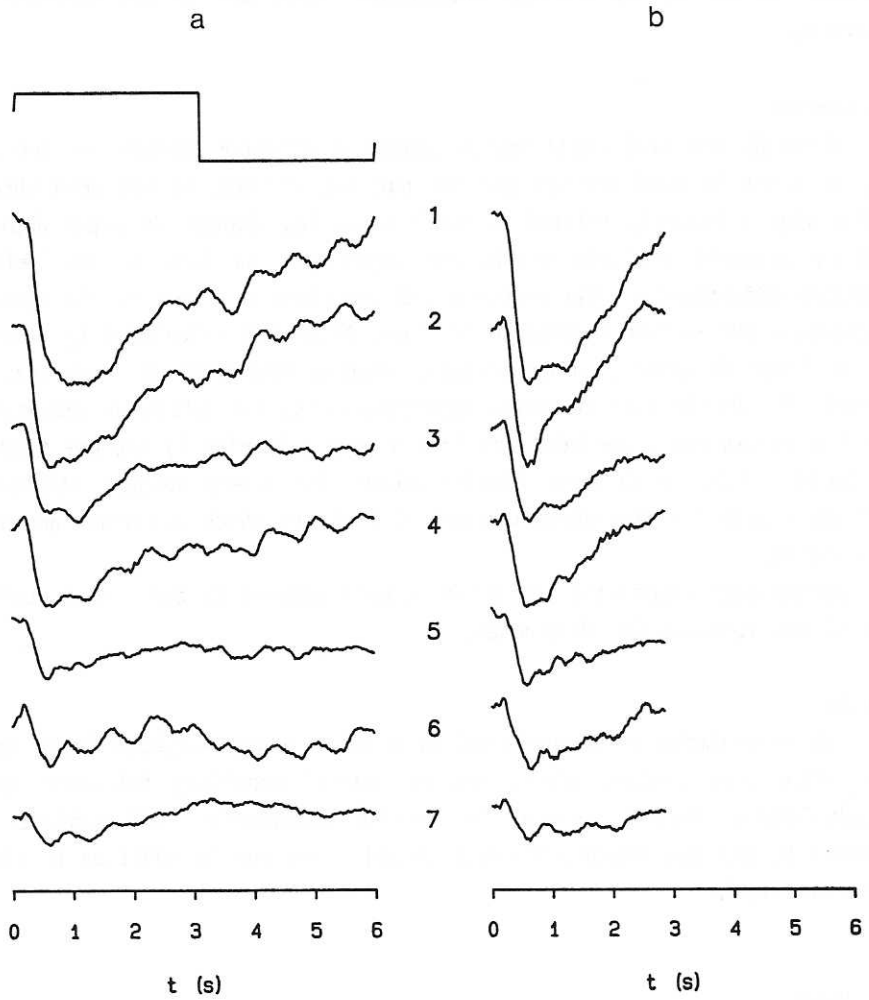


*Fig. 21. (a) Averaged responses of one subject to a single-reversal stimulus (left upper trace) for seven different levels of the unmodulated set of checks. (b) After summation over the intervals I (0 to 3 s) and II (3 to 6 s), only the even harmonic responses result.*

Closer examination of a subset of these trials, in which an appearance-disappearance stimulus is generated, may further clarify the problem posed in this chapter. Basically there are two conditions in which an appearance-disappearance stimulus is generated, one in which the contrast arises at luminance increase (Fig. 23a, upper trace at t = 0 s), and one in which contrast arises at luminance decrease (Fig. 23b, upper trace at t = 3 s). Fig. 23 shows pupil responses at the four different check sizes, for both conditions. In all

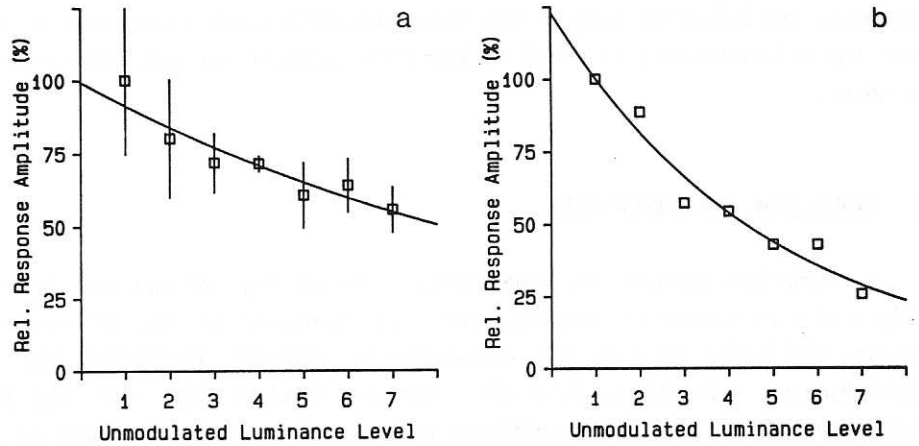


*Fig. 22. Amplitudes of the even harmonic responses from constriction onset to peak amplitude, plotted versus the unmodulated luminance level. Maximum amplitude per subject was set at 100%. (a) Average results at 50% modulation depth for seven subjects (±SD, interindividual), (b) results for one subject at 8%-modulation depth.*

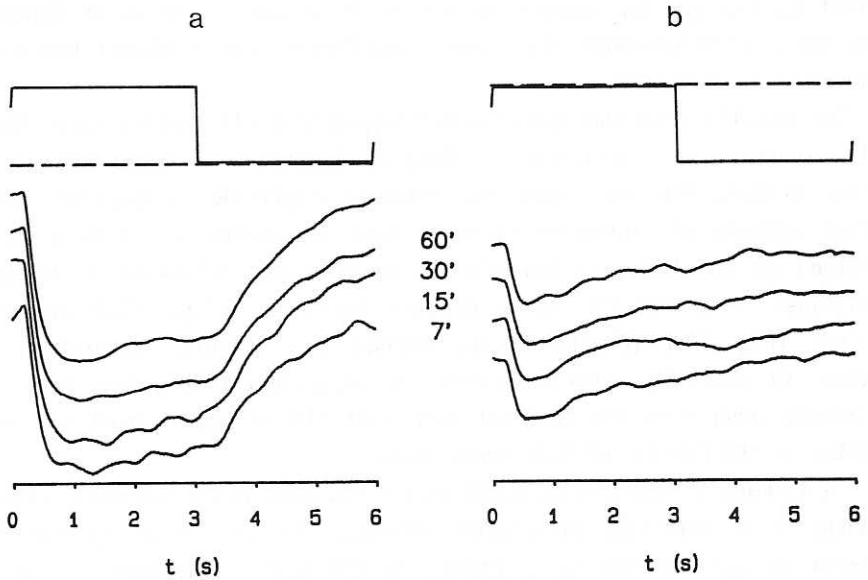


*Fig. 23. Appearance-disappearance stimulus responses. (a) Four responses at different check sizes as indicated of one subject when contrast comes about at luminance increase. (b) Four similar responses when contrast appears at luminance decrease.*

experiments the basic features of the responses under these circumstances were equal: constrictions only occurred at luminance increase and not when contrast came about.

## 3.5 DISCUSSION AND CONCLUSIONS

A comparison between the experimental data of Fig. 22 and the theoretically predicted curves for contrast and local luminance in Fig. 19 reveals a striking similarity between the even-harmonic response amplitudes and the local-luminance modulation. Even when contrast changes most and luminance changes least (Fig. 22b), no significant peak in response amplitude around the middle luminance level of the unmodulated checks was found. This indicates that the pupillary system does not follow the contrast modulation but only follows the luminance modulation.

It can further be concluded that the luminance responses behave as predicted. The steeper descent at the smallest modulation depth is due to the larger range of unmodulated luminance levels over the different trials as compared to the greater modulation depth. At a small modulation depth, and taking the true illuminances as a power function of the mentioned levels, this range necessarily expands.

The results from the appearance-disappearance stimulation show that the pupil responds with a constriction only at luminance increase and not when contrast arises. The fact that the response amplitude is smaller when the contrast appears at luminance decrease than at luminance increase, can be understood on the basis of Weber's law because of difference in background intensities. This results in a capture behaviour (Fig. 23a) and escape behaviour (Fig 23b) (Chapter 1.3). Because all authors, describing pupil reactions to spatially structured stimuli, reported constrictions only, their conclusions concerning the contrast origin of the reflex in question are not supported by the results of this experiment.

In summary it can be concluded that a recorded pupil response, evoked by a change in a spatially structured stimulus, is originated by the local-luminance change and not by a change in contrast. A decrease in response amplitude can be fully explained by blurring of the retinal image by the optic system of the eye.

With respect to the experiments described in this thesis, these findings do not necessitate the use of spatially structured stimuli for investigating

the pupil light reflex. Therefore, a homogeneous stimulus was used throughout the further experiments.

**Model**

To explain the local-luminance origin of pattern-induced pupil constrictions some considerations can assist in composing a model. Stark and Sherman (1957) and Stark (1959) originally used a linear third-order lag of a flux error signal, with disturbance as the effective input for the pupil system. Their model mainly served to explain the high-pass filter (AC) properties of the pupil system, for the pupil mainly reacts to changes in light intensity. Parallel to the AC path a DC gain term was later included to explain the tonic control of the pupil size (Sobel and Stark, 1962; Sandberg and Stark, 1968; Shimizu and Stark, 1977; Sun et al., 1983a). The most important modification of the model consisted of the addition of a rectifier in the AC path to explain the unidirectional rate sensitivity (Clynes, 1961; Sobel and Stark, 1962; Sun et al, 1983a). The model was finally reorganized by Sun et al. (1983) to give it a more sound intuitive representation. Because the pupil reflexes measured within the pattern-reversal experiments showed escape behaviour only, the DC path is not of interest here. Of concern is the AC path, which starts with a log transducer to resolve the Weber-Fechner law. Signals are transported next to a high-pass filter and a rectifier embodying the unidirectional rate sensitivity. In their model the signal is finally transported via the nerves and transformed by the iris muscles as described by a low-pass filter with a time delay. The nature of the pupil responses to checkerboard reversal requires many parallel AC paths. Every path is then originated by a specific part of the retina. For simplicity, the model can be reduced to only two parallel AC paths, each representing the inputs of one of the sets of checks. These considerations lead to the model given in Fig. 24.

When dispersion by the optical system of the eye spreads the image of the sets of checks more evenly over the retinal parts originating the parallel AC paths, the inputs will be diminished. This results in a smaller response amplitude. Normally, this dispersion becomes evident for check sizes smaller than 12' (Ukai, 1985). From 12' downwards the response amplitude decreases, and by the limited resolving power of the eye no responses can be measured for check sizes smaller than 1'. For subjects with a visual acuity of less than 1, illustrated by Fig. 17a, these limits may be higher. This can fully explain the decrease in response amplitude for decreasing check size. Therefore, no extra parameter control is thought to be needed for amplitude regulation.

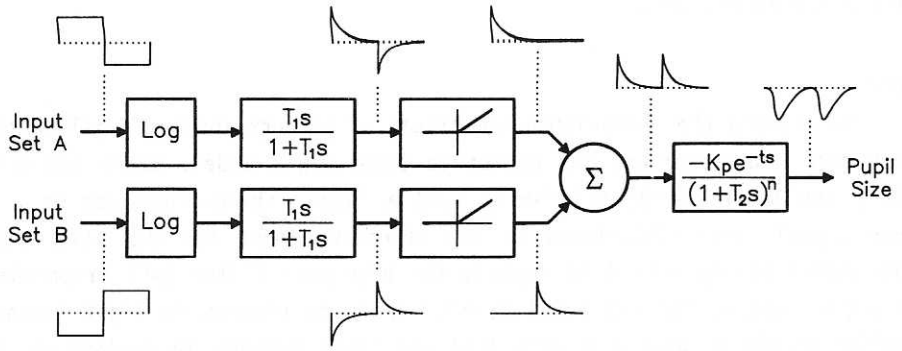


*Fig. 24. Model to explain the pupil response to a spatially structured stimulus.*

**Pupil acuity**

A clinical aim of pupillometry was the objective assessment of visual acuity. This could be of importance for patients suffering from low vision while no defects can be detected clinically. However, the high correlation between psychophysically assessed visual acuities and the thresholds in measuring pupil responses on decreasing check sizes, as described by Slooter (1981), does not justify the use of 'pupil acuities'. The absence of a contrast-sensitive pupil reflex, as demonstrated here, is not in support of a hypothesis in which the visual cortex is involved in the reflex arc. It is possible in a patient with a lesion beyond the geniculate body, to show a normal pupil response to spatially structured stimuli. Therefore, pupillometry cannot asses visual acuity, but it can help in locating the defect.

# 4. OSCILLATIONS INDUCED BY HIGH-GAIN AND EXTENDED-DELAY

## 4.1 INTRODUCTION

An important mechanism to regulate neural activity is feedback. An undeniable feature of human neural-feedback mechanisms in health and disease is their propensity to generate oscillations and other complex dynamical behaviour, such as tremors and the electrical activity of the cortex (Mackey and Milton, 1987; Stark, 1987; Milton et al., 1989). It is well known that the pupil reflex exhibits many features inherent in feedback mechanisms (Stern, 1944; Stark and Cornsweet, 1958; Stark, 1959, 1962a; Milton et al., 1988). The pupil light reflex is a negative feedback control system (Fig. 1). An increase in retinal light flux due to an increase in light intensity is compensated by a decrease in flux due to pupil constriction, and vice versa. An important feature of the pupil reflex is the ease by which it can be manipulated and monitored non-invasively, as alleged before. This chapter deals with a kind of manipulation that results in an instability of the pupil system producing oscillations.

One such manipulation is called edge-light pupil stimulation, which was first described by Lambert in 1760 (Loewenfeld, 1966), and has since been applied widely. A narrow slit of light is focussed on the pupil edge (Fig. 25). Due to pupil constriction the stimulus light is shielded from the retina by the iris and the pupil will dilate. Due to this dilatation the light re-enters the pupil, returning the system in its original state, and the process repeats.

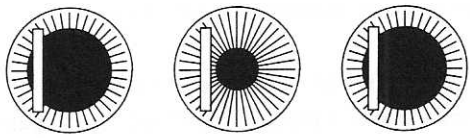

*Fig. 25. Pupil-oscillation induced by edge-light stimulation. Initial state with dilated pupil (left), fully constricted state (centre), and pupil returned to the dilated state (right).*

In edge-light pupil cycling, a change in feedback is accomplished, which, however, is not specified precisely. The edge-light stimulation technique does therefore not suit experimental research. Despite a difference in stimulus conditions, cycle periods reported, referred to as the pupil cycle time (PCT), vary by less than 10% on the average. Reported normative PCTs (in ms) are: 752

(Stern, 1944), 870 (Campbell and Whiteside, 1950), 980 (Wybar, 1952), 822 (Miller and Thompson, 1978a), 862 (Ukai et al., 1980), 806 (Manor et al., 1981), 738 (Gadoth et al., 1983), 814 (Hamilton and Drewry, 1983), 930 (Martyn and Ewing, 1984), 931 (Sood et al., 1985), 946 (Martyn and Ewing, 1986), and 788 (Alio et al., 1987). These periods average to about 850 ±80 ms.

So far, pupil cycling in diseases has only been tested based on this edge-light induction method (apart from the above cited references, see also Miller and Thompson, 1978b; Weinstein et al., 1980; Safran et al., 1981a,b; Manor et al, 1982a,b; Blumen et al., 1986).

Another way of manipulating the feedback of the pupil system is called environmental clamping (Stark and Sherman, 1957, Stark, 1962b). Clamping refers to an experimental technique in which the feedback loop of the reflex is first opened by applying Maxwellian view, and then reclosed with an external device relating changes in pupil size to changes in light intensity. This principle is outlined in Fig. 26.

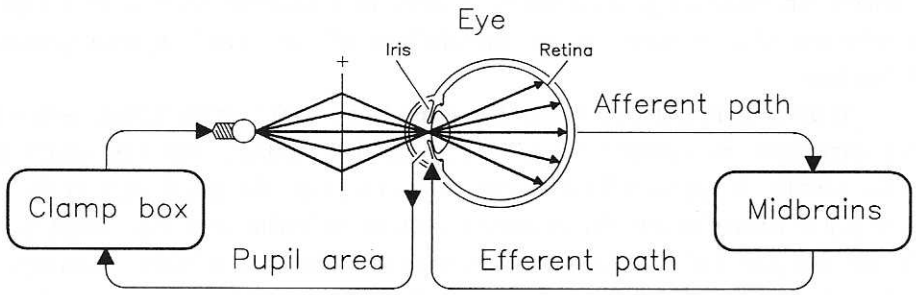


*Fig. 26. Pupil clamping. The pupil reflex arc is opened by presenting light to the eye under Maxwellian view conditions. The reflex arc is reclosed again by relating stimulus intensity to measured pupil size.*

By external clamping a precisely specified feedback can be inserted into the reflex. In this manner Stark (1962b) verified that pupil size oscillations could occur once the gain became sufficiently large. Here, the stimulus intensity was a smooth monotonous function of measured pupil size, referred to as smooth negative feedback (SNF). The period of these oscillations can be estimated from the experimentally measured open-loop transfer function for the reflex (Stark and Cornsweet, 1958). Moreover, it has been demonstrated that linear properties determine the frequency of these oscillations, whereas the shape and amplitude depend on the nonlinearities (Stark, 1962b).

Recent studies have emphasised clamping of the pupil light reflex with piecewise-constant negative feedback (PCNF). Here the stimulus light is either

on or off, depending on the value of the pupil area relative to certain area thresholds (Milton et al., 1988, 1989, Longtin and Milton, 1989a,b). The main advantages of PCNF over SNF are said to be the much easier experimental control (Milton et al., 1988) and the better analytical understanding of the oscillations so induced (an der Heiden and Mackey, 1982; Longtin and Milton, 1988; Milton et al., 1989). For high SNF gains, SNF and PCNF converge. Longtin and Milton (1988, 1989) proposed a model to explain these pupil oscillations, based on neurophysiological and anatomical considerations. A bifurcation analysis of the resulting non-linear delay-differential equation (DDE) is used to characterize its dynamical behaviour and to examine the influence of parameter variation. PCNF and SNF are similar in their monotonous decrease in feedback, wherefore only simple oscillations at one basic frequency occur. A third way of external clamping is provided by piecewise-constant mixed feedback (PCMF), where the feedback function consists of a decreasing as well as an increasing part (Longtin and Milton, 1988). In PCMF, chaotic behaviour can be induced (Hofstadter, 1981; an der Heiden and Mackey, 1982, 1987; an der Heiden, 1983). In PCNF and PCMF, the induced oscillations are determined mainly by certain area thresholds and delay. A slight change in these parameters, especially the threshold values, results in a large change in oscillatory behaviour. SNF oscillations are determined mainly by the gain of the system and delay (Longtin et al., 1990).

So far, theoretical and experimental results have only been obtained by changing area thresholds and gain, and not by changing the delay of the system. Alterations of the oscillation period have been demonstrated in diseases in which an increase in reflex delay is suspected (see above). However, as yet, the actual relationship between the pupil cycle time and the system's delay is not known both for edge-light pupil cycling and environmental clamping. Also, the SNF model, proposed by Longtin and Milton (1989a,b) and Longtin et al. (1990), has only been validated for spatial and not yet for temporal aspects.

It is the aim of the study described in this chapter to determine high-gain as well as extended-delay SNF-induced pupil-oscillatory properties. Then, the relationship between the system's delay and the oscillation period can be determined and the validity of the model proposed by Longtin and Milton (1989a,b) and Longtin et al. (1990) can be verified, also when the delay of the system is changed.

Experiments are limited to SNF, because the pupillometer on hand introduces an uncontrollable drift (Appendix C), whereas PCNF- and PCMF-induced oscillations are highly dependent on pupil size, and a stable signal is then a prerequisite.

## 4.2 THEORY

### 4.2.1 *FIRST-ORDER MODEL*

The first model of the pupil light reflex was proposed by Stark and Sherman (1957). This model was based on experiments, in which stimuli with sinusoidal modulated intensities of small amplitude were applied. From the resulting Bode-plots, the gain, order and delay of the system were determined. This model, however, captures only the linear properties. Most recently, Longtin and Milton (1989a,b) and Longtin et al. (1990) proposed a model for the pupil light reflex based on neurophysiological and anatomical considerations, involving nonlinear properties as well. I chose this model to be further evaluated, because it has shown to predict pupil-oscillatory properties very well, while it is of a marked simplicity. For a better understanding and appreciation, the basic assumptions leading to this model will next be recapitulated briefly.

The variable controlled by the pupil light reflex is the retinal light flux, which equals the product of illuminance and pupil area under normal conditions. Light fluxes are compressed logarithmically in the transduction process at the retina with a delay. The resulting number of neural action potentials travelling along the optic nerve per unit time is thus assumed to be logarithmically related to the light flux. It is assumed that the number of efferent and afferent action potentials per unit time are directly proportional with only an extra delay introduced in the midbrain. At the neuromuscular junction the neural activity is transduced into tension in the constrictor muscle. This process, involving the release of acetylcholine, introduces a further delay. The relationship between the number of efferent action potentials per unit time ($N_e$) and muscle tension ($x$) is first-order approximated, i.e. $N_e(t) \approx \mu(dx/dt + \alpha x)$. Changes in muscle tension finally result in changes in iris and thus pupil area. Pupil area is limited by a minimum and a maximum which are attained smoothly. Taking into account these assumptions, Longtin and Milton (1989a,b) and Longtin et al. (1990) proposed the following nonlinear DDE describing negative feedback in the pupil system:

$$\alpha^{-1}\frac{dA(t)}{dt} + A(t) = \frac{c\theta^n}{\theta^n + A^n(t-\tau)} + k, \qquad [1]$$

with the difference that here the model is rewritten so that k and c+k represent minimum and maximum pupil area, whereas in the original model c and k were

dimensioned as area per second. Equation 1 describes pupil behaviour in terms of pupil area A as a function of time t, A(t), and a time $\tau$ prior to t, A(t-$\tau$), where $\tau$ represents the total delay of the reflex arc. Furthermore, $\alpha$ is a rate constant determining constriction and dilatation velocity, $\theta$ is a constant at which pupil size is mid-range and n is a parameter defining the gain of the system. The right-hand side of [1] is called a Hill-type function, which is plotted in Fig 27.

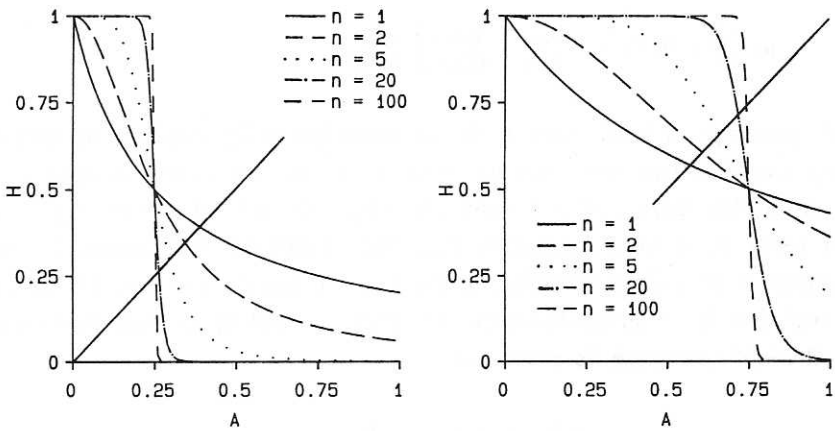


*Fig. 27. Hill function (H) for $\theta$ = 0.25 (left) and $\theta$ = 0.75 (right), with c = 1 and k = 0 arbitrarily chosen and varying n.*

The Hill function serves two major purposes here. Firstly, at small values of n, it accounts for a logarithmic decrease in pupil area if the light intensity is increased. Secondly, it identifies the upper (c+k) and lower (k) boundaries to which the pupil size is restricted. Equation [1] has been used before as a general paradigm for SNF systems with delay (Mackey and Glass, 1977; Mackey and Milton, 1987) and exhibits one bifurcation, which means that an oscillation occurs with one fundamental frequency, as the parameter n or $\tau$ is increased past a certain value.

When steps in light intensity are presented to the human eye, $\alpha = \alpha_c$ for constrictions differs from $\alpha = \alpha_d$ for dilatations. But it has been found experimentally that pupil cycling occurs even when the sympathetic supply to the iris is cut surgically (Milton et al., 1988) or blocked pharmacologically (Martyn and Ewing, 1986). It can therefore be concluded that pupil oscillations are controlled mainly by the parasympathetically regulated constrictor muscle. The role of the sympathetic nervous system seems to be primarily one of

determining the average pupil size (Longtin and Milton, 1989b). Consequently no distinction will be made here between $\alpha_c$ and $\alpha_d$.

**Piecewise-constant negative and mixed feedback**

Likewise, two other types of feedback based on [1] have been described (Longtin and Milton, 1988; Milton et al., 1989): piecewise-constant negative feedback (PCNF) and piecewise-constant mixed feedback (PCMF). If in [1] the right-hand side (RHS) is replaced by a piecewise-constant function according to

$$\alpha^{-1}\frac{dA}{dt} + A(t) = \begin{cases} A_{off}, & A(t-\tau) < \theta \\ A_{on}, & A(t-\tau) > \theta \end{cases}$$

the PCNF paradigm arises. Here $\theta$ is an experimentally adjustable threshold. Resulting oscillations are bounded from above by an upper asymptote $A_{off}$, towards which the pupil dilates when the light is off. When the light is on, the area tends to a lower asymptote $A_{on}$. This function is attained by setting the parameter n in [1] to infinity, where $A_{on} = k$ and $A_{off} = c+k$. If the RHS of [1] is replaced by a mixed-feedback function, depending on two thresholds $\theta_1$ and $\theta_2$, the PCMF paradigm is given by:

$$\alpha^{-1}\frac{dA}{dt} + A(t) = \begin{cases} A_{off}, & A(t-\tau) < \theta_1 \\ A_{on}, & \theta_1 \le A(t-\tau) \le \theta_2 \\ A_{off}, & A(t-\tau) > \theta_2 \end{cases}$$

The clamp function, relating stimulus intensity to pupil area, and the matching RHSs of [1], for the different types of feedback, are plotted in Fig. 28.

All parameters in these models can be estimated from experimentally obtained data, and the resulting oscillatory solutions have been shown to be in good agreement with the observed oscillations, except for the dependency on delay (Longtin and Milton, 1988; Milton et al., 1988; Longtin and Milton, 1989a,b; Milton et al., 1989; Milton and Longtin, 1990). SNF and PCNF exhibit simple oscillations only, while chaotic behaviour is also seen in PCMF due to the nonmonotonic feedback function (Mackey and Glass, 1977; Glass and Mackey, 1979). Where analytical solutions are available for the first-order PCNF and PCMF models, pupil behaviour according to the SNF model, as I am interested in, will have to be obtained by numerical integration.

**Smooth negative feedback**

For numerical analysis of the behaviour of SNF-induced pupil oscillations, I will alter [1] slightly for a normalizing purpose, by replacing

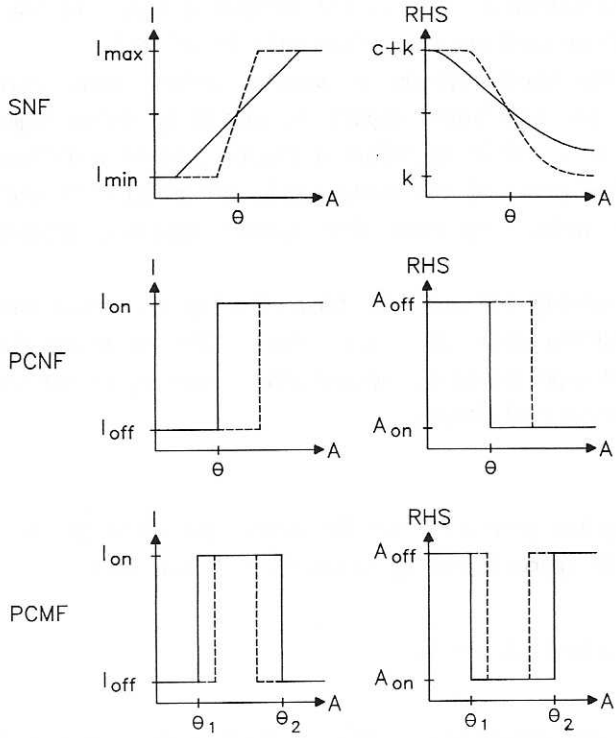


*Fig. 28. Clamping functions relating stimulus intensity (I) to pupil area (A) and the matching right-hand sides (RHS) of equation [1] for SNF, PCNF and PCMF. In SNF the ratio between stimulus intensity and pupil area can be changed, whereas in PCNF and PCMF the thresholds θ can be changed, as is depicted by the dashed lines (see text).*

$A^n(t-\tau)$ in the denominator of the Hill function by $A_\tau$-k, where $A_\tau$ equals A(t-$\tau$). Then [1] can be normalized by substituting A' = (A-k)/c and θ' = θ/c, resulting in $0 \leq A' \leq 1$ and $0 \leq \theta' \leq 1$, and c and k will vanish:

$$\Rightarrow \alpha^{-1} c \frac{dA'}{dt} + cA' + k = \frac{c(c\theta')^n}{(c\theta')^n + (cA_\tau' + k - k)^n} + k$$

$$\Rightarrow \alpha^{-1} \frac{dA'}{dt} + A' = \frac{\theta'^n}{\theta'^n + A_\tau'^n} . \qquad [2]$$

The advantage of this normalization is twofold. Firstly, the dynamics are described by two parameters less (c and k) and secondly, numerical (digital) calculations will be less prone to overflow. Once the actual minimum and maximum pupil areas are known, true values of pupil area A can be calculated by

the inverse substitution $A = cA'+k$. For further analysis of the SNF paradigm, equation [2] will be used and the primes will be omitted.

Normally the pupil system is stable, which means that, apart from hippus, it does not oscillate. Hippus is caused by noise injected somewhere into the reflex arc, and is therefore a fluctuation of a different nature. By clamping, both the gain and the delay can be manipulated to exceed a critical value as given below, to make the system unstable producing sustained oscillations.

The relationship between the theoretically and experimentally imposed delay is straightforward: they are equal. The relationship between the theoretically and experimentally imposed gain, however, is not straightforward, and needs a separate evaluation.

**Gain**

To identify the gain G of the SNF model, the question has to be answered whether [2] can be approximated by an equation of the form

$$\alpha^{-1}\frac{dA}{dt} + A = G\bullet(A_\tau - A_1) + A_2, \qquad [3]$$

where $A_1$ and $A_2$ are constants. If $H(A) = \theta^n/(\theta^n+A^n)$ is the Hill function, by [2] and [3] it follows that $G\bullet(A_\tau-A_1) + A_2 = H$. Then

$$G = \frac{dH}{dA} = \frac{-n\theta^n A^{n-1}}{(\theta^n+A^n)^2}. \qquad [4]$$

The gain thus defined, exhibits a maximum in the neighbourhood of $\theta$, as can be deduced from setting $dG/dA = 0$, or as can be seen from a plot of G versus A in Fig. 29. It is an experimental datum that G shows a maximum for A somewhere mid-range (Usui and Stark, 1982; Longtin and Milton, 1989b). This effect has been explained in terms of an 'expansive range nonlinearity' operating at the neuromuscular level, and is related to the nonlinear length-tension diagrams of the iris muscles.

For the approach given by [3], a linearization point should next be chosen. At first sight there are two possibilities: $A = \theta$ and $A = A_o$, where $A_o$ is given by the unique equilibrium of [2] when $dA/dt = 0$. By [2], $A_o$ is then given by

$$A_o = \frac{\theta^n}{\theta^n + A_o{}^n}.$$

Solutions of this equation are provided by the intersections of y = A and y = H(A) as given in Fig. 27. Because the maximum of G was observed to be located somewhere midrange, and midrange can be identified by $\theta = \frac{1}{2}$ in the normalized model, a proper choice for the linearization point is $A = \theta = \frac{1}{2}$. Moreover, if $\theta = \frac{1}{2}$, it follows that $A_o = \frac{1}{2}$. I will therefore take $A_o = \theta = \frac{1}{2}$ for numerical calculations, in which case $G = -\frac{1}{2}n$. This means that the gain is directly proportional to the parameter n in the SNF model.

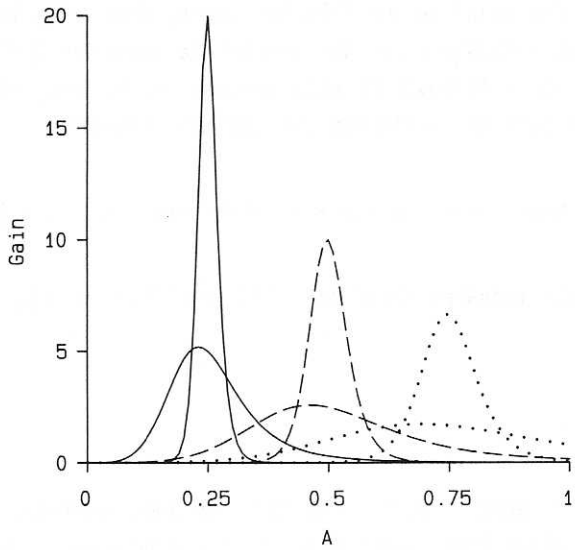


*Fig. 29. Plot of SNF gains according to [4] as a function of pupil area (A) for n = 5 and n = 20, with θ = 0.25 (solid lines), θ = 0.5 (dashed lines) and θ = 0.75 (dotted lines).*

Based on small-signal analysis, Stark (1959) has argued that the open-loop gain can also be expressed as:

$$G = \frac{dA \bullet I}{dI \bullet A} = \frac{dA/A}{dI/I}.$$

Here I and A represent average intensity and pupil area respectively. The numerator describes the change of flux due to a change of pupil area in response to a change of flux due to the change in light intensity in the denominator. A theoretical analysis reveals that this is in accordance with [3] (Longtin and Milton, 1989a). If, in the experiment, the pupil system is clamped by relating stimulus intensity linearly to pupil area with $dI/dA = \beta$, the nominator, and

thus G, is changed by a factor β. In a serial circuit, as the clamped pupil system is, the total gain is the product of all individual gains. Therefore, the total gain under the given circumstances is directly proportional to β.

In combining these findings about the gain, it can be concluded that the experimentally regulated external gain β = dI/dA determines the total system's gain in the same way as the parameter n in the SNF model.

**Oscillation onset and noise**

To predict the point of oscillation onset, depending both on gain and delay, the Laplace transform can be applied to equation [3]. The open-loop transfer function (y = OLTF•x) is then derived as follows, while $A_o$ will be omitted because it does not influence the system's dynamics:

$$\alpha^{-1}\frac{dA}{dt} + A = G \bullet A_\tau \quad <=> \quad \alpha^{-1}sy + y = G \bullet e^{-\tau s} \bullet x \quad => \quad y = \frac{\alpha G e^{-\tau s}}{s + \alpha} \bullet x,$$

and the closed-loop transfer function (CLTF = OLTF/(1+OLTF)) is consequently given by:

$$\frac{\alpha G e^{-\tau s}}{s + \alpha + \alpha G e^{-\tau s}}.$$

If the denominator equals zero, the CLTF becomes unstable. Therefore the characteristic equation (CLTF-denominator ≡ 0) can be used to look for periodic solutions, i.e. by equating s = iw, where w = 2π/P, P is the oscillation period, and index 0 refers to oscillation onset:

$$iw + \alpha + \alpha G_o e^{-\tau_o iw} = 0 \quad <=> \quad iw + \alpha + \alpha G_o(\cos(w\tau_o) - i \bullet \sin(w\tau_o)) = 0,$$

$$=> \begin{cases} \alpha + \alpha G_o \cos(w\tau_o) = 0 & => \quad G_o = -1/\cos(w\tau_o) \\ w - \alpha G_o \sin(w\tau_o) = 0 & => \quad w^2 = \alpha^2(G_o^2 - 1). \end{cases}$$

At present, these derivations are only of theoretical interest. It has been shown that the introduction of noise postpones the point of oscillation onset as predicted by the above equations (Longtin et al., 1990). Moreover, at oscillation onset, period and amplitude are hard to determine in practice. At higher gains and/or longer delays, the impact of noise on the predicted oscillatory behaviour can be neglected. Therefore the present study will not deal with pupil behaviour at oscillation onset. The experimentally imposed gain

and delay will be chosen such that distinct oscillations occur, and the SNF model can be verified without concern about noise.

### 4.2.2 *MODEL PREDICTIONS*

To evaluate pupil behaviour according to the SNF model, numerical integration of [2] was performed by means of the digital simulation programme PSI/e, running on a personal computer. Initial pupil area was set to zero. According to Longtin et al. (1990) $\alpha$ = 3.2 was chosen, and $\theta = \frac{1}{2}$ as suggested above. The parameter n was varied between 4 and 40, which showed to comprise the range of oscillation onset up to a state with almost constant behaviour. The parameter $\tau$ was varied between 0.2 and 1 s, which comprises approximately the minimum latency found experimentally (this thesis) and the maximum delay at which sustained oscillations could be induced experimentally. A Runge-Kutta 2 integration algorithm was selected, with the integration interval set to 5 ms. Traces of A versus time were calculated, as examplified in Fig. 30. The postulated constancy of the pupil size during the interval from $-\tau$ to 0, results in an onset instability. Peak to trough amplitude and interpeak period were determined only when a sustained oscillation developed and the stable limit cycle was reached. Oscillation amplitude and period so obtained are plotted in Fig. 31, both for varying gain (n = 4, 5, 6, 7, 8, 9, 10, 15, 20 or 40) and varying delay ($\tau$ = 0.2, 0.22, 0.24, 0.26, 0.28, 0.3, 0.35, 0.4, 0.45, 0.5, 0.6, 0.8 or 1 s).

The predicted oscillation amplitude grows fast shortly after oscillation onset (Fig. 31a), whereas it remains virtually constant at higher gains. It was verified that shortly after oscillation onset the oscillation amplitude is linearly related to $\sqrt{(\tau-\tau_o)}$, where it levels off when $\tau$ and $\tau_o$ deviate (Fig. 31b). According to the model, there is no substantial influence of gain on oscillation period (Fig. 31c), whereas the period (P) shows an extremely high linear correlation (r = 1.00) with the total delay (Fig. 31d) according to $P = 257 + 2.23 \cdot \tau$ (ms).

The relationship between oscillation period and delay showed to be relatively insensitive to variations in $\alpha$ and $\theta$. If $\alpha$ was varied between 2 and 20, the slope of the predicted period-versus-delay curve varied from 2.0 to 2.7 only, whereas the slope changed by less than 0.2 when $\theta$ varied between 0.25 and 0.75. This analysis justifies once more the choice of taking $\alpha_c = \alpha_d$ for SNF.

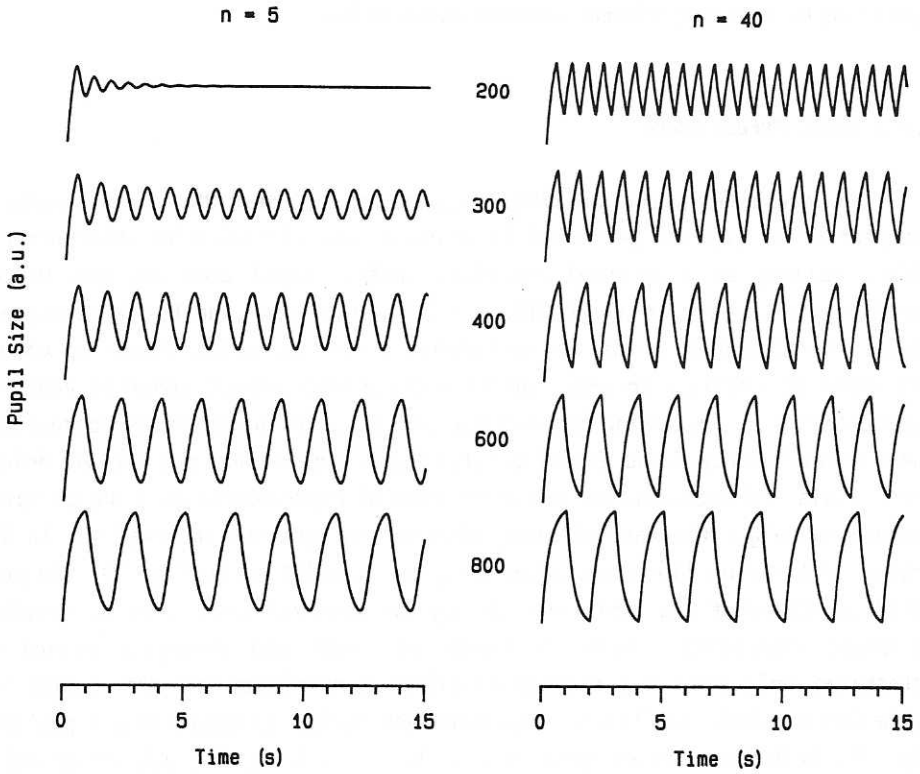


*Fig. 30. Solutions of [2] under conditions as described in the text at n = 5 (left) and n = 40 (right) for different delays (ms) as indicated.*

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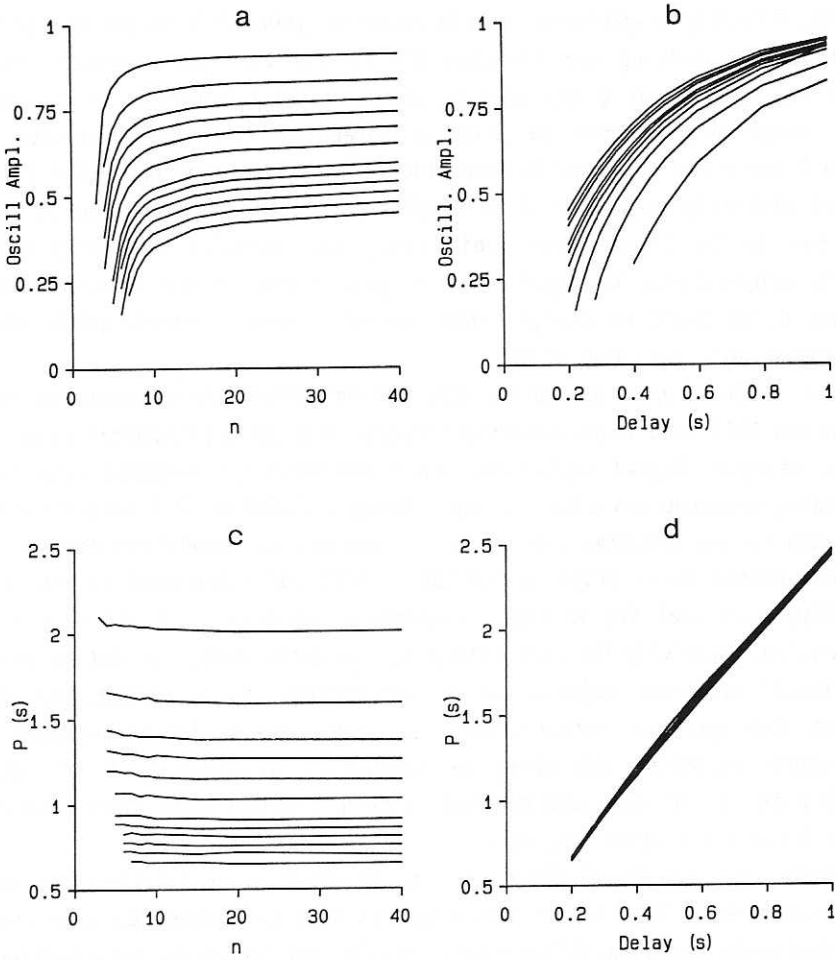
*Fig. 31. (Right page) Predicted SNF oscillation amplitude and period. In (a) and (b) the oscillation amplitude is plotted versus gain and delay, for increasing delay and gain respectively (from bottom up). In (c) and (d) the oscillation period (P) is plotted under the same conditions.*




## 4.3 EXPERIMENTS

### 4.3.1 *METHODS*

For the SNF experiments, the IRIS pupillometer and Maxwellian view stimulator were used as described before. In fact, the clamping as shown in Fig. 26, had already been outlined in Fig. 11, illustrating the principle of the IRIS pupillometer. The SNF was synthesised using a personal computer with A/D and D/A facilities. Every 2 ms, a sample from the IRIS pupillometer signal was digitized and stored in a ring buffer. Also every 2 ms, a previously stored value was retrieved and output to the D/A-convertor after multiplication by a

constant, directly proportional to the external gain. This output was converted into a current driving the stimulus LED in the IRIS pupillometer. The LED-current was limited by 0 and some maximum, in which interval it was approximately linear proportional to stimulus intensity. Stimulus intensity varied between 0 and 4 Log Td. Signals proportional to LED-current and pupil size were recorded digitally at a rate of 50 samples per second. Because the IRIS signal has shown to be linear with pupil area, the stimulus intensity was thus directly proportional to pupil area as prescribed in the previous section. Gain and delay could be changed interactively. Delays introduced by the IRIS pupillometer were less than 2 ms.

Per subject, gain and delay were varied within one session. During each session the IRIS head frame remained fixated, and the pupillometer-gain setting was not changed. Signal amplitudes could therefore be compared mutually. The pupil being measured was also the pupil being stimulated. A fixation target was conjugated to the subjects far point to suppress accommodation and facilitate fixation. Within every trial, a constant offset was introduced to the stimulus intensity, such that the average illuminance was equal for all trials within each session. Initially for every subject, the extra delay was set to zero, and the external gain was adjusted so an oscillation was suspected, but was not distinct. This gain was referred to as 1. Gains were varied accordingly from 1 to 6 where possible, depending on successive saturation of the stimulus intensity at its minimum and maximum. External delays were varied thereafter between 0 and 0.5 s where possible.

Peaks and troughs of those periods in which an oscillation was manifest were first marked. Oscillation amplitude was then calculated by averaging over all marked peak-to-trough differences. Oscillation period was provided by twice the averaged peak-to-trough and trough-to-peak intervals. Differences due to eventual asymmetrical reflexes were thus cancelled. Periods in which the LED-current clipped were disregarded.

Four healthy male subjects participated. Their ages were 22 (WS), 23 (RM), 26 (JC) and 46 (RH) year.

### 4.3.2 *RESULTS*

Fig. 32 shows some time series of the oscillations in pupil size that occur with SNF. The oscillations have a nearly sinusoidal shape with a fluctuating amplitude and period. All spectra are dominated by the fundamental mode (0.5 - 1.25 Hz), and there is little harmonic content (spectra not shown, see

also Longtin et al., 1990). Data set lengths were often virtually unlimited, which means they could persist for several minutes. Sometimes the oscillations were interrupted by stimulus-signal saturation or persistent eye blinking. These saturations appear to be due to uncontrollable baseline drift, which in turn is caused by pupillary noise, and by the IRIS pupillometer (Appendix C). The oscillations were found not to be significantly affected by occasional eye blinking.

Mean amplitudes and mean periods of SNF oscillations were plotted per subject as a function of feedback gain and externally imposed delay. A typical example is given in Fig. 33. The gain has been normalized so that G ≈ 1 when the pupil starts to oscillate. Accordingly, not all amplitudes and periods could be determined at G = 1.

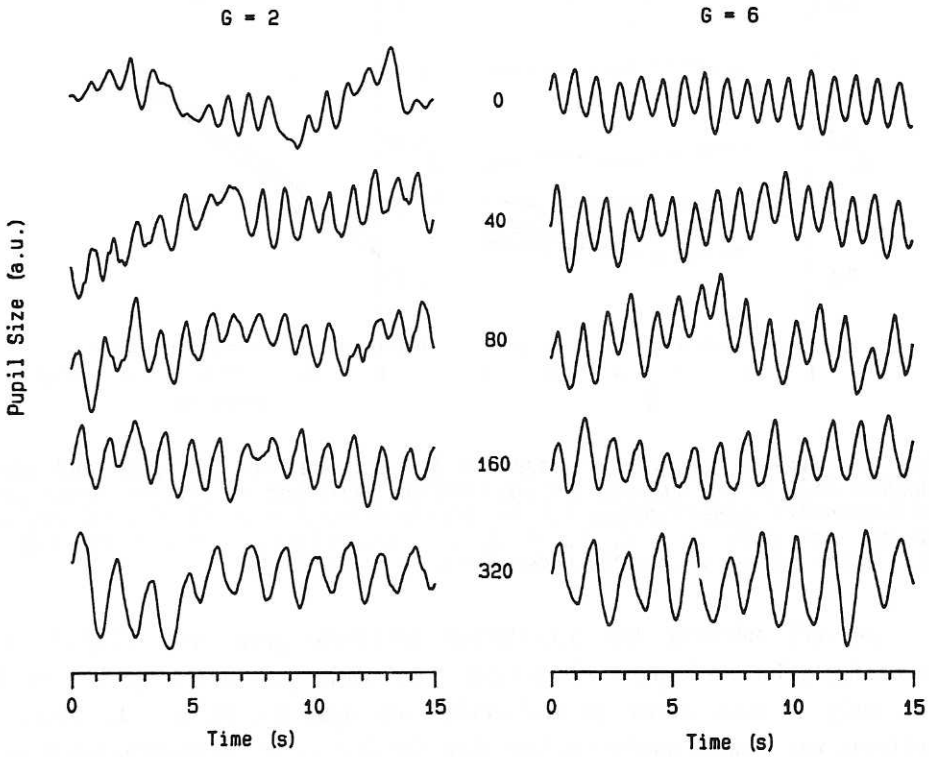



*Fig. 32. Examples of observed pupil oscillations for subject WS at G = 2 (left) and G = 6 (right) for different external delays as given (delays in ms).*

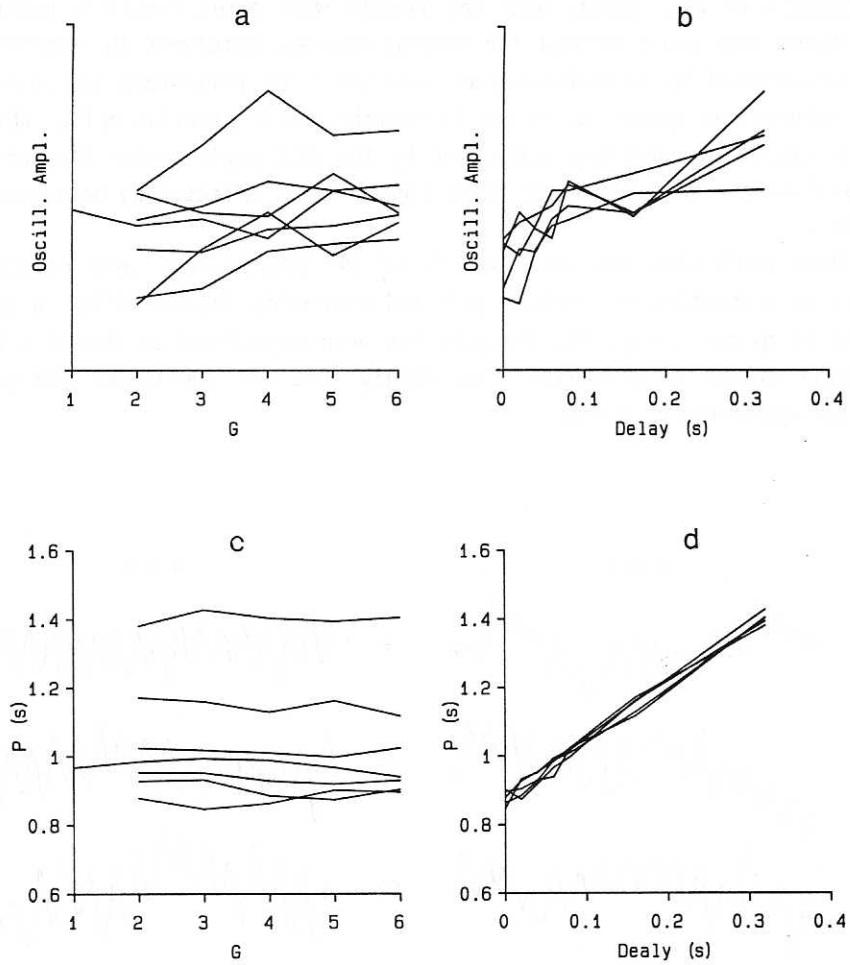



*Fig. 33. Typical example of observed SNF oscillation amplitude and period (subject WS). In (a) and (b) the oscillation amplitude is plotted versus gain, and externally imposed delay, for increasing delay ($\tau$ = 0, 20, 40, 60, 80, 160, 320 ms) and gain (G = 2, 3, 4, 5, 6), respectively. In (c) and (d) the oscillation period is plotted under the same conditions.*

In all subjects the oscillation amplitude grew only slightly with increasing gains at higher values (e.g. Fig. 33a). Due to the definition of G = 1, only in some cases an oscillation was apparent at G = 1. Then, the amplitude was significantly smaller than for G > 1. An increasing oscillation amplitude was also observed in all subjects when the delay was increased (e.g. Fig. 33b).

No significant differences in oscillation period were observed between the different gain settings (e.g. Fig. 33c). This was a constant observation for all subjects. As a result, the period versus external-delay curves coincided at different G-values (e.g. Fig. 33d). In all subjects these period-versus-delay curves showed an extremely high linear correlation. Regression parameters of the period-versus-delay curves are tabulated separately in Tab. I.

| Subject | a | b | r |
|---|---|---|---|
| WS | 875 | 1.66 | 0.99 |
| RM | 844 | 1.67 | 0.99 |
| JC | 903 | 2.10 | 0.97 |
| RH | 1016 | 1.97 | 0.96 |

*Tab. I. Linear regression parameters of oscillation period versus delay (a = intercept in ms, b = slope, r = correlation coefficient).*

## 4.4 DISCUSSION AND CONCLUSIONS

Oscillations in the human pupil light reflex could be produced by external feedback which modifies the normal functional dependence of retinal light flux on light intensity and pupil size. The type of external feedback under consideration was smooth negative feedback (SNF). In SNF, the gain and delay of an external device, the clamp box regulating stimulus intensity to pupil area, were varied. A slightly modified model for pupil functioning, proposed by Longtin and Milton (1989a,b) and Longtin et al. (1990), was evaluated numerically, and experiments were performed. The system's gain was shown to be directly proportional to the parameter n in the model and to the factor dI/dA determining the ratio of stimulus intensity to measured pupil size in the experiment. The modelled delay is equal to the sum of the internal delay of the pupil system itself and the delay externally imposed by the clamp box.

Within the range of parameter settings under consideration, model predictions and observed oscillations are in good agreement, apart from some minor differences. The gain was changed up to a factor 6, and the total delay ranged from 0.2 - 0.8 s. The most striking difference concerns the oscillation shape at higher gains, i.e. the predicted extremities show sharp peaks due to the first-order approximation (Fig. 30), while all experimentally gathered traces had a sinusoidal shape (Fig. 32). However, no asymmetries concerning

constriction and dilatation were observed, justifying once more the choice of setting $\alpha_c = \alpha_d = \alpha$ in the model.

Asymmetric responses, however of an opposite nature, were observed in the unnormalized model as described by [1], i.e. the constriction was slower than the dilatation, especially for values of $\theta$ below midrange. Besides simplifying the model, the normalization thus also serves an essential purpose.

Notwithstanding the linearization of the model for determining the system's gain, also for larger gains the predicted and observed oscillatory behaviours are alike (see also Stark, 1962b).

Additional to the gain dependency, which was partly described before (Longtin et al., 1990), here also the dependency on delay is investigated. Qualitatively, observed extended-delay-induced oscillatory behaviour is also predicted adequately by the model. The most striking resemblance between the predicted and observed behaviour concerns the relationship between the oscillation period and delay. In all cases an extremely high linear correlation was found ($r > 0.95$), independent of the gain. At this point, however, a second difference between the predicted and observed behaviour appears. All observed slopes (b) of the period-versus-delay curves showed to be significantly less (b = 1.85 on the average) than the predicted slope based on the first-order model (b = 2.23). It was verified that the predicted slope does not change significantly due to the modification of the model allowing normalization. The only remaining parameters that may influence this behaviour, are $\alpha$ and $\theta$. Of these two, $\theta$ was of least significance, and increasing $\alpha$ to 20 only resulted in a decrease of b to b = 2.0. I therefore hypothesise that at least a second-order term should be included in the model to give an oscillation period less dependent on delay as is observed. The slopes may then be brought into agreement with each other.

Then also, the internal pupil-system's delay $\tau_i$ can be estimated from the predicted and observed oscillation period (P) as a function of delay. If the predicted intercept is represented by $a_0$, the observed intercept by $a_1$ and the external delay by $\tau_e$, then:

$$\left.\begin{array}{l} P = a_0 + b\bullet(\tau_i+\tau_e) \\ P = a_1 + b\bullet\tau e. \end{array}\right] \quad \Rightarrow \quad \tau_i = \frac{a_1 - a_0}{b}.$$

If, at first approximation, b is set to b = 2, with $a_0$ = 257 and the average observed intercept $a_1$ = 910, $\tau_i$ = 327 ms would follow. This value will be discussed further in Chapter 6 with final remarks.

**SNF and edge-light pupil cycling**

The independence of oscillation period of gain suggests that the type of feedback is not as important for the observed cycle times as it may seem, as long as the feedback does not result in a piecewise-constant type. For PCNF it has been shown that the period fluctuates with fluctuating system's parameters, such as $A_{off}$ (Longtin et al., 1990). Based on the small observed variability in edge-light PCTs (see introduction), I therefore hypothesise that edge-light pupil cycling is closely related to SNF oscillatory behaviour. In fact, also in edge-light stimulation the total gain is increased. This increase is due to the possible reduction of retinal flux to zero. Then, the edge-light pupil cycle time (PCT) mainly depends on the system's delay, and not on the chosen stimulus intensity and on the precise shape of the spot focussed on the pupil margin. This is in agreement with findings reported by Miller and Thompson (1978a) and Manor et al. (1981). Therefore, the PCT is a reliable parameter for determining abnormal delays.

# 5. CONSTRICTION LATENCY

## 5.1 INTRODUCTION

One of the most direct and obvious ways to demonstrate delays is the observation of the output of a system when presenting a step to the system's input. It is also the best applicable way in clinical practice. Constrictions so elicited in the pupil system, both in healt and disease, are the subject of investigations described in this chapter. A thourough analysis with respect to the temporal parameters of these constrictions is of great importance, and will be dealt with first.

## 5.2 THEORETICAL CONSIDERATIONS

### 5.2.1 *LATENCY DETERMINATION*

The importance of an accurate and objective CL determination, as pointed out in the Introduction, is generally neglected. No more than 30% of the authors describing some CL-related item mentioned their way of determining the CL. Only 15% took more effort than just looking for a subjectively observable deflection. Methods used so far consist of amplitude threshold crossing (Fig. 34b) as employed by Borgmann (1972a), or velocity threshold crossing (Fig. 34c) as employed by Feinberg and Podolak (1965) and Friedman et al. (1967). Alpern et al. (1963) used the intersection of two straight-line fits, one during the latency period and one through that part of the constriction in which the velocity seems constant (Fig. 34d). A mathematical curve-fitting procedure (Fig. 34e) has been applied by Lee et al. (1969). A procedure using velocity deflections from zero (Fig. 34f) was described by Pfeifer et al. (1982). Most of these approaches, however, have their disadvantages. The subjective method, by looking for observable amplitude deflections, may give different results when applied by different analysts. The threshold crossing methods do not indicate the real constriction onset, but some moment thereafter, depending on the amplitude of the constriction. The method using the intersection of two fitted lines is also doubtful. Pupil velocity during constriction is not constant, as shown by Fig. 34c, for example. However, the method applied by Lee et al. (1969), and the one applied by Pfeifer et al. (1982) use objective criteria approximating the real constriction onset moment.

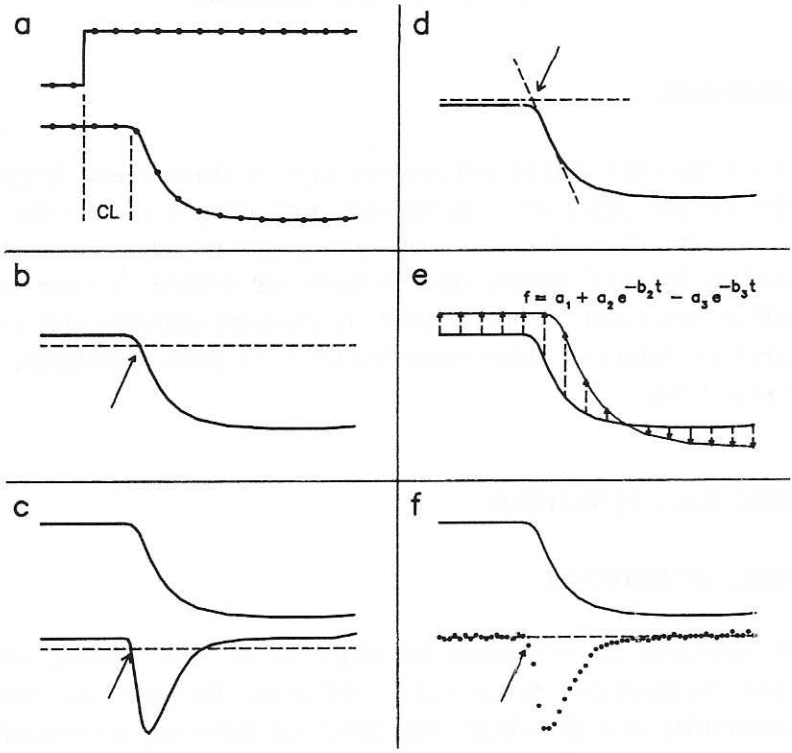



*Fig. 34. Example sketches of the pupil reflex induced by a sudden increase in light intensity ((a), upper trace) for determination of the constriction latency (CL). Shown are five criteria ((b) to (f)) for the determination of the constriction onset (oblique arrows) as described in the text. Where appropriate, possible sample moments are depicted by dots ((a) and (f)). Pupil velocities are shown in (c) and (f) (lower traces). Auxiliary lines are dashed.*

To reduce the influence of noise on pupil constrictions, thwarting the detection of the onset moments, averaging of several responses has been performed by some authors, e.g. Lee et al. (1969), Semmlow et al. (1975), Ellis (1979), and Beaumont et al. (1987). Based on a discussion concerning the standard deviation of the averaged signal, Lee states that "physiological latency variation, if present, is probably less than 25 ms", so averaging is allowed. But in view of possible pathological differences of less than 25 ms, the discrepancy between averaged latencies of individual constrictions and the latency of averaged responses has to be reexamined.

It is the aim of this section to consider the accurate determination of the moment of pupil constriction onset. It is assumed that data are digitized for recording and analysis. Three relevant major problems will be dealt with:

- to what extent does the sample rate play a role,
- which algorithm should be used, and
- what is the influence of averaging of raw pupil data upon the measured constriction latency?

**Influence of sample rate on latency**

Concerning the determination of the CL, two moments have to be detected. One moment is provided by the stimulus, the second by the pupil signal. The interval between these two moments yields the latency. Both determinations contribute to an inaccuracy of the CL.

First the stimulus onset, if recorded together with the pupil signal, has to be determined. If the stimulus is not locked to the sample moments, the inaccuracy resulting from the sample process is evidently proportional to the sample interval itself (see also Fig. 34a). Because a step function consists of an infinite series of frequencies according to Fourier, the sample rate should

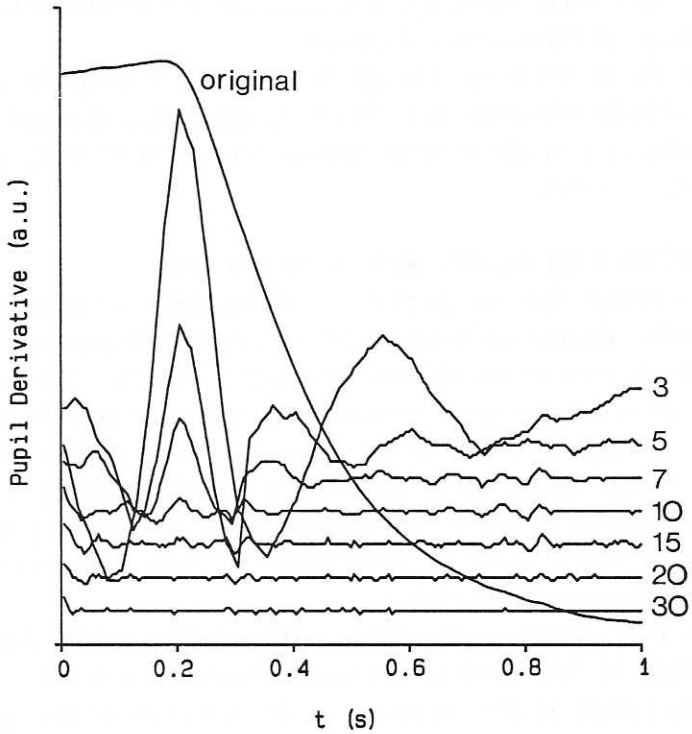



*Fig. 35. Band-pass-filtered constrictions. Of interest at present are the lower cut-off frequencies, which are (top down) 0 (the original example, sampled with 200 samples per second), 3, 5, 7, 10, 15, 20 and 30 Hz, respectively.*

be chosen as high as possible.

Secondly, the moment of constriction onset has to be detected. As will be discussed further on, this can be performed accurately and simply by a method based on velocity deflections from zero, as proposed by Pfeifer et al. (1982). Here again, a high sample rate is necessary for high accuracy. High sample rates are incompatible with TV pupillometers where the pupil size is only delivered about every 20 ms. But high accuracy can be achieved at lower sample rates. With sinusoidal stimulation, Stark and Shermann (1957) showed that the cut-off frequency of the linearized pupil system is somewhere below 4 Hz. This implies a sample rate of 8 Hz to be sufficient for recording data without loss of information. However, it is now known that the pupil system is highly non-linear and the question might arise whether higher frequencies are contained in a pupil constriction. To sort this out, a comparison was made between high-rate sampled constrictions filtered with different band-pass filters, as shown in Fig. 35. This comparison shows that a pupil constriction does not consist of frequencies above 10 Hz. Thus, sampling the pupil signal with 20 Hz should be sufficient to reconstruct the original signal. But the reconstruction requires an adequate model to explain the phenomenon.

Two methods to tackle the problem of accurately finding the constriction onset moment will be discussed next. The first and most simple way is based on deflections from zero in the velocity signal. The second is based on a second-order mathematical model.

**A simple algorithm using velocity deflections from zero**

Velocity rather than the position signal enables a reliable detection of the onset moment, because velocity deviations are more pronounced (Fig. 34c). Digital differentiation of the A/D-converted pupil recordings allows the search for a series of velocity samples consecutively less than zero (Fig. 34f). The first sample in such a series of at least 100 ms marks the onset of the constriction. Linear trends during the latency period, resulting in a velocity offset, can eventually be taken into consideration. The search process can be (re)started after a step in the stimulus signal is found. This gives a fast and simple programmable search algorithm.

For the first sample in the series with velocities less than zero, the temporal accuracy is determined by the sample interval and noise (Fig. 34f). A noisy velocity sample at the beginning of the constriction may have a value greater or less than zero, delaying or advancing the onset moment. Filtering may reduce the noise, but will generally also advance an event onset, such as that of the constriction. Therefore, filtering should not be performed, and,

with the assumption that the noise is gaussian, averaging of individually determined latencies will approximate the true latency. The optimum resolution is the sample interval time. Sampling at 200 Hz, for example, can thus give an inaccuracy of 5 ms for the individual onsets at its best.

There is a discrepancy between this way of detecting the CL and the subjective way based on the pupil size as performed by most authors. The first observable change of pupil size is generally put at a certain moment after the real constriction onset. This discrepancy was demonstrated by having a number of measurements analysed by several naive subjects/analysts, using velocity deflections from zero as well as applying the subjective method based on pupil size. The subjective way yielded systematically longer latencies, with an average difference of about 20 ms. Moreover, the spread in latencies was about four times as high as compared to the analysis based on velocity deflections from zero.

Using the velocity signal, detection of the moment of peak constriction-velocity (PCVL) can be performed by a simple extremum search algorithm. Object to CL determination, digital filtering can be applied to eliminate eventual noise here, because the velocity near the extremum is fairly symmetrical.

Since the above algorithm does not involve the absolute velocity, but only deviations from zero (CL), and peak velocity (PCVL), the method is independent of signal amplitude. This implies that pupillometers yielding a relative measure, such as most analogue infrared-reflecting techniques, are applicable for the determination of pupil latencies, both CL and PCVL, without the need of calibration.

**Analysis of a second-order model**

With infrared TV pupillometers, giving the pupil size every 25 ms, for example, or in general on low-rate sampled data, the above method will give insufficiently accurate results. Another algorithm based on curve-fitting should then be applied. When applying curve-fitting, the problem is to find a function which approximates best the pupil light reflex. Some constraints may simplify the problem. Validity is only required in the interval of stimulus onset up to the end of the constriction. In most circumstances pupil constrictions show capture behaviour (Usui, 1974), and will come to an end within approximately one second after stimulus onset. Two assumptions can further simplify the problem. Firstly, the model should permit at least a linear trend of pupil size during the latency period. Psychological influences or mental activity (Drischel, 1957) may cause changes despite a constant retinal illuminance. Higher-order trends during the latency period are negligible because the

interval of interest is less than 500 ms and the mentioned disturbances are all rather slow. Secondly, it is assumed that during the constriction itself no other mechanisms but the light reflex are active, i.e. during the constriction trends are absent. With T representing the constriction onset moment, the model (f) can be separated into a part with only a linear trend for t<T, and a part describing the constriction for t>T. For the latter, the exponential decay is most striking (e.g. Fig. 2 or Fig. 34), for which an exponential function will be used. At least one additional exponential function will have to be incorporated for the bending of the curve at the constriction onset. By first approximation this all results in

$$f(t<T) = a_0 + b_0 \cdot (t-T) \text{ and}$$
$$f(t>T) = a_1 + a_2 \cdot \exp(-b_2 \cdot (t-T)) - a_3 \cdot \exp(-b_3 \cdot (t-T)).$$

Together with the constraints of continuity of f and its first derivative in t=T, this results in $a_0=a_1+a_2-a_3$ and $b_0=-a_2 \cdot b_2+a_3 \cdot b_3$. The substitutions $t-T=(t-T-|t-T|)/2$ for t<T and $t-T=(t-T+|t-T|)/2$ for t>T will next result in one equation for f, which describes the constriction in the interval of stimulus onset till the end of the constriction:

$$f(t) = a_1 + (a_3 \cdot b_3 - a_2 \cdot b_2) \cdot \frac{t-T-|t-T|}{2} +$$
$$+ a_2 \cdot \exp(-b_2 \cdot \frac{t-T+|t-T|}{2}) - a_3 \cdot \exp(-b_3 \cdot \frac{t-T+|t-T|}{2}).$$

If the pupil size during the latency period is constant, this implies that $a_2 \cdot b_2=a_3 \cdot b_3$ (or $a_3=a_2 \cdot b_2/b_3$). In system-analytical terms this means that f stands for a pure second-order step response.

Curve-fitting, using this second-order model preceded by a linear trend, has been applied to a number of constrictions, viz. a typical example, data with blink artefacts and noise, data sampled at a low rate, and weighed data-sets over certain intervals. Results are shown in Fig. 36. The accuracy of the fit as far as the CL is concerned, is expressed in terms of the CL's standard error ($SE_{CL}$). Using all 200 samples of a 'typical example', an accuracy of 0.4 ms was found. Weighing the data set of this series (i.e. by including less samples out of the region of interest, Fig. 36f) gave an accuracy of 0.5 ms. Using a 0.5-s interval enclosing the onset only, the accuracy slightly increased to 0.7 ms. Fig. 37 shows how the accuracy changes when the sample frequency is reduced by using subsampled series. Accuracy was plotted by means of latencies-plus-error bars as well as just by the standard error itself.

Inaccuracies of these samples were all within the acceptable range, i.e. < 25 ms, on the condition that more than 20 samples per second are involved. At lower sample rates, accuracy decreased rapidly to far over 2 ms. This means that when using a TV-pupillometer, giving the pupil area every 25 ms for example, curve-fitting can help well in getting accurate latencies, provided the stimulus is locked to the sample moments.

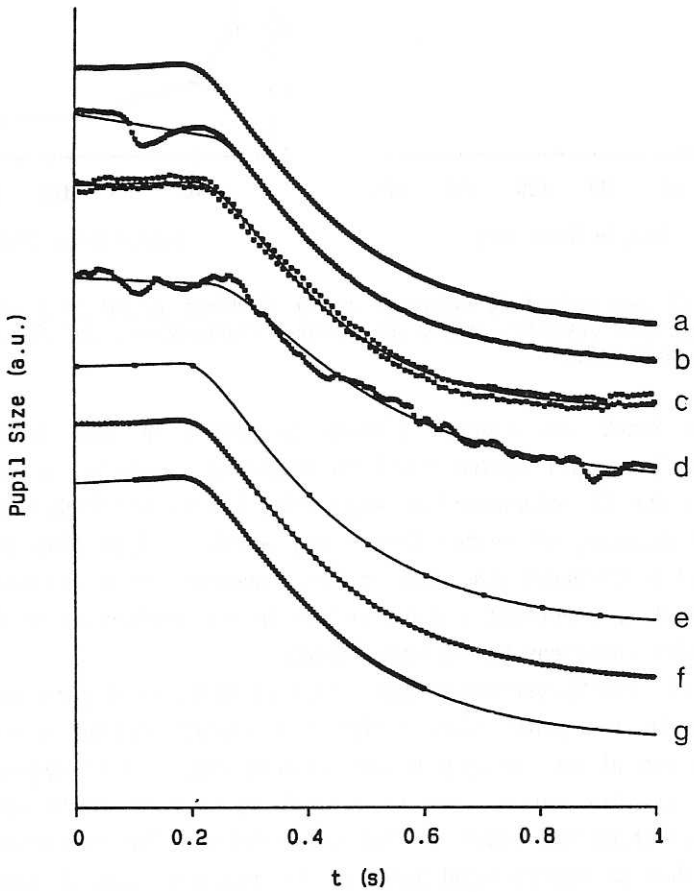



*Fig. 36. Examples of computer-fitted pupil constrictions, based on the second-order model described in the text. Stimulus onset is at t=0. Sampled data are given by dots, model results by solid lines. (a) Typical example (200 Hz, $SE_{CL}$=0.4 ms ). (b) Little (blink) artefact during the latency period (200 Hz, $SE_{CL}$=6 ms). (c) Noisy signal (200 Hz, $SE_{CL}$=7 ms). (d) Bad signal (200 Hz, $SE_{CL}$=12 ms). (e) Typical example sampled at 10 Hz ($SE_{CL}$=6 ms). (f) Typical example with a sample rate of 200 Hz applied in the interval around constriction onset (0.1 to 0.3 s), and 100 Hz outside ($SE_{CL}$=0.5 ms). (g) Typical example of curve fitting applied on only an interval of the constriction (200 Hz, $SE_{CL}$=0.7 ms).*

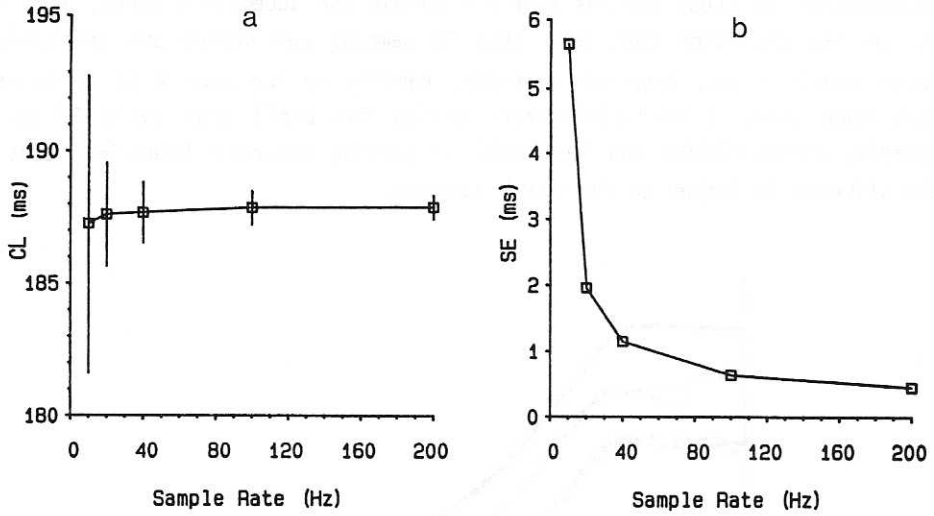



*Fig. 37. (a) CL determined by means of curve fitting, based on a second-order model, on a typical example sampled at various frequencies. (b) Standard error (SE) versus sample rate.*

Although Stark and Sherman already suggested in 1957 a third-order description of the pupil system based on Bode-plot analysis, a second-order model suffices for CL determination. Neglecting third- and higher-order terms results in an accuracy of better than 5 ms, which is less than the average intraindividual differences described so far. Moreover, if a third-order model would be applied, a practical problem arises in the estimation of two of the three exponential functions having equal signs.

Lee at al. (1969) performed curve fitting based on a pure second-order model, i.e. with the pupil size during the latency period assumed to be constant. The use of an averaged signal allowed omission of eventual random trends. Next, as many curves were calculated by them as there were samples reasonably indicating the start of the constriction. The constriction onset moment could then be approximated based on the residual sums of squares (Fig. 34e). Here, two modifications of this method are employed. Firstly, a linear trend during the latency period is allowed, and secondly, t-T is substituted by $(t-T\pm|t-T|)/2$. Then only one curve-fitting process is needed to estimate the parameter T.

The use of the second-order model also enables detection of the PCVL. Once all the parameters of the model f are known, the PCVL can simply be derived. At the peak constriction-velocity t>T, and the second derivative equals zero, for which one may write

$$\frac{d^2f}{dt^2} = a_2 \cdot b_2^2 \cdot \exp(-b_2 \cdot (t-T)) - a_3 \cdot b_3^2 \cdot \exp(-b_3 \cdot (t-T)) = 0.$$

If at stimulus onset t=0, this results in T=CL and

$$PCVL = CL + \frac{\ln(a_2 \cdot b_2) - \ln(a_3 \cdot b_3)}{b_2 - b_3} + \frac{\ln(b_2) - \ln(b_3)}{b_2 - b_3}.$$

The dependency of the CL and PCVL on signal amplitude can also be evaluated according to this model. In general, T can be searched for by a curve-fitting process so as to minimize the residual sum of squares of the actual data points and the mathematical model f. This process is independent of the amplitude of the signal. Changing the amplitude will only result in an equal change of the parameters $a_1$, $a_2$ and $a_3$, leaving all the time-dependent parameters ($b_1$, $b_2$ and T) unchanged. Like the CL, the PCVL is independent of the signal amplitude, but for one aspect. Subtraction of an equal change in $a_2$ and $a_3$ in the second term of the above equality will eliminate the amplitude dependency. The PCVL, however, does depend on the absolute values of the coefficients $a_2$ and $a_3$, determining the trend during the latency period. The PCVL is therefore only amplitude-independent if no trend is present, i.e. when $a_2 \cdot b_2 = a_3 \cdot b_3$, leaving a dependency on the time factors $b_2$ and $b_3$ which describe the constriction. If, however, several constrictions are measured under the same circumstances, averaging of PCVLs will eliminate the influence of normally distributed trends.

A comparison, made between subjective determination using observable deflections in pupil amplitude and the two objective methods described above, yielded significantly longer latencies and a far greater variance for the first. Therefore, the subjective method should be rejected for application. Comparison of CL's of a series of constrictions, elaborated by both above-described detection methods, revealed a difference of less than 5 ms on the average. At high sample rates the method employing velocity deflections from zero has preference, for it is easier and faster to apply. Because of the availability of a high-temporal-resolution pupillometer, CLs will therefore now be determined in this way. At low-rate sampled data and if the stimulus is locked to the sampling, however, the curve-fitting method should be applied.

**Averaging**

Averaging of individual responses is a general procedure to eliminate noise, including hippus in pupillometry, but not necessarily leaves the signal

characteristics unchanged. The difference between the latency of an averaged response and the average latency of the individual responses was therefore evaluated. Eleven copies of a pupil-constriction signal were only shifted in time with normally distributed latencies and were averaged (mean ±SD = 200 ±15 ms). In Fig. 38 results are given with the interval of interest lifted out. This figure shows that the onset moment of the averaged signal is shifted to the left with respect to the mean of the individual latencies. This was confirmed by curve-fitting applied to the averaged response.

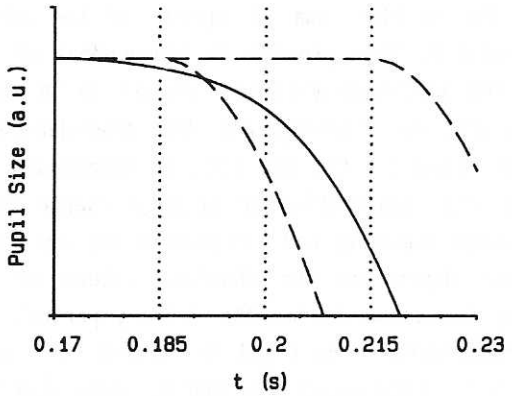



*Fig. 38. Example of averaged and individual pupil constrictions, in which the latency of the averaged signal does not equal the mean latency of the same individual constrictions. The solid line represents the average of 11 identical constrictions only shifted in time with normally distributed latencies (200 ±15 ms). The dashed lines represent constrictions at the mean ±SD.*

The reason for this shift is obvious when considering averaging as a summation. The first sample in the averaged response with a value falling beyond the range of sample values of the pre-constriction period will be determined by the single constriction with the smallest latency. When looking for latency differences of less than 25 ms, this advancement will become important. In the present investigations CLs are therefore determined based on individual constrictions.

### 5.2.2 *DEFECT LOCATION*

**ANS defects**

The pupil constriction is innervated by the parasympathetic nervous system as is pointed out in Chapter 1. This pathway, as shown anatomically in Fig. 4, is schematically redrawn in Fig. 39.

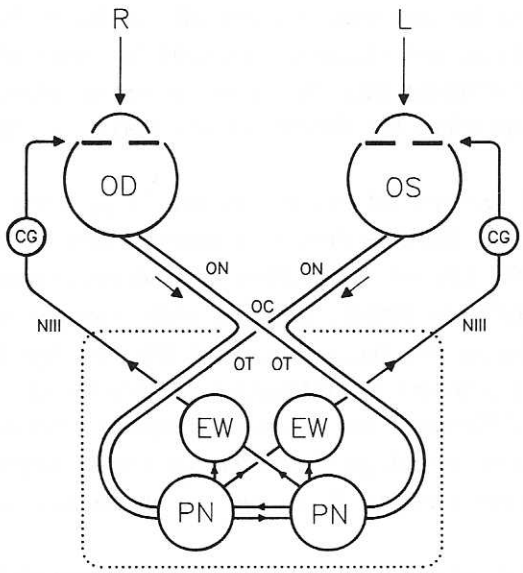



*Fig. 39. Schematic representation of the parasympathetic pupil reflex arc. OD = right eye, OS = left eye, ON = optic nerve, OC = optic chiasm, OT = optic tract, PN = pretectal nucleus, EW = Edinger-Westphal nucleus, NIII = oculomotor nerve, CG = ciliary ganglion, R = stimulus for right eye, L = stimulus for left eye.*

With reference to this simplified model, the CL of each eye is affected by three major contributing factors. The afferent part results in a delay $\tau_A$. The central processing time between the optic chiasm and the efferent arc, including the three partial crossings, leads to a delay $\tau_C$. The efferent part, including the delay at the nerve muscle transition, results in an efferent delay $\tau_E$. In the following, the direct and consensual reflexes are denoted by the subscripts OD and OS, while the subscripts R and L will denote the eye that has been stimulated. There are four essential latencies, which are consequently given by $CL_{R,OD}$, $CL_{R,OS}$, $CL_{L,OD}$ and $CL_{L,OS}$. As an example, $CL_{R,OS}$ represents the constriction latency of the left eye, when the right eye has been stimulated. The four latencies are then equal to:

$$CL_{R,OD} = \tau_{A,OD} + \tau_C + \tau_{E,OD} \quad [1]$$

$$CL_{R,OS} = \tau_{A,OD} + \tau_C + \tau_{E,OS} \quad [2]$$

$$CL_{L,OD} = \tau_{A,OS} + \tau_C + \tau_{E,OD} \quad [3]$$

$$CL_{L,OS} = \tau_{A,OS} + \tau_C + \tau_{E,OS} \quad [4]$$

Subtraction of [1] and [2] results in $CL_{R,OD}-CL_{R,OS} = \tau_{E,OD}-\tau_{E,OS}$, where $\tau_{A,OD}$ and $\tau_C$ cancel. The same result is obtained when the left eye is stimulated, i.e. [1]-[2] = [3]-[4]. This means that differences between the direct and the consensual reflex latency imply differences in efferent delays. If, for

whatever reason, one eye is known to be impaired, and not OD but OS is the impaired eye, the sign of $CL_{R,OD}$-$CL_{R,OS}$ and $CL_{L,OD}$-$CL_{L,OS}$ will be inverted. The difference is then identified by Efferent dCL. The latter inversion always yields the latency of the impaired eye minus the latency of the healthy fellow eye.

If on the other hand [1] and [3] are subtracted, $\tau_C$ and $\tau_{E,OD}$ cancel, leaving $CL_{R,OD}$-$CL_{L,OD}$ = $\tau_{A,OD}$-$\tau_{A,OS}$, and the same result is obtained when OS is examined, i.e. [1]-[3] = [2]-[4]. This implies that reflex differences between stimulus sides point to a relative afferent defect. The same sign inversion as mentioned above, applied to $CL_{R,OD}$-$CL_{L,OD}$ and $CL_{R,OS}$-$CL_{L,OS}$ if the left eye is impaired in stead of the right, results in the identification by Afferent dCL.

These considerations allow a differentiation between unilateral efferent and afferent impairments. Another major advantage of the comparison of intra-individual latencies is that a dependency of the CL on age and gender will be set aside.

Furthermore, if in addition to CL determination, visual evoked potentials are measured, also bilateral impairments may be detected. Analogous to the CL, the VEPL may be thought to be affected by separate contributing factors. However, for the VEP there are only two major components and these are afferent only. The first part of the nerve conduction, up to the optic tracts where pupil fibers split, is equal to $\tau_A$ for the pupil system. The second part consists of some central processing delay and the radiation toward the striate cortex ($\tau_S$). Two VEP latencies can then be measured, one for stimulation of the right eye ($VEPL_R$) and one for stimulation of the left eye ($VEPL_L$). These two latencies are thus equal to:

$$VEPL_R = \tau_{A,OD} + \tau_S$$
$$VEPL_L = \tau_{A,OS} + \tau_S.$$

When comparing CLs and VEPLs, bilateral impairments resulting in abnormal delays, can thus also be differentiated.

In comparing CLs with VEPLs, care has to be taken, because differences in retinal function leading to both evocations have not been taken into account in the above. VEPs are mostly measured using structured contrast stimuli, while the pupil is measured using luminance stimuli. It was already concluded in Chapter 3 that the pupil system does not respond to contrast stimuli. Nevertheless, it is reasonable to assume that both evocations will be delayed if the impairments under consideration result in a reduced conduction velocity.

**Effector defects**

The neural pathways of the parasympathetic system result in a delay between stimulus and constriction onset. Once a constriction has begun, its course will be influenced by the rigidity of the iris. In case of an iris stiffness it may be reasonable to assume that the moment of peak constriction-velocity is delayed with respect to the constriction onset moment, especially if the iris musculus sphincter is impaired. These considerations lead to the hypothesis that in case of a nerve-conduction impairment, as in ON and DM, the CL is prolonged, while the difference between PCVL and CL remains unaffected. However, when the iris muscle structure is impaired, as possibly in MyD, the CL is hypothesised to remain unaffected, while the difference between PCVL and CL is prolonged.

### 5.2.3 *STATISTICS*

Data will be explored by simple statistical testing only, i.e. by applying student-t testing and (multiple) linear regression. To evaluate the significance of possible differences between one statistic and a scalar or another statistic, double-sided student-t tests will be applied. When using (multiple) linear-regression analysis, yielding two (or more) p-values related to the intercept and slope(s), only the latter will be given. With respect to my purposes the intercept is of minor interest, and the corresponding p-value is extremely small. Because of the complexity of the underlying phenomena, higher-order dependencies are thought to be too speculative. That is why only linear regression is used.

## 5.3 EXPERIMENTAL CONDITIONS

One of the major interests of this chapter is the differentiation between afferent and efferent impairments. Both pupil and VEP testing has therefore to be performed, as indicated above. Most of the pupillometry has already been described, except for the choice of stimulus parameters. VEP testing is a common clinically applied method, and will therefore be described only briefly here.

### 5.3.1 *RATIONALE OF CHOSEN STIMULI*

To assess the latency of the pupil system, a specific stimulus was used. This concerns spatial as well as temporal aspects. Furthermore, it has to be remembered that patients with neurologic and ophthalmologic deficiencies not evident to the investigator, may be present in the examinations to be described below. To avoid erroneous interpretation of the results, and to restrain any inconvenience for the patients as much as possible, a set of stimuli was defined as follows.

The origin of the pupil light reflex was shown to be one of local luminance in Chapter 3. Moreover, if, as here, geographical variations in retinal functioning are not of interest, a large retinal area should be stimulated (Pfeifer et al., 1982). Due to the dependency of pupil behaviour on retinal illuminance rather than eye illumination, stimuli should be presented under open-loop conditions. A homogeneous stimulus was therefore used presented by the Maxwellian view stimulator as described in this thesis.

Stimulus intensities may be chosen in the scotopic or the photopic range. Presenting scotopic stimuli has several disadvantages. The smaller the presented intensities, the larger the latencies and their (relative) variations. Small latency differences are therefore harder to detect under scotopic than under photopic circumstances. Moreover, under scotopic conditions the smallest change in background illuminance can already have great impact upon the pupil reflex, resulting in large changes in the measured parameters. Also, under scotopic conditions relatively long dark-adaptation times are required, but this is hard to realize at the patient's bedside and is not appreciated by most subjects. Hence, it was thought rational to limit stimulus intensities to a photopic range, which is from about 1 to 4 Log photopic Troland (cf. Walraven et al., 1990). Under these circumstances mainly the cones and the parasympathetic nervous system are involved, and the influence of fatigue is suppressed (Lowenstein and Loewenfeld, 1951, 1952a,b; Aguilar and Stiles, 1954; Lowenstein et al., 1964). Higher light-pulse intensities result in frequent eye blinks at stimulus onset, making detection of the constriction onset impossible.

Although to a minor degree, the temporal stimulus parameters are important as well. When pulse durations shorter than the CL are chosen, the sympathetically induced dilatation may already start during the unfinished constriction. Especially the latency related to the moment of peak constriction-velocity (PCVL) will then be highly dependent on the stimulus duration. That is why pulse durations longer than 250 ms were chosen.

### 5.3.2 *MEASUREMENT AND STIMULATION OF THE VEP*

For visual elicitation of a cortical response mostly one of two types of spatially structured stimuli is applied, both using a checkerboard as the basic pattern. Either the checkerboard is reversing, so that the black and the white fields change places, or the checkerboard pattern appears or disappears on top of a uniformly illuminated field. In both cases the overall illuminance remains constant in time. Especially in patients with a pathological delay in the visual neural trajectory, the pattern-reversal method was proven to be more sensitive than the pattern-presentation method (van der Poel, 1985). Hence, in the present investigations only the pattern-reversal stimulus was applied. This was accomplished by projecting a slide with a checkerboard pattern via a twisting mirror (response time less than 5 ms) onto a translucent screen. By connecting a square-wave modulated voltage to the electromagnetically activated mirror, the gain could be adjusted in such a way that the fields precisely overlapped after each reversal. The periphery of the screen was masked from the projection.

Responses are led off from silver-silver-chloride electrodes fixed to the subject's scalp with collodium. The active electrode is usually placed between approximately 1 and 2.5 cm above the inion. Because of the low signal-to-noise ratio, signals are coherently averaged over 50 to 150 periods.

The VEP is generally quantified in terms of the amplitude and latency of the major response peak, which occurs about 100 ms after the stimulus onset, e.g. the checkerboard reversal. The latency of this P-100 peak is called the visual evoked potential latency (VEPL).

## 5.4 NORMATIVE DATA

### 5.4.1 *INTRODUCTION*

Because of the great variety in stimuli applied so far (Appendix A), and the dependency of the CL on stimulus conditions, a set of normative data has been gathered by using the techniques and according to the rationale described above. Where appropriate, these normative data will be used for comparison with data from patients to be described further on.

First interest now concerns interocular differences. The dependency of the CL on the stimulus intensity will be investigated next. The dependency of the CL on the time between two pulses is both of experimental and of

theoretical interest. A maximum number of constrictions has to be recorded preferably in the shortest possible time. The theoretical interest concerns the short-term adaptation, as first mentioned by Link and Stark (1988). Based on these normative data, the yet obscure dependency of the CL on age and gender, can also be examined. Finally, a possible relationship between the PCVL and CL will be searched for.

### 5.4.2 *EXPERIMENTAL CONDITIONS*

**Pupillometry**

Stimuli applied consisted of square-wave modulated light with background and pulse illuminances, varying between 1.3 to 4.1 Log Td. Interval and pulse durations were varied between 0.25 and 8 s.

To look for interocular differences and age dependency, different intensities of background (1.4 and 1.8 Log Td) and pulse (2.9 to 4.1 Log Td) were applied. Pulse duration was kept constant at 1.2 s and the interval at 5 s. An additional group of subjects covering a wide age range was tested, using a fixed stimulus of 1.2 to 3.7 Log Td, with pulses of 1.2 s duration, 5 s apart.

| $I_{off}$ | $I_{on}$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1.34 | 1.45 | 1.78 | 2.18 | 2.55 | 2.87 | 3.34 | 3.71 | 4.03 |
| 2.49 | 2.61 | 2.93 | 3.34 | 3.71 | 4.03 | | | |
| 2.93 | 3.05 | 3.38 | 3.78 | 4.03 | | | | |
| 3.34 | 3.46 | 3.78 | 4.03 | | | | | |
| 3.71 | 3.83 | 4.03 | | | | | | |

*Tab. II. Applied background- ($I_{off}$) and pulse- ($I_{on}$) retinal illuminances in Log Td. $T_{off}$ is set at 5 s, $T_{on}$ at 1.2 s.*

| $T_{off}$ | $T_{on}$ | | | | | |
|---|---|---|---|---|---|---|
| 0.25 | 1.00 | 0.50 | | | | |
| 0.50 | 2.00 | 1.00 | 0.50 | | | |
| 1.00 | 4.00 | 2.00 | 1.00 | 0.50 | | |
| 2.00 | | 4.00 | 2.00 | 1.00 | 0.50 | |
| 4.00 | | | 4.00 | 2.00 | 1.00 | 0.50 |
| 8.00 | | | | 4.00 | 2.00 | 1.00 |
| | └ light pulse ┘ | | | └ dark pulse ┘ | | |

*Tab. III. Applied interval- ($T_{off}$) and pulse- ($T_{on}$) durations in seconds. Note that when $T_{off}$ is less than $T_{on}$, this effectively results in a dark pulse. $I_{off}$ is set at 2.49 Log Td, $I_{on}$ at 3.34 Log Td.*

For explicit investigation of the dependency of the CL on the stimulus parameters, these were varied according to Tabs. II and III. Because the CL is more or less related to changes in pupil size, and changes in pupil size are known to be related to the Weber fraction, stimulus intensities were chosen accordingly, i.e. $(I_{on}-I_{off})/I_{off}$ = constant at different levels. The temporal parameters were set arbitrarily, with $T_{on}/T_{off}$ = constant at different values.

Whether male and female CLs differ or not was tested using the stimulus parameters given in Tab. II.

The relationship between the PCVL and CL was tested over a variety of these stimuli.

In every trial it was strived for to measure six unjammed constrictions per eye. Subjects were dark-adapted for 5 min in an incompletely darkened room. Longer adaptation times were thought to be unnecessary because the cone system was of main interest, and complete dark adaptation was thought to be needless because the first light pulse presented to the subject would undo the adaptation.

**Subjects**

None of the participating subjects had any neurologic or ophthalmologic disfunction, except for wearing spectacles. Subjects normally wearing spectacles were tested without correction for a better IRIS signal quality. The fixation target of the Maxwellian view stimulator could account for accommodation. Subjects did not use any medicine known to influence visual and pupil function.

### 5.4.3 *RESULTS*

**Interocular differences**

During the experiments the reflexes of the right and left eye were recorded simultaneously, while the right and the left eye were stimulated separately. This gave the opportunity to look for differences between the direct and consensual reflex, and for a possible effect of the choice of eye stimulated.

A group of 15 healthy subjects, 11 males and 4 females in the range of 22 to 45 years of age participated. None of the experiments yielded a statistically significant difference between the CL of the direct and the consensual reflex. Nor did it make any difference whether the right or left eye was stimulated.

The average intraindividual CL variability (within subjects) amounted to 16 ms. Interindividual differences (between subjects) came to 20 ms on the average.

**CL dependency on stimulus**

To investigate the influence of the stimulus intensity on the CL, 11 subjects, 7 males and 4 females in the range of 21 to 31 years old, were examined. Twenty-two different combinations of the background intensity $I_{off}$ and pulse intensity $I_{on}$ were presented as tabulated in Tab. II, whereas the pulse and interval duration were kept constant. Fig. 40a shows the averaged latencies for all subjects (22 eyes) arranged according to the background intensity $I_{off}$. In joining the five curves into one multiple linear regression analysis, a relation appeared according to $CL = 264 - 29*(\mathrm{Log}(I_{on})-\frac{1}{2}\mathrm{Log}(I_{off}))$. In Fig. 40b the mean CLs of all 22 experiments are accordingly plotted versus $\mathrm{Log}(I_{on})-\frac{1}{2}\mathrm{Log}(I_{off})$.

To investigate the dependency of the CL on the temporal stimulus parameters, a group of 11 subjects in the same age range as above was submitted to another series of experiments. The background and pulse intensity were kept constant, but the interval $T_{off}$ and pulse time $T_{on}$ were varied according to Tab. III. The CL was measured and plotted versus $T_{off}$ and $T_{on}$, as shown in Fig. 41. Within the first two seconds after stimulus offset, the CL decreased rapidly, while it remained constant afterwards. Pulse duration did not show to influence the CL.

**CL versus age**

In a group of 15 subjects in the age range of 22 to 45 years (mean ±SD = 31 ±7 year), the CL dependency on age was tested at different stimulus intensities. In another group of 56 subjects in the age range of 12 to 75 years (mean ±SD = 39 ±17 year), the age dependency was tested at fixed intensities.

The age dependency at different stimulus intensities is shown in Fig. 42a-d. Linear regression revealed intercepts of 148 ±16, 165 ±10, 165 ±17 and 153 ±16 ms, respectively. The slopes were 0.98 ±0.5, 1.07 ±0.3, 0.84 ±0.5 and 0.92 ±0.5 ms/year, respectively, but statistical significance was only marginal.

The age dependency in the group covering an extended age range is shown in Fig. 42e. This revealed a slight, but statistically significant age dependency according to $CL = 192 + 0.36 \cdot age$ (intercept ±5 ms, slope ±0.11 ms/year, $p<0.003$).

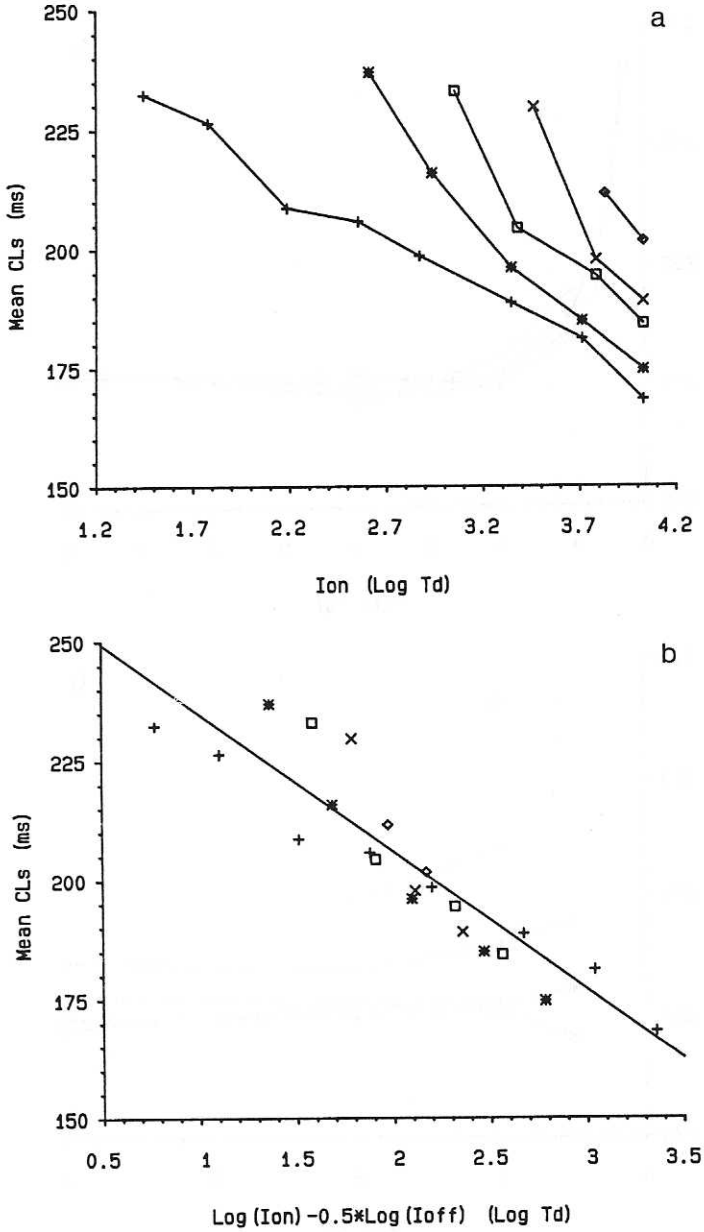



*Fig. 40. Mean CLs of eleven subjects (a) plotted versus the logarithm of the pulse intensity $I_{on}$, at $I_{off}$= 1.3 (+), 2.5 (*), 2.9 (squares), 3.3 (x), and 3.7 (diamonds) Log Td.; (b) plotted versus the difference of $Log(I_{on})$ and $\frac{1}{2}Log(I_{off})$. The best fit is given by the solid line.*

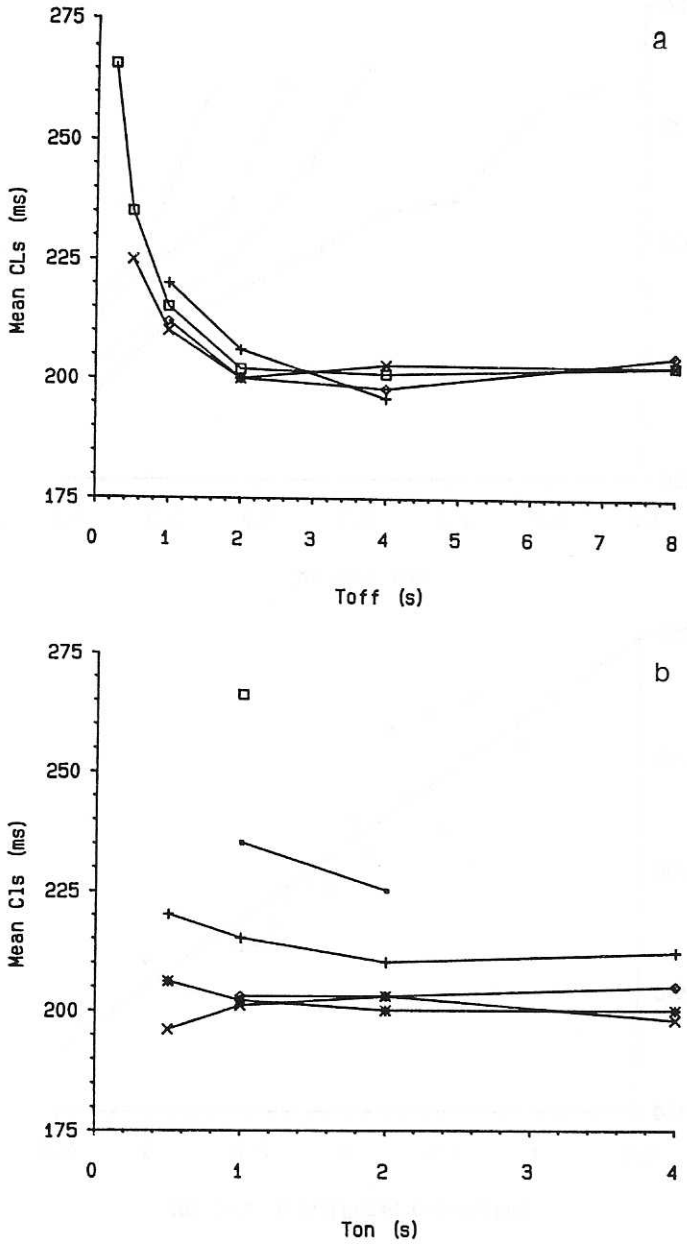



*Fig. 41. Mean CLs (a) plotted versus the interval duration $T_{off}$ at pulse durations $T_{on}$= 0.5 (+), 1 (squares), 2 (x) and 4 (diamonds) s.; (b) plotted versus the pulse time $T_{on}$ at interval durations $T_{off}$ = 0.25 (squares), 0.5 (dots), 1 (+), 2 (*), 4 (x) and 8 (diamonds) s.*

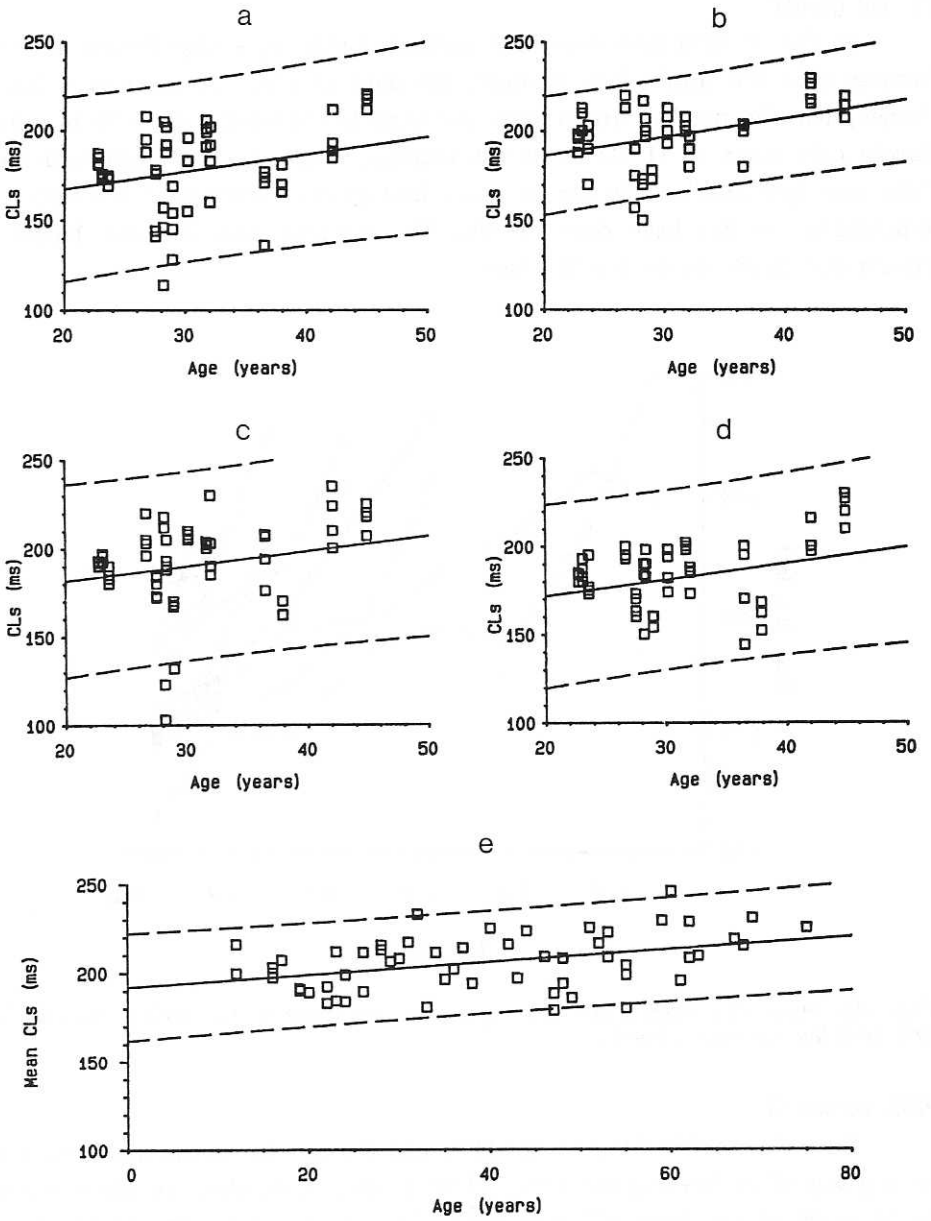



*Fig. 42. CL dependency on age. CLs averaged per eye versus age of 15 subjects for four different stimuli: (a) 1.8-4.1, (b) 1.4-2.9, (c) 1.4-3.3, (d) 1.4-3.8 Log Td. (e) Mean CLs of 56 subjects plotted versus age (1.2-3.7 Log Td). Linear regression lines are shown by solid lines (parameters given in the text), 95%-prediction levels by dashed lines.*

**CL and gender**

So far no data have ever been presented showing a significant difference between male and female CLs. However, the data of Fig. 40, where male CLs last longer, do. The matched differences per stimulus between the isolated male and female data comes to 11 ±11 ms on the average, which is significantly different from zero ($p<0.0002$). This can be visualized by replotting male and female data separately, as has been done in Fig. 43. Averaged male CLs are larger than female CLs in 20 out of the 22 cases.

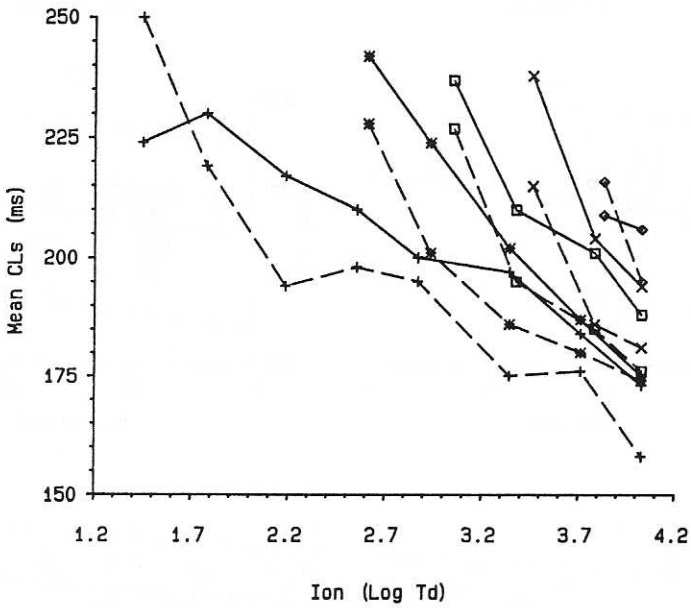



*Fig. 43. Mean CLs from Fig. 40, replotted separately for males (solid lines) and females (dashed lines).*

**PCVL versus CL**

The relationship between the PCVL and the CL was investigated explicitly in a group of 24 healthy subjects, 12 males and 12 females, in the range of 23 to 63 years of age (mean ±SD = 44 ±21). The stimulus consisted of light pulses from 1.2 to 3.7 Log Td, 1.2 s of duration, 5 s apart. Multiple regression analysis showed no significant relationship other than between PCVL and CL. This implies that age and gender are not involved in the relationship between PCVL and CL. The prevailing significant relationship between the PCVL and CL appears in Fig. 44. Linear regression showed an intercept of 102 ±13 ms and a

slope of 1.08 ±0.07 (r=0.87). Within the range of errors this result was in line with results from experiments performed at different stimulus intensities.

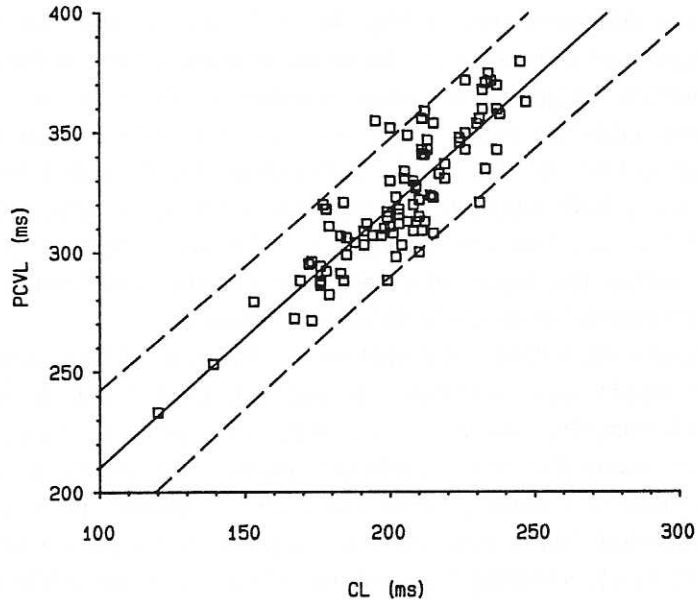



*Fig. 44. Peak constriction-velocity latency plotted versus constriction latency. Data are averaged per eye. The solid line represents the best linear fit, and the dashed lines represent the 95%-prediction levels.*

### 5.4.4 *DISCUSSION*

**Interocular differences**

The finding, that stimulation of the right or left eye does not make any difference, is in accordance with that of Müller-Jensen (1976). Further data presented in this thesis therefore consist of the averaged latencies of the simultaneously recorded direct and consensual reflexes, unless stated otherwise. No constraints were laid upon the choice of the eye that was stimulated. A distinction will be made, however, when investigating abnormalities of the pupil light reflex in patients, because latency differences may then help to discriminate between afferent and efferent lesions.

The average intraindividual latency difference (16 ms) found now is in agreement with data presented by Drischel (1957). Müller-Jensen and Hagenah (1976) and Müller-Jensen (1978) found a much smaller intraindividual difference

of 5 ms. This small intraindividual variability may well have been calculated from the pooled latencies, whereas current data were not pooled.

**CL dependency on stimulus**

From the data presented in Fig. 40 it is evident that the CL decreases with some logarithmic function of the pulse intensity. Most authors describing this relationship suggested a linear dependency, for example Cibis et al. (1977). Others suggested higher-order relationships, such as quadratic (Ellis, 1981) or exponential (Ellis, 1979). Furthermore, Feinberg and Podolak (1965) and Lee et al. (1969) suggested a linear relationship between the CL and the logarithm of $I_{on}$-$I_{off}$ (see also Appendix A). The data presented in this thesis may further define the logarithmic behaviour and the relationship between the CL and the difference in pulse-to-background intensity.

The experimental conditions applied by Lee et al. (1969) were closest to the present conditions. Therefore, it was attempted first to verify their suggested relationship, i.e. $CL = a + b \cdot Log(I_{on}) + c \cdot Log(I_{on}-I_{off})$. This, however, was not successful and a different approach was explored. As suggested by Fig. 40a there is a roughly linear relationship between the CL and $Log(I_{on})$. Therefore, multiple linear regression was applied to the pooled data (i.e. for all values of $I_{off}$), yielding a dependency of the CL on the difference between $Log(I_{on})$ and approximately half $Log(I_{off})$. The intercepts of the regression lines presented in Fig. 42 (CL versus age) are within the error levels of these data. Apparently, the CL does depend on the background or adaptation level, as opposed to the hypothesis of Lee et al. (1969). For photopic stimuli the suggested relationship results in a maximum latency ($I_{on} = I_{off}$) of 235 ms. At higher background intensities the CL versus $Log(I_{on})$ curves tend to a more exponential behaviour according to $CL = CL_o + A \cdot \exp\{ -B \cdot [Log(I_{on}) - Log(I_{off})] \}$. When excluding the data from the lowest background intensity, regression analysis revealed a minimum latency of 175 ±12 ms and a maximum latency of 238 ms. The latter is in good agreement with the previously found maximum, and with the data presented by Lee et al. (1969), although to a minor degree. This value differs much from the maximum CL of 500 ms found by Alpern et al. (1963), or 400 ms as found by Cibis et al. (1977), but these latter values referred to measurements just above intensity threshold, i.e. in the scotopic range. Apparently there is a difference in scotopic and photopic CL limits. This can be understood on the basis of the functional difference between rods and cones, which also accounts for the discontinuity in visual and pupil sensitivity versus adaptation time (Ohba and Alpern, 1972).

From the above it can also be concluded that, in contrast to the reflex amplitude, the latency does not behave conform the Weber and Weber-Fechner laws. The CL may thus indeed provide additional information on pupil functioning.

So far, only Link and Stark (1988) attempted to determine the effect of the repetition rate of a square-wave-modulated light stimulus upon the CL. However, they did not look for separate control by pulse and interval duration. Their finding of latency increasing with increasing repetition rate, i.e. decreasing pulse and/or interval duration, is now confirmed, and it is also demonstrated that this mainly results from the decrease in interval duration. In Fig. 41a the exponential decay in $T_{off}$ is most striking, especially with respect to the relatively small dependency on the pulse time $T_{on}$. This suggests a very fast adaptation process, i.e. within 2 s. The relatively small differences in the CL resulting from the different pulse times is not very surprising, because most pulses lasted longer than the CL itself. The stimulus cannot control the constriction onset after its occurrence. Differences, though occurring, probably resulted from adaptation to the previous pulse. This will be further enhanced by a small interval time. When using pulse times longer than one second and interval times longer than two seconds, the constriction latency was found to be independent of the previous pulse. In system-analytical terms, temporal pupil behaviour must therefore be controlled by a leaking integrator with only a small time constant, which is regulated by the afferent number of action potentials per unit time. This short-term adaptation can also be held responsable for differences between the light and dark reflex of the pupil system.

**CL versus age**

The CL dependence on age has been discussed by several authors. Kumnick (1956), Borgmann (1972a), Beaumont et al. (1987), and Grünberger (1987) found no significant age dependency. Petersen (1956), Feinberg and Podolak (1965), Müller-Jensen (1978), and Pfeifer et al. (1983), however, did. Only Feinberg and Podolak (1965), and Pfeifer et al. (1983) presented the dependency as a regression of the latency on age, and reported an increase of 0.77 and 0.89 ms per year, respectively.

The present investigations show little difference in the increase of CL with age between the different stimuli, up to about 50 years, i.e. 0.95 ±0.10 ms per year on the average. This increase in latency agrees well with that found by Pfeifer et al. (1983), although the age ranges do not match. However, the data of Fig. 42e, belonging to a larger age range comparable to that

reported of by Pfeifer et al. (1983), reveals a smaller increase of the CL with increasing age. An assumption that the latency increases only up to the age of 50, whereafter it remains constant, could not be confirmed by statistical analysis and would contradict the data of Pfeifer et al. (1983). It is therefore now concluded that the CL does increase with increasing age, but the extent of this increase remains uncertain.

Müller-Jensen and Hagenah (1976) have shown that CL prolongation during life is not caused by a reduction in visual acuity due to reduced refraction and differences in pupil sizes. It must therefore result from a reduced nerve conduction. Because SNS activity diminishes with increasing age (Pfeifer et al., 1983), the increase in CL certainly is an effect of reduced PNS functioning. Dorfman and Bosley (1979) have demonstrated a NCV reduction in the CNS of approximately 0.24 m/s per year. If the total parasympathetic reflex arc length is in the order of 0.2 m, and if the latency caused by the nerves is 20 ms (the average overall latency is 220 ms, minus 200 ms for the innervation of the iris muscle according to Loewenfeld, 1966), this results in a reduction by 2.4% per year. The average latency increase according to this thesis is 0.4 ms per year, which comes to 2%. This might seem to be in agreement, but it should be emphasised that these calculations are based on three questionable assumptions: (1) delays at synapses are neglected, (2) the innervation of the human iris sphincter takes as long as for the iris sphincter of the cat as studied by Loewenfeld (1966), and (3) NCV decays are the same for all nerves in the CNS.

In contrast to the knowledge about the development of the CNS, little is known about its degeneration. Axonal degeneration, diminution in number and density of nerve fibers, increase of connective tissue, and myelin loss are possible causes (Dorfman and Bosley, 1979). Hypothetical morphological models have not yet been verified histologically, however (Lang et al., 1985).

**CL and gender**

Drischel (1957) and Grünberger (1987) were the only authors so far to mention a difference between male and female CLs, but without presenting data. Müller-Jensen and Hagenah (1976) looked for such a difference, but could not detect any. All experiments performed during the present study revealed slight CL differences between men and women. For example, the data concerning the influence of the stimulus parameters on the CL were separated for males and females in Fig. 43. In this figure, the female subjects show a clear and persistently shorter CL than the male subjects do, except for two points only. Although the separate cases show no statistically significant differences, the significance of the average difference over the pooled data is high (11 ms,

$p<0.0002$). Because statistical significance is not always this obvious, the practical impact of this finding is of minor importance. Further data will therefore not be separated for gender beforehand. Why male and female latencies differ, may be of scientific interest, however.

The literature hardly gives an explanation for this phenomenon. In studies on the BAEP, SEP and NCV, latency differences between males and females have been reported, with the male data showing consistently longer latencies than female data. The most striking gender differences have been demonstrated in children, independent of body length and temperature, with much emphasis laid upon the formation of myelin (Lang et al., 1985; Kimura, 1987).

The difference demonstrated in this thesis may be explained by inevitable differences in brain weights or sizes (Dekaban and Sadowski, 1978). The difference in nerve lengths then explains a difference in latencies, as for the VEP and BAEP (Allison et al., 1983). However, the pupil system is more complicated, by, among other factors, the innervation of the iris muscles. If this is ignored at first, and if constriction latencies are proportional to the lengths of the reflex arc, and if these lengths are in proportion to the lengths of the average male and female skull (glabella-opisthocranion distance according to Lusted and Keats, 1972), i.e. 21.2/20.1, then female CLs would indeed be about 10 ms shorter than male Cls. Also this calculation is questionable, because here the innervation of the iris muscles must be neglected, whereas its involvement is an essential supposition in the analysis of age dependency. Furthermore, if the innervation time is disregarded and the reflex arc length is again approximated by 0.2 m, the NCV would be in the order of 1 m/s, while it would be about 10 m/s if the innervation is taken into account. For CNS-NCV, 10 m/s is a more realistic value. Therefore, differences in NCV may play their part in causing different male and female CLs, but they do not explain the total observed gender differences in latency.

**PCVL versus CL**

The high correlation between the PCVL and CL (exemplified by Fig. 44), an intercept of approximately 100 ms, and a slope approximating 1 suggest that the peak constriction velocity always occurs some 100 ms after the constriction onset. This was independent of age, gender, and stimulus conditions.

By theoretical analysis of a second order approximation of pupil constrictions, it was shown that the difference between PCVL and CL is independent of constriction amplitude. It is now also demonstrated experimentally that this difference is independent of stimulus intensity and thus of constriction amplitude. These two facts strongly support the theory of a simple stepwise

neural input to the iris sphincter. On that basis the difference between PCVL and CL is a reliable parameter for muscular function and the CL for neural function. This finding can contribute to the differentiation between muscular and neural disfunction in diseases with abnormal pupil behaviour, such as myotonic dystrophy. As a consequence, CL variation is only due to variation in neural activity and not in muscular functioning. An explanation for the variation, still existing in the PCVL-versus-CL relationship, might be given by the variation in trends during the pre-constriction period. As indicated in the description of the second-order model, the PCVL does depend on this trend.

Because of the constant difference between the PCVL and the CL, further investigations concerning normative data were limited to the CL.

## 5.5 OPTIC NEURITIS AND MULTIPLE SCLEROSIS

### 5.5.1 *INTRODUCTION*

Optic neuritis (ON) is broadly defined and may encompass all affections of the eye, without any abnormality of the media or fundus, in which a sudden decrease of visual acuity is experienced by the patient. Some of the most important causes of ON reported in the literature are listed by van der Poel (1985). There are some similarities between visual pathological expressions in ON and diabetic neuropathy. The latter is an item of interest which will be described separately in the next section. Multiple sclerosis (MS) remains the major cause of ON. But there are still no parameters that may predict the risk of developing MS.

In ON and MS, disfunctioning of the afferent visual pathway is a known phenomenon. The afferent pathway is thought to be identical in mediating both pupil and VEP responses, at least up to the posterior third of the optic tracts (Chapter 1). The availability of a high-temporal-resolution pupillometer enabled a more detailed study of the relationship between VEPLs and CLs. In ON, unilateral impairments are often present, and Section 5.2 of this chapter hypothesised a way to differentiate between unilateral afferent and efferent defects. Müller-Jensen and Zschocke's (1979) statement about the fact that it is not possible to differentiate between afferent and efferent impairments based on pupillometry only, is therefore of special interest.

Besides general neurological and ophthalmological findings, special attention will be paid to the recovery time (RT) elapsing after the acute phase of the ON.

### 5.5.2 *EXPERIMENTAL CONDITIONS*

As far as the ON/MS group is concerned, every patient was subjected to a standard eye examination, completion of a general questionnaire, VEP measurement and pupillometry. Examination by an ophthalmologist excluded other eye diseases than ON and MS. Where necessary, patient data were compared with the data presented in the previous section.

All tests were performed during morning hours in order to diminish possible diurnal pupillary effects (Tiedt, 1963; Utsumi et al., 1976, 1978).

**Pupillometry**

The stimulus consisted of light pulses, 1.2 s long with an illuminance of 4.1 Log Td, at intervals of 5 s with an illuminance of 1.8 Log Td. The aim was to measure at least 6 uncontaminated (without eye blinks) constrictions per eye.

**VEP**

VEP measurements were performed using checkerboard reversals presented by a projection of a checkerboard slide via a rotating mirror. A translucent screen was seen at a distance of 1 m, spanning a total visual angle of 30°. The individual squares spanned 1° at a mean over-all luminance of 13 and 127 cd/m$^2$ and 0.5° at 13 cd/m$^2$ only. Modulation depth was approximately 80%. The stimulus frequency was 1 Hz (i.e. 2 reversals per second). Eventual refraction errors were corrected. Both eyes were stimulated separately. Patients were asked to look at a fixation spot at the centre of the stimulus field.

**Eye examination**

The standard eye examination consisted of Snellen visual-acuity testing, Goldmann perimetry, slitlamp examination, and ophthalmoscopy.

**Patients**

Forty-nine patients, known to suffer from ON either in the acute or recovery stage, were willing to participate in an experiment to assess the CL and VEPL. Patients were selected from the referrals to an electrodiagnostic department. Lesions of the conductive system other than those of ON and MS, such as by Leber's disease, compression, or toxic neuropathy, were excluded as far as could be ascertained by history taking and clinical examination. Three patients were examined during both the acute and the recovery phase. The group consisted of 12 males and 37 females, ranging from 18 to 74 years of age (mean

±SD = 39 ±10). The RT ranged from 0 (acute) to 9 years (mean ±SD = 1.2 ±1.5). The right eye was impaired in 13 patients, the left eye in 26, while 7 patients suffered from a bilateral impairment. In 3 cases, bilateral impairment was doubtful. Twenty-two patients were diagnosed as multiple sclerosis (MS) in one of three catagories: possible, probable, or definite MS.

None of the patients used any drugs known to influence the parameters of interest.

### 5.5.3 *RESULTS*

**General**

In four patients mean CL could not be determined because of too frequent eye blinking. In seven patients no VEP at all was recorded. In one patient neither CL nor VEP could be obtained. Per subject four average latencies were determined, $CL_{R,OD}$, $CL_{R,OS}$, $CL_{L,OD}$ and $CL_{L,OS}$ (see Section 5.2.2). However, in two cases 3, and in one case only 2 of the 4 CLs could be determined. Fourteen patients complained of painful eye movements. Standard eye examination showed seven Marcus Gunn pupils and in nineteen patients partial scotoma or impaired fundi were found.

**Pupillometry**

The left part of Fig. 45 (Figs. 45a,b,c) shows the results of the pupillometry. In order to get around possible influences of e.g. age and gender on the CL, in the first instance intraindividual, i.e. interocular, differences were analysed. Consequently, only patients with a unilateral impairment were involved. The interocular latency difference between the affected and the healthy fellow eye (dCL) was calculated with respect to the direct and consensual reflex (Fig. 45a), as well as with respect to the eye that had been stimulated (Figs. 45b,c).

Fig. 45a shows a symmetrical distribution of the dCLs around zero (mean ±SD = 2 ±27 ms), and statistical testing yielded no significant difference from zero. For reasons to be mentioned further on, the CLs of MS patients were tested separately. A slight increase of 6 ms on the average in dCL between the direct and consensual pupil reflex of the MS patients only could be established.

The latency difference between stimulation of the impaired and the healthy fellow eye of the unilateral ON patients (Fig. 45b) was significantly different (mean ±SD = 15 ±34 ms, $p<0.0006$). The difference between the CL of

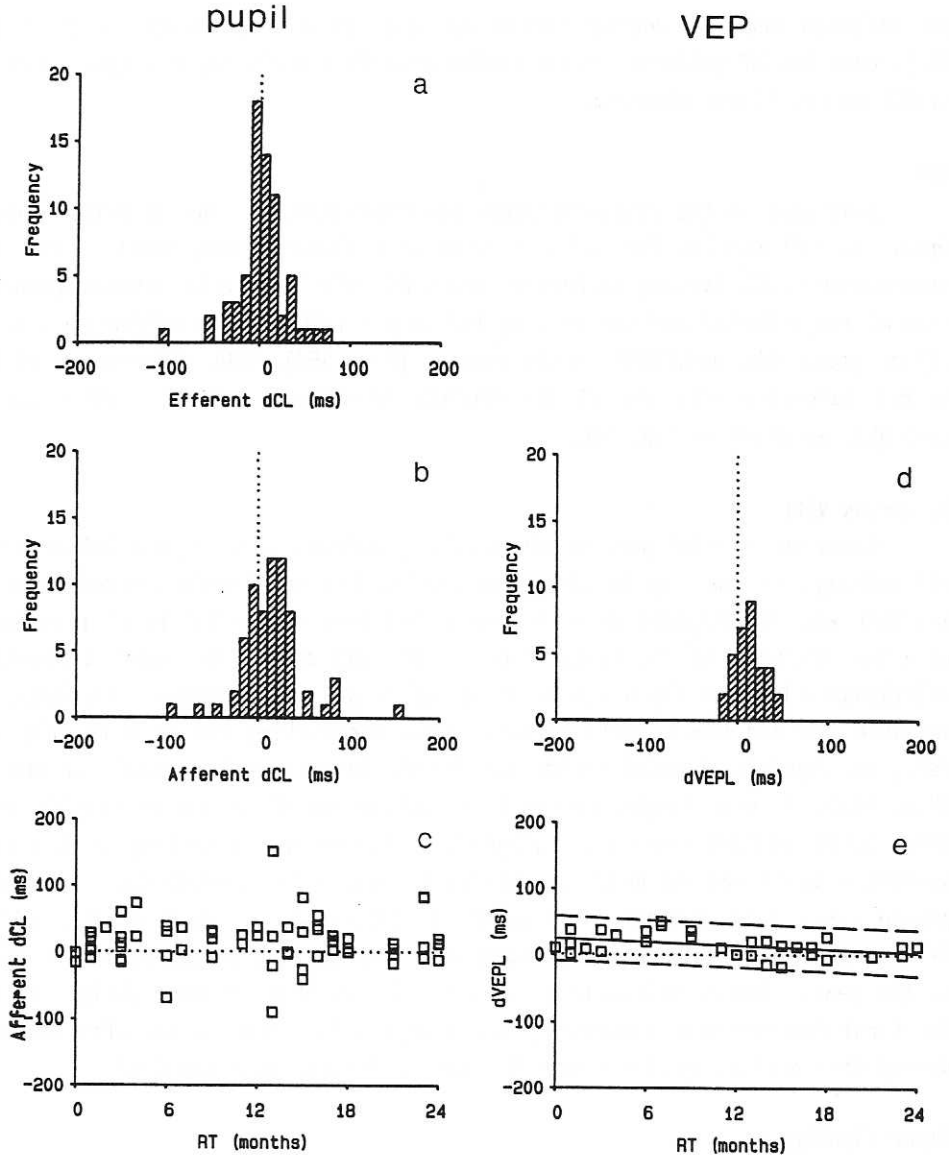



*Fig. 45. Latency measurements of the pupil constriction (left), and the P-100 peak of the VEP (right). (a) Interocular CL differences between the direct and consensual reflexes of the impaired and healthy fellow eyes, both for stimulation of the right and left eye. (b) CL differences between stimulation of the impaired and healthy fellow eyes, both for the direct and consensual reflex. (c) Scatterplot of the differences shown in b versus the time between the acute phase of the neuritis and the investigation (RT in months). (d) VEP-latency differences between the impaired and healthy fellow eyes. (e) Regression of the differences in VEPL on the recovery time (solid line) with 95%-prediction levels (dashed lines).*

the affected and the healthy fellow eye did not decrease with the RT (Fig. 45c). When the MS patients were excluded from this analysis, a slight decrease in dCL versus RT was observed.

**VEP**

Analogous to the aforementioned, the right part of Fig. 45 (Figs. 45d,e) shows the VEP results for patients with an unilateral impairment. Here, the interocular P-100 latency difference could be calculated only between stimulation of the affected and the healthy fellow eye (dVEPL). The difference was 14 ±17 ms (mean ±SD, $p<0.0002$) on the average (Fig. 45d). VEPL differences (dVEPL in ms) decreased with the RT (in months) according to $dVEPL = 25 - 0.9 \cdot RT$ ($p<0.05$), as shown in Fig. 45e.

**CL versus VEPL**

Since the initial part of the pupillary pathway runs in parallel with the VEP pathway, at least up to the optic tracts, the relationship between the CL and VEPL was investigated in more detail. Patients with a bilateral impairment were not included in the calculation of dCL and dVEPL. To reveal a possible relationship between the absolute values of CL and VEPL, however, all acquired absolute CLs and VEPLs could be used. When correlating the CL with only the VEPL, no significant relationship was found. Because dVEPL depends on the RT (Fig. 45e), it was thought rational to include the RT in correlating CL with VEPL. Using multiple regression analysis, a relationship according to $CL = a_0 + a_1 \cdot VEPL + a_2 \cdot RT$ was found to be significant ($a_0 = 114$, $p<0.00005$; $a_1 = 0.57$, $p<0.002$; $a_2 = 1.11$, $p<0.02$). In Fig. 46 the dependency of the CL on VEPL and RT is demonstrated by separating the data into four equally spaced RT intervals up to two years. The relationship between the CL and VEPL is most obvious during the first few months of recovery. This is especially true for the affected eyes during this period, in which case $CL = 62 + 0.97 \cdot VEPL$ with $p<0.0003$.

**Other findings**

Both the CL and the VEPL correlated well with visual acuity. The CL appeared to be related according to $CL = 210 - 20 \cdot acuity$ ($p<0.02$), and the VEPL according to $VEPL = 129 - 14 \cdot acuity$ ($p<0.002$).

When the presence of scotomas, MS, Marcus Gunn pupils, abnormal fundi, and painful eye movements were taken into account in all above-mentioned tests, no significant dependency on these quantities whatsoever could be demonstrated. For this, again multiple regression analysis was used.

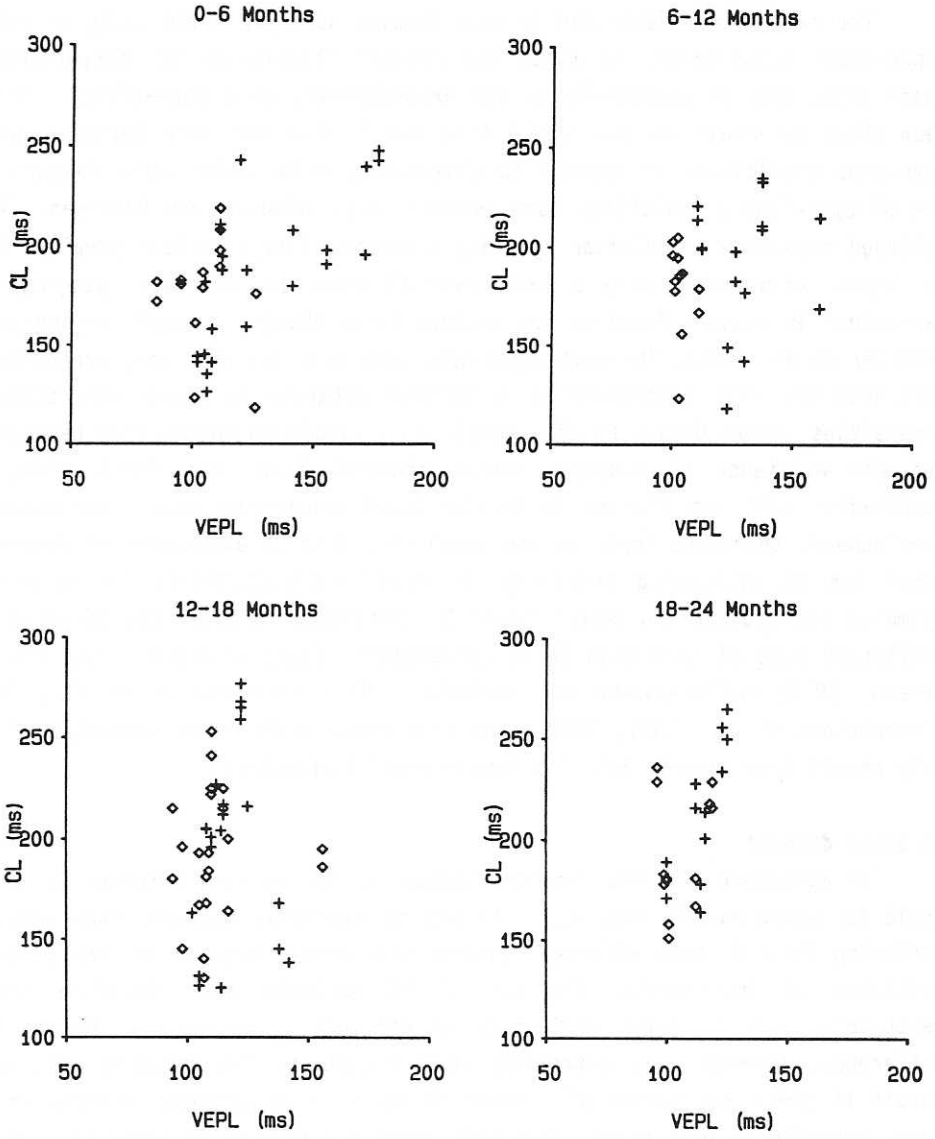



*Fig. 46. Scatterplots showing the relations between CL, VEPL, and recovery time. Data are divided into four equally spaced recovery periods of six months each, separately for the unaffected eyes (diamonds) and the impaired eyes (+).*

## 5.5.4 *DISCUSSION*

**Retinal influences**

The experiments described in this chapter were performed using so called 'open-loop' stimulation, in which the retinal illuminance is independent of pupil size, and is adjustable by the experimenter. As a side-effect, it has been shown by Utsumi et al. (1978) that the CL does not vary diurnally under open-loop conditions, as opposed to closed-loop stimulation which favours the use of open-loop stimulation. Some authors (e.g. Jakobsen and Pedersen, 1985) achieved open-loop stimulation by using a narrow (1 mm diameter) parallel beam of light, stimulating only a small retinal area. In this way, geographical variations in retinal function may produce large changes in pupillary response (Pfeifer et al. 1982). The wide-angle open-loop stimulus used now, subordinates this problem. This subordination is further enhanced by using high stimulus intensities (Alexandridis et al., 1981). As a result no correlation between CL and the existence of scotomas and/or abnormal fundi was found. This, in combination with the finding in Section 5.4.4 concerning neural and muscular involvement, therefore leads to the conclusion that CL abnormalities described above can be attributed primarily to neural disfunctioning. The choice of stimulus may explain why others found CL differences between the affected and unaffected eyes of more than 50 ms (Lowenstein, 1954; Thompson, 1966; Müller-Jensen, 1978; Müller-Jensen and Zschocke, 1979; Argyropoulos et al., 1980; Alexandridis et al., 1981, 1982), and that these differences probably did not only result from neural, but also from retinal disfunction.

**Efferent defects**

In agreement with the clinical picture of ON, no mean Efferent CL defect could be demonstrated (Fig. 45a). It may be expected, however, that patients suffering from MS show efferent defects more often, because of the multiple existence of impairments. The CLs of MS patients were therefore tested separately, and a slight difference in efferent latencies was found. This difference, however, was determined with regard to the affected eye. As a result of the distribution of chances in MS to have efferent defects on the same, opposite or both sides, this difference was only of marginal statistical significance.

**Afferent defects**

It was possible to show a statistically significant mean Afferent dCL in patients with a unilateral ON (Fig. 45b). The hypothesis, that unilateral

afferent defects can be differentiated without referring to the VEP, can thus be confirmed.

A persistent damage to the afferent pupil pathway has been demonstrated in ON, based on the persistent CL prolongation during recovery. The persistent prolongation of the CL, using high stimulus-intensities, is in agreement with the results of Alexandridis et al. (1982) who used a high-intensity pre-stimulus adaptation. However, the present difference was possibly influenced by yet other impairments than that of the optic nerve and retina in some of the patients with MS. This is analogous to the aforementioned efferent dCL in MS. The influence of lesions other than in the afferent arc, as in MS, was confirmed by the slight decrease of dCL versus RT when excluding the MS patients from the analysis.

It is worth noticing that the relatively small differences in CLs could stand out thanks to the high time-resolution of the pupillometer in combination with the way of detecting the CL.

The significance of pure afferent pupil disfunctioning in ON and MS, as opposed to muscular disfunctioning, is further enhanced by the fact that in this group of patients the difference between the PCVL and CL appeared to be normal.

**CL versus VEPL**

As mentioned before, both the CL and the VEPL depend partly on the conductivity of the afferent pathways. In ON the fibers leading to both evocations (if they differ), are equally damaged (e.g. by demyelination of the fibers in the optic nerve, chiasm, and/or tract). This was suggested by Ellis (1979) based on amplitude information, and is confirmed by the prolongation of both the CL and VEPL shown in Figs. 45b,d. Especially during the first 6 months of the recovery period, a highly significant relationship was demonstrated between CL and VEPL, with a slope approximating 1 (Fig. 46a), suggesting that the conductivity reduction is indeed the same for both evocations.

However, during recovery the CL remains prolonged, while the VEPL returns to normal. This can be explained partially as follows. The pupillary pathway consists of several synapses after the trajectory shared with the VEP. I hypothesise that these additional synapses enhance the delay as a consequence of the temporal summation leading to supra-synaptic threshold potentials. After recovery, a slight decrease in NCV in the afferent pathway may then remain unnoticed in the VEP, but does appear in the pupil light reflex. It can be furthermore explained by the different origins of the reflexes under consideration. If both the retina and the afferent neural trajectory are affected in

the acute phase of ON, both the CL and the VEPL will be abnormal. The CL is then affected by reduced neural functioning as discussed earlier, while the VEPL also depends on retinal damage. The VEP is known to be a mere contrast response (Riemslag et al., 1985), and contrast is preprocessed in the retinal ganglion layers. Furthermore, the VEPL and the visual acuity correlate well, and therefore the retina and the optical system of the eye have a great impact upon the pattern-reversal VEP. Conform the fast recovery of both visual acuity and VEPL, retinal damage recovers within a few weeks, while neural damage persists. There is, however, one confounding result, i.e. the relationship between CL and visual acuity. Because under the present stimulus conditions the CL was concluded to depend primarily on neural disfunction, the decrease in acuity has to be partially caused by neural disfunction as well.

Since earlier data have not been discriminated according to the RT, others (Ellis, 1979; Jakobsen and Pedersen, 1985) have not been able to show such a relationship.

If both pupil and VEP data could be divided into normal and abnormal, a comparison could further contribute to locate the lesion (Müller-Jensen and Zschocke, 1979). Unfortunately, the CL differences found in the present study are too small as compared to the standard deviation. Hence, nearly all latencies fall within the normal range (Section 5.4). This makes a further location of the impairment impossible, and is likely to keep doing so under the chosen stimulus conditions which led to the unambiguous neural cause of CL prolongation.

## 5.6 DIABETES MELLITUS

### 5.6.1 *INTRODUCTION*

Abnormalities in pupil behaviour have also been observed in patients suffering from diabetes mellitus (DM). Main abnormalities are a reduced resting pupil diameter and sluggish light reactions (Smith et al., 1978). Because the nerve supply of the iris is exclusively autonomic, assessment of pupil behaviour is also a suitable way of obtaining information on ANS functioning in this type of disease.

Measurement of the amplitude of pupillary constrictions has been shown to provide information about parasympathetic function (Smith and Smith, 1983; Hreidarsson and Gundersen, 1985). However, the presence of miosis due to increasing age will influence the reflex amplitude. This also holds for sympa-

thetic disfunction and/or afferent pupillary defects caused by optic nerve disease or retinopathy.

Measurement of the latency of the pupillary constriction has also been shown to provide information about parasympathetic function (Pfeifer et al., 1982). Results presented in the literature are conflicting, however, and increased latencies (Friedman et al., 1967; Pfeifer et al., 1982; Lanting et al., 1988) as well as normal latencies (Hreidarsson and Gundersen, 1985) have been reported in DM patients.

If nerve conduction is impaired in DM, the site of impairment, addressed by Hreidarsson and Gundersen (1985) only, is still questionable. Comparison of visual evoked potential latencies and pupil latencies may again help in determining whether the defect is afferent or efferent. Because there is no specific reason to assume unilateral impairments in DM patients, interocular comparison of differences is of no use. Latencies of both the pupil constriction and the VEP must therefore be compared for their absolute values.

This section intends to investigate whether CL prolongation can be determined in DM, and to determine whether eventual defects are afferent or efferent.

### 5.6.2 *EXPERIMENTAL CONDITIONS*

**Pupillometry**

Pupillometry as described above was performed by use of a background retinal illuminance of 1.2 Log Td, and a pulse intensity of 3.7 Log Td. Pulse duration was set to 1.2 s, and the interval to 5 s. Both eyes were stimulated separately, while the direct and consensual reflexes were recorded simultaneously.

Normal values for the CL and PCVL under the present circumstances were determined in a group of 56 healthy subjects in the range of 12 to 75 years of age (mean ±SD = 39 ±17).

**VEP**

VEPs were recorded by presenting a pattern reversal at a frequency of 1 Hz. This stimulus was generated by projecting a checkerboard on a translucent screen via a rotating mirror. The square screen was viewed at a distance of 43 cm, encompassing a total visual angle of 30 degrees. The check size was 1°20'. A small fixation target was placed at the centre of the screen. Each subject

was dark adapted for 5 min before examination. Eventual refraction errors were corrected. Both eyes were stimulated separately.

Normal values for the latency of the P-100 peak in the VEP (VEPL) were obtained in 40 healthy subjects in the range of 25 to 70 year of age (mean ±SD = 46 ±12).

**Patients**

Forty-two DM patients were examined with ages ranging from 28 to 70 years (mean ±SD = 52 ±12). The duration of DM ranged from 1 to 54 years (mean ±SD = 19 ±14). Twenty-seven patients were insulin dependent and 28 had clinical evidence of neuropathy. There were no causes of neuropathy other than of diabetic origin. Patients with a proliferative retinopathy were excluded. None of the patients had cataract, and visual acuity was at least 0.7.

### 5.6.3 *RESULTS*

**Pupillometry**

In the control group, the mean CL was 206 ±15 ms. A slight but significant dependency of the CL on age could be demonstrated (CL = 192 + 0.36•age, $p<0.003$), which is indicated by the drawn line in Fig. 47.

In the DM patients, the mean CL amounted to 248 ±26 ms. This latency differed highly significantly from the CL in normals ($p\ll0.0001$). The prolongation was longer in this group than within the optic neuritis group. It therefore made sense to divide patient data into normal and abnormal values, taking into account the age dependency. For all patients, the CL of both eyes was either in the normal or abnormal range. No statistically significant differences between the direct and consensual reflexes were found. Twenty-three patients showed an abnormal CL, as visible in Fig. 47.

The CL showed no correlation with the duration of DM, and the difference between the PCVL and CL appeared to be normal.

**VEP**

Examination of the control group revealed a mean VEPL of 95.7 ±4.0 ms (±SD). VEPLs were considered abnormal when their values were 2.5 times the SD above mean, i.e. greater than 105.7 ms.

The average VEPL in the patient group was 99.1 ±11.1 ms. In eight patients an abnormal prolongation of the VEPL was demonstrated (1 bilateral, 7

unilateral). The distribution of latencies within the patient group is shown in Fig. 48.

The VEPL showed no correlation with the duration of the disease.

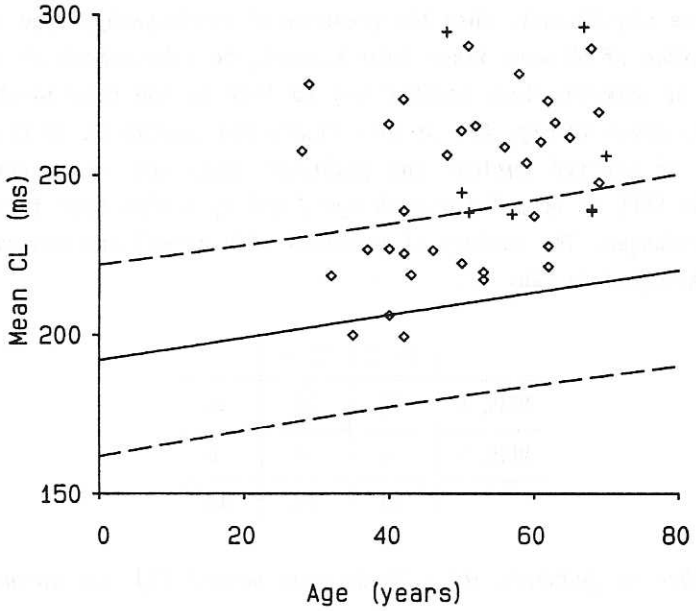



*Fig. 47. Pupil constriction latency versus age of 42 patients suffering from diabetes mellitus. The normal range lies in between the dashed lines, with the mean as a solid line. Patients with a VEPL in the normal range are denoted by diamonds, whereas those with an abnormal VEPL are denoted by plus signs.*

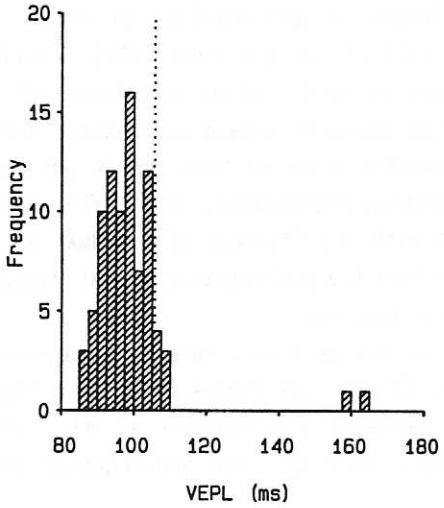



*Fig. 48. Frequency distribution of the VEPL for both eyes in 42 diabetic patients. Values beyond 105.7 ms (dotted line) are considered as prolonged.*

**CL versus VEPL**

Latencies of the pupil constriction and the VEP showed a correlation according to CL = 167 + 0.81•VEPL ($p<0.002$), separately for both eyes. However, if the eyes at the right in Fig. 48 are considered as outliers, this correlation is no more significant. When the presence of retinopathy, type of DM (I or II), and duration of DM were taken into account, no enhancement of this correlation could be demonstrated. Another way to look at the relationship between CL and VEPL is given in Fig. 47. In this figure the average CL of both eyes for all patients is plotted against the patients' age, and it is denoted by a diamond if the VEPL is normal for both eyes, and by a plus sign if the VEPL is abnormally prolonged. The numbers of patients with normal and abnormal CLs and VEPLs are summarized in Tab. IV.

| | CL ≤ | CL > | |
|---|---|---|---|
| VEPL ≤ | 15 | 19 | 34 |
| VEPL > | 4 | 4 | 8 |
| | 19 | 23 | 42 |

*Tab. IV. Number of patients with DM showing normal (≤) and abnormal (>) CL and/or VEPL.*

### 5.6.4 *DISCUSSION*

In DM a significant, relatively large, CL prolongation of 42 ms was found, which is abnormal for more than half of the patients (55%). Possible iris disfunction can be excluded, because no prolongation was found of the difference between PCVL and CL. None of the patients showed any retinal influence on pupil functioning, and it is therefore believed that the CL prolongation in DM for patients without a proliferative retinopathy, is entirely due to nervous disfunction. This is in agreement with the findings of Friedman et al. (1967) and Pfeifer et al. (1982), who ascribed the prolongation to ANS disfunction, especially to reduced parasympathetic function.

The VEPL was prolonged in only 19% of the patients. This is in agreement with the observations of Pozzessere et al. (1988). But others observed a higher percentage of VEPL prolongation in DM patients (Puvanendran et al., 1983; Cirillo et al., 1984; Algan et al., 1989). Moreover, the prolongation shown

now is far less pronounced. It has, however, to be borne in mind that different kinds of populations under different circumstances are considered.

In the present study only a small difference in VEPLs between DM patients and the controls is detected, while the difference in CLs is much larger, especially with respect to the ON group. Moreover, the (cor)relation between CL and VEPL is ambiguous, and the majority of patients with an abnormal CL showed a VEPL within the normal range (Tab. IV). Based on these considerations, the main slow-down of neural pupil pulse transmission cannot be attributed to the afferent arc. The CL prolongation in DM then reflects an efferent defect.

## 5.7 MYOTONIC DYSTROPHY

### 5.7.1 *INTRODUCTION*

So far, the literature does not provide any evidence about abnormal ANS function concerning pupil behaviour in myotonic dystrophy (MyD). Quantitative latency data presented, show large variations resulting in ambiguous differences between normals and MyD patients. This, however, may not be the only reason leading to indistinct results. If abnormal muscular function is involved, then the detection of the true moment of constriction onset is of extreme importance. Because iris constriction results from neural innervation, the true constriction onset cannot be influenced by muscle rigidity. The assumed onset moment, however, may be influenced by muscle rigidity for example, if a criterion is used yielding some moment after the true onset moment resulting in an amplitude- and velocity-dependent latency. These latter parameters are known to be different in MyD, and therefore care has to be taken in determining the CL.

It has already been pointed out that the difference between PCVL and CL depends mainly on muscular function. This difference is therefore also of interest with respect to MyD. Thompson et al. (1964) described a tendency of peak contraction speed to be less, to occur later, and to last longer in MyD than normally, but exact figures were not given. Moreover, the presented CL data were determined subjectively. Bird et al. (1984) presented CL data determined objectively, approximating the true onset moment, but they did not present PCVL data. It is therefore relevant to examine a group of MyD patients with respect to an objectively determined CL and PCVL.

### 5.7.2 *EXPERIMENTAL CONDITIONS*

**Pupillometry**

Square-wave-modulated light (1 to 4 Log Td) was used as a stimulus. Pulses lasted for 1.2 s at intervals of 5 s. For all subjects the CL and PCVL were determined for both eyes. To evaluate possible slow-down of constriction course, the difference between PCVL and CL was calculated for each subject separately.

**Patients**

Nine patients with characteristic clinical symptoms of MyD, and a positive family history for the disease, were studied with their informed consent. The group consisted of three females and six males from 20 to 61 years of age (mean ±SD = 39 ±13 year). Patients had neither signs nor symptoms suggestive of other neurological disorders. Medication had not been changed for at least three months preceding the test. Patient data were compared with data obtained from nine healthy age- and sex-matched controls.

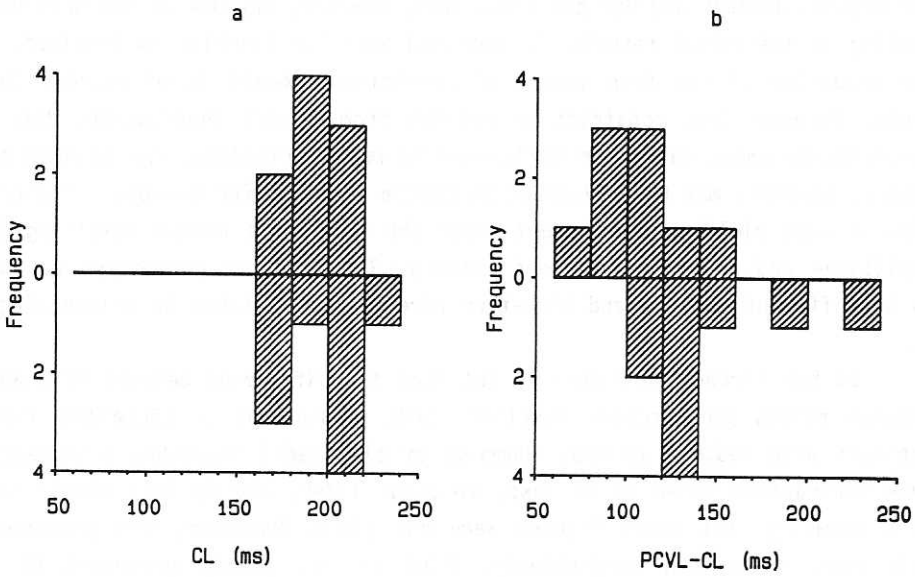



*Fig. 49. Frequency histograms of the CL (a) and the difference between PCVL and CL (b) in nine healthy controls (upper halves) and nine myotonic dystrophy patients (lower halves).*

### 5.7.3 *RESULTS*

No asymmetries in pupillary light-reflex latency between right and left eyes were found. That is why CLs and PCVL-CLs of both eyes were averaged per patient. The average CL did not differ for both groups (Fig. 49a, 195 ±21 ms in MyD and 193 ±19 ms for normals). It was, however, found that the interval between constriction onset and peak velocity was significantly prolonged in the MyD group (Fig. 49b, 145 ±41 ms versus 111 ±22 ms, $p<0.05$).

### 5.7.4 *DISCUSSION*

Possible CL prolongation in MyD has not been excluded by Thompson et al. (1964). That this issue remained in doubt is not only due to the small number of patients (although larger than the present number studied). The sluggish constriction results in an assumed delayed constriction onset, when determined subjectively, which further prevented the above authors from drawing a more firm conclusion. In the present investigations much effort has been taken to determine the very beginning of the constriction instead of a moment afterwards which is influenced by the course of the constriction. The corresponding CLs of the MyD patients and the controls are therefore no basis for indication of nervous involvement at this level. However, the duration between constriction onset and peak velocity is extended as compared to normal, thus pointing to muscle disfunction, i.e. a rigidity of the iris sphincter (Section 5.4.4). This also confirms quantitatively the supposition based on a qualitative analysis by Thompson et al. (1964). The sluggish reaction of the near reflex (Thompson et al., 1964) corroborates this supposition.

The observed sluggishness of the pupil constriction, without defects of the ANS, can be explained best by assuming iris smooth-muscle involvement. Experimental evidence of smooth-muscle involvement in the gastrointestinal and urinary tract in MyD has been given by Collins Harvey et al. (1965). Mechler and Mastaglia (1981) found indications of vascular smooth-muscle abnormalities. The abnormalities found at present fit well into this concept.

## 5.8 CONCLUSIONS

It was hypothesised that relative afferent and efferent defects can be differentiated by temporal parameters provided by pupillometry only. The

results of the investigations in patients with optic neuritis and multiple sclerosis, do not lead to renunciation of this hypothesis.

Furthermore, it was hypothesised that with pupillometry in conjunction with visual evoked potentials, afferent and efferent defects can be distinguished. The results of the investigations in patients with diabetes mellitus do not compel rejection of this hypothesis.

Lastly, it was hypothesised that impairments of the autonomic nervous system and effector impairments can be discriminated by constriction latencies. Based on the results of the investigations in patients with myotonic dystrophy, also this hypothesis can not be rejected.

# 6. FINAL REMARKS

In addition to the discussions concerning specific details presented in each chapter, there are some aspects that remain open for a general discussion.

The IRIS system satisfies the requirements of accurate measurement of the pupil reflex, in particular with respect to temporal resolution. The latter is of major concern with respect to constriction latency determination, and proved to be sufficient. Moreover, reflexes of both eyes can be measured simultaneously, allowing investigation of interocular differences. A disadvantage is that only a relative measure for the pupil size is provided. However, the signals are linear with respect to pupil size, and mutual relative amplitude changes can be compared reliably, under the condition of a fixed adjustment within one subject. The IRIS pupillometer therefore proved also convenient to the experiments in which the reflex amplitude was of interest, that is the experiments on contrast sensitivity and pupil oscillations.

The mean delay of the pupil system, deduced at first approximation from the oscillation experiments, amounted to more than 300 ms. The latency of light-step induced pupil constrictions amounted to less than 300 ms typically. Involvement of a short-term adaptation process, influencing latency with a time constant of less than 2 s, was already concluded. In the oscillation experiments, the pupil, and hence the stimulus intensity, oscillated in a sinusoidal fashion with a cycle time of less than 2 s. The sensitivity of the pupil system to respond on changes in light intensity is therefore much less in the oscillation experiments than in the light-step induced constriction experiments, and the observed delays and latencies may differ.

Under normal closed-loop conditions and constant illumination, the uncontrollable retinal illuminance may vary by a factor of over 16 ($= A_{max}/A_{min} \approx \pi \cdot 4^2/\pi \cdot 1^2$). The major advantage of the application of Maxwellian view stimulation, is that the retinal illuminance is independent of pupil size, i.e. the pupil system does not influence the stimulus, and the retinal illuminance is thus known at any moment. Moreover, Maxwellian view stimulation with wide-angle retinal illumination was proved reliable for avoiding influences due to geographical variations in retinal functioning. In both the ON/MS and DM group of patients, no bias due to minor retinal disorders was found.

Under the given circumstances, the CL depended roughly on the difference between the stimulus intensity and half the background intensity. Deviations from this behaviour were encountered, however, with special reference to different backgrounds. This suggests that a comparison between different groups of subjects should be based on parameters measured under identical conditions. Some of the patient data presented in this thesis were therefore compared with a separate control group. The importance of the latter is also demonstrated by the differences found in age dependency.

Information on the pupil latency cannot yet be expected to contribute to the diagnosis of ON, as a consequence of the small difference in CLs between the ON/MS group and healthy subjects as compared to the variation in normative CLs. With respect to this disease, temporal pupil parameters determined under photopic conditions will thus be of mere fundamental scientific interest for the time being. In DM the CL is prolonged much more. The CL can therefore contribute to the clinical diagnosis, particularly with respect to neural disfunctioning, of this disease.

It has been suggested recently that myotonic dystrophy may be a polymorphous disease with respect to the neural and muscular origin (meeting of the Ned. Neurol. Ver., Utrecht, January 11, 1991). This thesis has shown that application of the CL and PCVL has considerable potential for clinical diagnosis of MyD, especially with respect to a discrimination between the neural and muscular origins of this disease, or, in general, any other disease with doubtful neural and/or muscular origin. However, the small sample size used in this thesis should be extended to validate this statement.

Latency prolongation in demyelinating diseases, such as MS, is mostly contributed to a reduction in NCV (Rasminsky and Sears, 1972; Halliday and McDonald, 1977), i.e. due to the nerves themselves. It remains open for discussion, whether yet another phenomenon due to demyelination results in prolonged latencies. If, due to the loss of isolating tissue around the nerves, part of the neural pulses dissolve in the surrounding tissue, potential build-up in a posteriorly located synapse or node of Ranvier will take more time by temporal summation. Consequently, it will also take more time for this potential to exceed the synaptic threshold, thus increasing the latency of the track under consideration. This has more impact, the more synapses there are in a certain neural trajectory. I therefore hypothesise that besides a reduction in NCV, abnormal delay of a neural path enclosing synapses is induced partly by

a retarded potential build-up in the synapses due to demyelination of the preceding nerves. In case of the VEP, this effect may be negligible, whereas it might be important for the pupil light reflex, in which at least four consecutive synaptic relays have to be passed.

A quantitative theoretical analysis of this type of delay enhancement would require a detailed evaluation of the extremely complex equations involved (Hodgkin and Huxley, 1952; Taylor, 1963; Frankenhaeuser and Huxley, 1964; Goldman and Albus, 1968; Hutchinson et al., 1970; Koles and Rasminsky, 1972; Schauf and Davis, 1974; Brill et al, 1977; Waxman, 1978). Even then, a reduced NCV by demyelination cannot yet be explained satisfactorily (Riemslag, 1986). Any further discussion of this matter of great scientific interest would go beyond the subject of this thesis. I made an indirect tentative search for the impact of this effect. Several substances are known to decrease (e.g. caffeine and nicotine) or increase (e.g. alcohol) the synaptic threshold, above which neural pulses are relayed. In a trial, two subjects were tested pupillometrically just before and after administration of one of these drugs in the form of coffee. Within 30 minutes seven cups (regular Dutch strength) per subject were consumed, and a shortening of the latency period was indeed observed. Statistical testing made no sense due to the small sample size, but the experiment does indicate the value of the assumption. Further experiments are needed to reach a more firm conclusion.

The wide variety in (ab)use of substances as mentioned above may also explain some of the interindividual latency variability. It is therefore suggested that, besides age and gender, the drugging by more or less socially accepted stimulants has to be taken into account in order to minimize CL variability.

Yet another reason for the interindividual CL variability might be the existence of differences in nerve lengths. This has already explained differences in latencies of several evoked responses (pupil, BAEP and SEP) up to a certain level as addressed in Chapter 5.4. The nerve lengths cannot be measured directly, but they do correlate in some way with brain-weight, brain-size and body-weight and -size. It is therefore recommended that some of these parameters, preferably body-weight and -size, be taken into account in future experiments involving CL analysis.

## APPENDIX A: TABULAR SUMMARY OF LATENCY-RELATED LITERATURE

(For explanation of symbols and abbreviations see page 116)

| | method | color of light (dg) | $I_{off}I_{on}$ units | $T_{off}T_{on}$ (s) (s) | N age (yr) | CL (ms) |
|---|---|---|---|---|---|---|
| Donders 1866 | En | C 20 | | | 1 | No subjective observable latency difference between right and left eye. |
| Arlt 1869a,b | En | C | sunlight | | 4 | 492. [1] |
| Vintschgau 1881 | En | C W Y | oil-lamp | 300 600 | >1 | 550-565. No differences between right and left eyes. [1] |
| Garten 1897 | UK 4 | 1 | arc-lamp flash | | 1 | 500. |
| Piltz 1904 | Ky | C W | >0 | 2 | >1 40 50 | 200-300. CL decreases with increasing I. |
| Weiler 1910 | Ky | | | | | 150-280. |
| Gradle 1923,1932a,b | Ci 16 | C W 20 3 | 0 arc-lamp 8 Amp | 1 | 12 <30 | 188. |
| Bender 1933 | Ci 24 | C 10 | 960 fcd | 5 10 | 19 18 32 | 188 (120-250). |
| Lowenstein 1933 | Ci <16 | C | >0 | 1 | | 60. CL independent of initial pupil size. |
| Gundlach 1934 | Ci 16 | C W 0 | >0 Lamp 100 W | »1 | 3 | >120. |
| Machemer 1935 | Ci 75 | C 15 | 0 6000 lx | 10 | 1 31 | 210-220. [2] |
| Talbot 1938a,b | Ci 16 | | | | | 200-350 ±30. |
| von Brunn 1941 | IR | C W | 0 | 0.1 | 7 25 76 | 250-300. |
| Machemer 1941 | Ci 75 | C W 30 5 | 500 500 lx 6000 6000 | >1 30 | 13 0.5 75 | 217 = constant. [2] |
| Matthes 1941 | IR | C | | 0.01 0.1 | | 200-260. |
| Lowenstein 1942 | IC 10 | C | | | | 200-280. |
| Tschirren 1947 | IR 40 | C | Lamp 25W | 0.05 | | 260-300. |
| Lowenstein 1949 | IC 10 | | | 4 1 | | 180-220. CL increases with number of stimuli presented. |
| Lowenstein 1951 | IC 10 | | 0 0.2 fcd 90 | | 20 | 200. CL increases with decreasing I. |
| Lowenstein 1954 | IC 10 | 6 | 0 15 fcd | 1 3 | 300 <50 | 200-300. Optic neuritis shows "low intensity" reactions. |
| Young 1954 | IP 20 | C 17.4 5 peri | 8800 mL | 2 300 | 10 21 29 | 220 ±35, 175 ±39. [2] |

| | meth d(Hz) | col opr | adap l (dg) | $I_{off}I_{on}$ units | $T_{off}T_{on}$ (s) (s) | N age (yr) | CL (ms) |
|---|---|---|---|---|---|---|---|
| Kumnick 1956 | IC 10 | C W | 10 | 12.2 fcd | 3 1 | 94 7<br>91 | 200-246. 219 for first 58, 243 for 59th and 60th consecutive constrictions, CL prop. to pupil rest diameter and independent of age. |
| Petersen 1956 | IC 25 | C W | >90 10<br>1.3Lux | 1.3 3600 lx | 1E-3 | 303 0<br>80 | 225 ±40. CL increases with age. [2] |
| van der Tweel 1959 | Ky 33 | C W | | <3E-4 lm | | >1 | 200 ±50 not depending on stimulus intensity. |
| Drischel 1957 | IR | C W | >15 | 30 lx | >4 0.01 | 493 10<br>22 | 234 ±32 males,<br>228 ±28 females.<br>±15 intraindividual diff.<br>±29.5 interindividual diff. [2] |
| Stark 1957 | IR | O | | | | | 180. CL determined with light steps as well as with sinusoidal stimuli resulting in "nonminimum phase shift". |
| Lowenstein 1959a | IS 60 | C | >25 | 0 | 1 | 5 16<br>38 | CL decreases gradually with Log(I), also over scotopic/photopic threshold. |
| Lowenstein 1959b | IS 60 | C W<br>R | | | 3 0.1<br>3.9 1 | 5 17<br>34 | 270-550. CL not depending on psychosensory stimuli or spontaneous thoughts. |
| Dolenek 1960 | IC 24 | C W | 20 | | | | 125-292. |
| Alpern 1963 | IC 13 | O | 13.5 30 | 7E-3 td<br>7E+5 | 180 4E-3<br>0.15 | 7 | 180-500. $CL^{-1}$ prop. to change in pupil size, differently for 180<CL<300 and 300<CL<500, the maximum CL=500. [3] |
| Künkel 1961,1963 | IR | | 10 | | <0.02 | | 245 (155-325). CL varies rhythmically every 110 sec., ±26 intraindividual diff. |
| Tiedt 1963 | IP 16 | C W | | 15 lx | >4 0.01 | 7 19<br>29 | 230. CL shortest around 15.00h, longest around 3.00h. |
| Lowenstein 1964 | IS 60 | WR 1<br>GB | | 0 | 1 | 3 | CL increases with wavelength (WRGB), with threshold compensation all curves coincide. [2] |
| Thompson 1964 | IS 60 | | | | 3 1 | 9 12<br>63 | 250. CL in myotonic dystrophy "slightly" prolonged to 325. |
| Feinberg 1965 | IS 60 | C | | 1E+6 cd | 1E-5 | 86 14<br>67 | 214-314. CL = 222.3 + 0.77•age<br>CL prop. to Log(I2-I1). [4] |
| Kristek 1966 | IC 24 | C | 20 | 0 55 lx | 4 | 64 10<br>60<br>5 | 204 ±25. No differences between right and left eyes.<br>Cl prolonged in optic neuritis. |
| Thompson 1966 | IS 60 | C | >3 | 15 fcd | 3 1 | 10 13<br>58 | 236 ±17 for healthy fellow eyes, 272 ±25 for impaired eyes in optic neuritis. |
| Friedman 1967 | IS 60 | | | 1E+6 cd | 1E-5 | 22 40<br>22 49 | Normal 226 ±4.<br>Diabetes mellitus 245 ±5. [4] |
| Lee 1969 | IS 1200 | O | 6 | -5 -2 Log<br>+4 +7 td | »1 | | CL = 312 - 18.6•Log(I2) - 5•Log(I2-I1). [5] |

| | method (Hz) | colour adaptation (dg) | $I_{off}$ $I_{on}$ units | $T_{off}$ $T_{on}$ (s) (s) | N age (yr) | CL (ms) |
|---|---|---|---|---|---|---|
| Borgmann 1972a,b | IS 17 | C 15 | 175 lx | 35 1 | 136 6 72 | 270 ±40 6-29 year, 280 ±40 30-49 year, 295 ±40 50-72 year, 282 myopes, 275 emmetropes. [6] |
| Müller-Jensen 1976,1978 | IR | C W 9 5 15asb | 0 3.75 asb 1500 | 0.125 | 101 15 89 | 232-313. CL decreases exponentially with Log(I), CL increases with age, no differences between right and left eyes, ±5 intraindividual diff. ±18 interindividual diff. |
| Utsumi 1976 | TV 100 | O 15 | 2 lx | >20 | 4 | 264-301. Pupil size changes diurnally, CL only interindividually. |
| Cibis 1977 | IR | C 0.5 45 2.0 | 0 100 Log cd/m² | 2.9 0.1 | | 240-400 ±10. Stepintensity measured from threshold, CL prop. to Log(I) and field angle, inv. prop. to angle of field, the threshold, a, from 400= a+b•Log(I). [2] |
| Utsumi 1978 | TV | O Y 25 C | 25 lx | | 6 | 233 ±8, constant under "open loop", diurnally variable under "closed loop" conditions. |
| Müller-Jensen 1979 | IR | C 9 | 15 asb | 7 0.125 | 1 2 | 250. 25 ms prolongation in optic neuritis (afferent), 50 in 3-rd nerve palsy (efferent). |
| Ellis 1979 | TV 50 | O W 14.5 30 | 0 1 Log 5.3 cd/m² | 7.9 0.1 | 19 18 48 22 16 48 | 220-500. Prolonged in optic neuritis. |
| Ellis 1981 | TV 50 | O W 14.5 30 | 0 1 Log 5.3 cd/m² | 7.9 0.1 | 19 18 48 | 220-500. CL = 445.7 - 22.9•Log(I) + 76.2•Log(I)². |
| Argyropoulos 1980 | IR | C 60 30 | 0 -6 Log +3 cd/m² | 0.1 | | 200-450 for healthy fellow eyes, prolonged for optic neuritis. |
| Namba 1980 | TV | O | photopic | | 24 | CL independent of age and sex, prolonged in 29% of diabetes. |
| Alexandridis 1981 | IR | C W 60 30 | 0 -7 Log +3 cd/m² | 0.1 | 31 <60 | 200-350 for healthy fellow eyes, prolonged in optic nerve diseases. |
| Alexandridis 1982 | IR | C W 60 | 0 -7 Log 1.6 +3 cd/m² | 0.1 | 17 | 200-500 for healthy fellow eyes. Prolongation of 50 ms in optic neuritis, permanent at light-adapted state, temporary at dark-adapted state. |
| Pfeifer 1982 | TV 30 | C W 10 light | 0.024 2.7fcd 12.5 | 90 10 | 7 | CL = 466 - 3.25•I, no differences between right and left eyes. Normal = 355 ±4, diabetes = 416 ±8. [7] |
| Pfeifer 1983 | TV 30 | C W 10 light | 0.024 32 fcd | 90 10 | 51 21 79 | CL = 314 + 0.89•age. |
| Bird 1984 | TV | 15 | 0 32 fcd | | 15 21 58 | 340 ±30. CL in myotonic dystrophy about the same (372). [7] |
| Pfeifer 1984 | TV 30 | C W 10 light | 0.024 32 fcd | | 33 | Insulin dependent CL = normal, non ins. dep. CL = prolonged. |

| | method (Hz) | color loop (dg) (mn) | $I_{off} I_{on}$ units | $T_{off} T_{on}$ (s) (s) | N age (yr) | CL (ms) |
|---|---|---|---|---|---|---|
| Grünberger 1985 | TV | C | 145 lx | 0.3 | 45<br>45 | 180 ±50 in normals,<br>150 ±80 in psycho-somatic diseases. [2] |
| Hreidarsson 1985 | TV | O Y <10 15 | 1 26 mu lm | 15 0.26 | 37 25<br>93 42 | Normal 231 ±33.<br>Diabetes mellitus 236 ±30. [8] |
| Jakobsen 1985 | TV | O ±2 | | | 14 | Normal 301 ±4.<br>Multiple sclerosis 325 ±7. |
| Beaumont 1987 | TV 50 | C G 7.2 10 | 0.5 387 cd/m² | 15 5 | 11 59 ±8.3<br>15 63 ±7.9 | Normal 240 ±18, CL independent of age,<br>256 ±17 in Parkinson's disease. [2] |
| Grünberger 1987 | TV | 3 160Lux | 145 lx | 0.3 | 107 18<br>49 | 150 ±50 male (18-31 year),<br>190 ±70 male (32-45 year),<br>170 ±50 female (18-29 year),<br>170 ±50 female (30-49 year).[2] |
| Link 1988 | TV 25 | O | 0.1 fL 10 | 0.59 10 | | CL = 253 - 14•Log(I) + 70•R - 29•R•Log(I), with R the repetition rate of the stimulus. |

*1-st column:* *author and year of publication.*

*2-nd column:* *measuring method and sample rate in (Hz)*

*En = Entoptic.*
*IR = Infrared Reflection, continuous detection with phototransistors.*
*TV = Infrared Television.*
*Ci = Cinematography.*
*Ph = Photography.*
*IP = Infrared Photography*
*IC = Infrared Cinematography.*
*IS = Infrared Scanning.*
*Ky = Kymography.*
*UK = Ultra-Violet Kymography.*

*3-rd column:* *loop, stimulus colour and field angle in degs (dg) and the adaptation time inminutes(mn)*

*C = Closed loop.*
*O = Open loop.*
*W = White.*
*B = Blue.*
*G = Green.*
*R = Red.*
*Y = Yellow.*

*4-th column:* *Iof and Ion, background and step intensities in units :*

*lm = lumen (flux)*
*cd = candela (intensity)*
*fcd = footcandle (illuminance)*
*lx = lux (illuminance)*
*fL = footlambert (luminance)*
*asb = apostilb (luminance)*
*L = lambert (luminance)*
*td = troland (retinal illuminance)*

*5-th column:* *Tof and Ton, interval and step duration in seconds (s)*

*6-th column:* *number, N, of subjects with the age range in years (yr)*

*7-th column:* *the constriction latency, CL, in msec. (ms), with a brief description of the author's findings concerning the CL. In the lower right corner in between square brackets [] the way of detection of the CL is given:*

*1 = Pushing a button on the constriction onset moment (corrected for the inherent manual latency).*
*2 = The subjective way, by hand.*
*3 = Intersection between straight lines through parts before and during the constriction.*
*4 = When the pupil velocity is more than 3.5 mm/s.*
*5 = Curvefitting over an average of 20 responses.*
*6 = When the pupil area changes more than 0.05 mm.*
*7 = Search for differences from zero in the average of the velocity of 5 constrictions.*
*8 = When 10% of maximum constriction amplitude is reached.*

## APPENDIX B: A NEW MAXWELLIAN VIEW DESIGN

A new stimulator was developed to replace the Maxwellian view stimulator described in Chapter 2. The latter is inhomogeneous in light emission and tends to dangle in shaky subjects, resulting in a tilt of the stimulator.

Fig. 13 shows that the tube and second lens in the stimulator act as a diaphragm, obscuring the outer LED rays, and leading to an inhomogeneous emission lobe. The new stimulator, therefore, had to consist of preferably one or otherwise two adjacently placed lenses. Because the LED has to be imaged into the pupil, its magnification had to be less than 1 (in contrast to about 2 in the old one). However, the field angle of the emission lobe is proportional to the magnification. To get the largest possible field angle, a magnification of about 1 was chosen. Furthermore, some sort of adjustable fixation target had also to be projected onto the retina. Both the LED and the fixation-target images had to be as sharp as possible. This all was sorted out by ray-tracing. These constraints, subject to commercially available lenses, resulted in a stimulator sketched in Fig. 50.

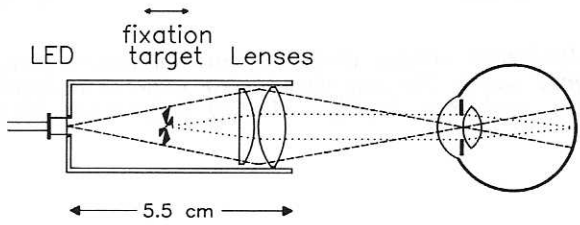



*Fig. 50. Diagram of the new Maxwellian view stimulator, showing good homogeneity of the emission lobe. The dashed lines show the outer rays of the illumination. The dotted lines indicate the projection of the fixation target on the retina.*

The preferable option of one lens was not feasible, but finally the Combined Optical Industries Limited provided two plastic lenses which together best approximated the needs. The lenses (COIL types 5723: aspheric biconvex with an equivalent focal length of 42.62 mm and 5584: plano-convex with an equivalent focal length of 45.92 mm) were placed as depicted in Fig. 50. The total field angle of the emission lobe is now 24°, and the diameter of the LED image (Stanley HAY5566X, 580 nm, 150 mcd typical) is only 1.7 mm. The total length of the stimulator has been reduced from 100 to 60 mm. The retinal illuminance, by

changing the distance d as measured with the original method of Nygaard and Frumkes (1982), now yields a difference of less than 5% (Fig. 51). So, the homogeneity of this emission lobe largely exceeds that of the current stimulator.

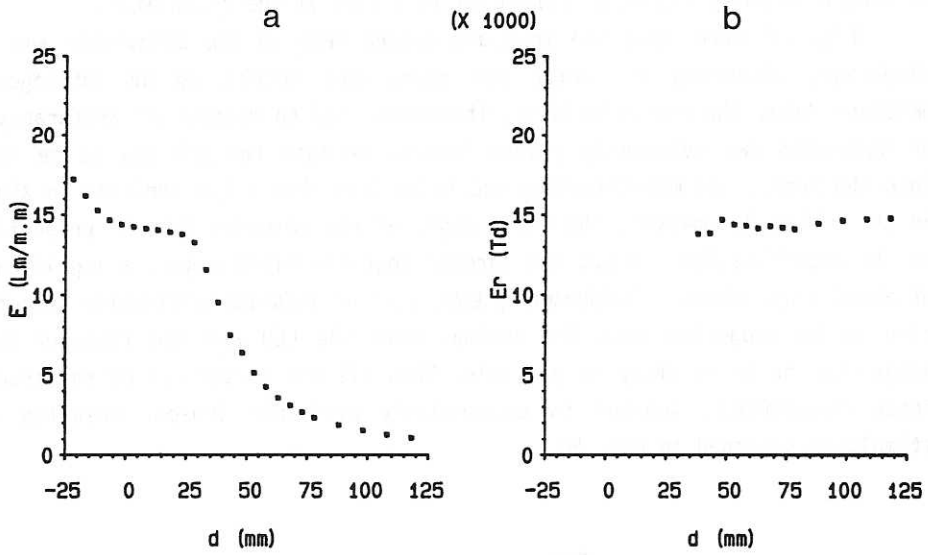



*Fig. 51. (a) Illuminance versus distance between source image and detection surface as measured using the new Maxwellian view stimulator according to a set-up of Fig. 12, analogous to Fig. 14. (b) Calculated retinal illuminances from (a).*

## APPENDIX C: CALIBRATION OF PUPIL SIZE

In an effort to relate the IRIS pupil signal to the absolute pupil size in mm or $mm^2$, a calibration procedure was contrived using an entoptic principle (Broca, 1924; West, 1988). Based on this principle, as depicted in Fig. 7, a device was constructed for fitting onto the IRIS frame, just as the stimulator for eliciting the light reflex. Instead of one stimulus LED, now an array of SMD-LED's were mounted. The LED's were grouped into pairs, symmetrically positioned around the optical axis of the calibration device. A lens projected a reduction of the light sources between 1 and 2 cm in front of the eye (Fig. 52a). A current could be switched to one of the LED pairs enabling the subject to view two images of his own pupil. The stimulator in front of the other eye was used to establish pupil sizes, so that the entoptically imaged pupil edges in the other eye could touch.

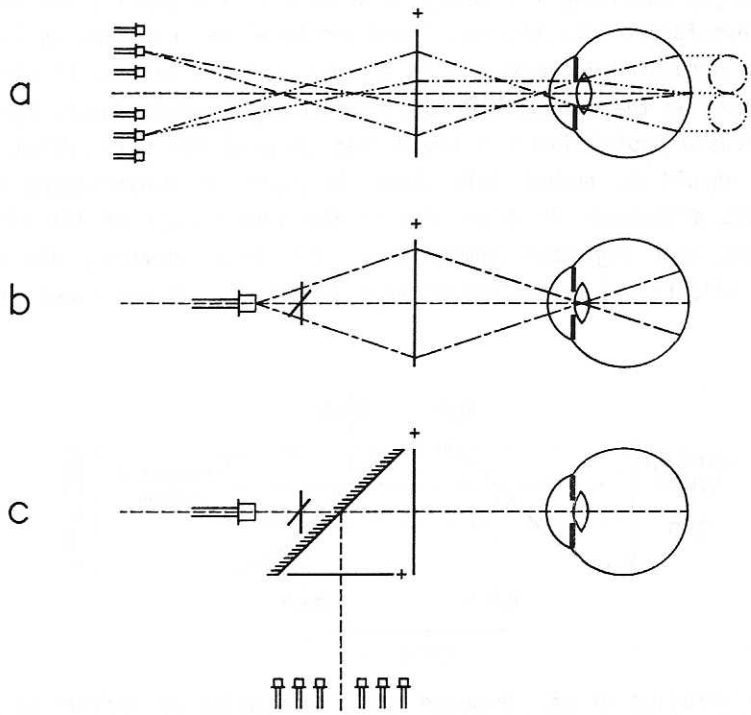



*Fig. 52. Device for pupil-size calibration. (a) Entoptical calibrator. (b) Pupil stimulator with fixation target. (c) Mergence of (a) and (b).*

This calibration device was meant to be incorporated into the newly developed Maxwellian view stimulator (Fig. 52b,c).

A calibration procedure should next cover a range of pupil sizes, as wide as physically possible, and include at least three values in order to verify linearity and reduce errors. It was originally hoped that by using the stimulator for the other eye, steady (static) pupil sizes in that range could be adjusted. This, however, did not work out. Large pupil areas could be obtained easily by dark adaptation. But small steady pupil sizes could not be obtained as a result of the limited visual stimulus angle and the limited stimulus intensity. Two other methods were therefore introduced. The first uses the dynamic range when a pupil reflex is elicited by a light pulse. The second uses a forced oscillation. With the dynamic pupil reflex elicited by a light pulse, the constriction does yield a sufficiently wide range of pupil diameters. But this range is completed too fast for the subject to indicate when the retinal images of his own pupil touch. Also, the much slower dilatation phase was inapplicable. For obtaining a wide range of diameters, stimulus intensity should be large, resulting inevitably in capture of the pupil size. The second option employs forced oscillations. These can be either elicited by light with sinusoidally modulated intensity or by successive light pulses. If the subject pushes a button at the moments when the retinal pupil images touch, the average of the indicated pupil diameters could then be used for calibration. Because the button should be pushed both when the pupil is constricting and when dilating, the difference in sizes due to the time delays of the visual and manual reflex arcs may then cancel (Fig. 53). This, however, did not work either. For eliciting a wide range of pupil diameters, it was found experimen-

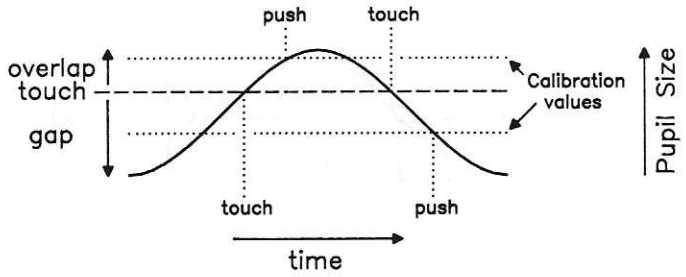



*Fig. 53. Calibration of the measured pupil size using an entoptical pupillometer in front of one eye and a sinusoidally modulated stimulus in front of the other eye. The solid line represents pupil size, the dashed line the pupil diameter at which the images actually touch. After a certain delay a button is pushed to indicate the moment when circles touch.*

tally that the frequency of the stimulus should be about 0.5 - 1 Hz. The button should then be pushed with a frequency of up to 2 Hz, which is practically too fast. Moreover, the responses obtained at lower frequencies were asymmetric, which means that the constriction is faster than the dilatation, and the difference in sizes due to the different delays thus do not cancel.

For a small range of pupil sizes the static and the first dynamic method have been applied in a series of experiments, although with much effort. The linearity between the IRIS signal (in arbitrary units) and the pupil size ($mm^2$), as verified by Reulen et al. (1988), was thus confirmed. It appeared, however, that the IRIS signal continuously drifts due to reflection of infrared light by the eyelids, although relative changes in pupil size did remain constant. The IRIS pupillometer is apparently not suitable for measuring the absolute value of the pupil size, and calibration of the IRIS pupil signal was not further attempted.

## REFERENCES


**Aguilar M, Stiles WS. (1954)** Saturation of the rod mechanism of the retina at high levels of stimulation. Optica Acta 1:59-65.

**Alexandridis E. (1967)** Pupillographische Untersuchung der Netzhautempfindlichkeit des Taubenauges. Albr. v. Gr. Arch. klin. exp. Ophthal. 172:139-151.

**Alexandridis E. (1968)** Bestimmung der Dunkeladaptionskurve mit Hilfe der Pupillenlichtreflexe. Ber. Dtsch. Ophthalmol. Ges. 68:274-277.

**Alexandridis E. (1970)** Räumliche and zeitliche Summation pupillomotorisch wirksamer Lichtreize beim Menschen. Albr. v. Gr. Arch. klin. exp. Ophthal. 180:12-19.

**Alexandridis E. (1971)** Pupillographie. Anwendungsmöglichkeiten als objektive Untersuchungsmethode der Netzhautsinnesfunktion. Hüthig, Heidelberg.

**Alexandridis E. (1973)** Lichtsinn und Pupillenreaktion. In: Die normale und die gestörte Pupillenbewegung. Symposion der D.O.G. 10-12 März 1972 in Bad Neuheim. Dodt E, Schrader KE (eds), J.F. Bergmann Verlag, München :58-71.

**Alexandridis E. (1985)** The pupil. Springer-Verlag, New York.

**Alexandridis E, Argyropoulos T, Krastel H. (1981)** The latent period of the pupil light reflex in lesions of the optic nerve. Ophthalmologica 182:211-217.

**Alexandridis E, Gärtner RL, Krastel H, Hagenlocher HU. (1982)** Latenz der Pupillenlichtreflexe und des VECP im Verlauf der retrobulbaren Neuritis. Fortschr. Ophthalmol. 79:356-357.

**Alexandridis E, Krastel H. (1972)** Ein tragbares Infrarot-Reflex-Pupillenmeter. Ber. Dtsch. Ophthalmol. Ges. 71:652-654.

**Algan M, Ziegler O, Gehin P, Got I, Raspiller A, Weber M, Genton P, Saudax E, Drouin P. (1989)** Visual evoked potentials in diabetic patients. Diabetes Care 12:227-229.

**Alio J, Marcos M, Millan A, Urrutia M. (1987)** The pupil cycle time test. Neuro-ophthalmology 7:27-37.

**Allison T, Wood CC, Goff WR. (1983)** Brain stem auditory, patter-reversal visual, and short-latency somatosensory evoked potentials: latencies in relation to age, sex, and brain and body size. Electroencephal. Clin. Neurophysiol. 55:619-636.

**Alpern M, Campbell FW. (1962)** The spectral sensitivity of the consensual light reflex. J. Physiol. (Lond.) 164:478-507.

**Alpern M, Kitai S, Isaacson JD. (1959)** The darkadaptation process of the pupillomotor photoreceptors. Am. J. Ophthalmol. 48:583-593.

**Alpern M, McCready DW, Barr L. (1963)** The dependance of the photopupil response on flash duration and intensity. J. Gen. Physiol. 47:265-278.

**Argyropoulos Tr, Krastel H, Alexandridis E. (1980)** Latenz der Pupillenlichtreflexe bei Erkrankungen des Sehnerven. Ber. Dtsch. Ophthalmol. Ges. 77:373-377.

**Arlt F. (1869a)** Beitrag zur Kenntniss der Zeitverhältnisse bei den Bewegungen der Iris. Graefe Arch. Ophthal. 15:294-317.

**Arlt F. (1869b)** Tijdsbepalingen ten aanzien der bewegingen van den oogappel. Ned. Arch. Genees- en Natuurk. 4:481-501.

**Barbur JL, Forsyth PM. (1986)** Can the pupil response be used as a measure of the visual input associated with the geniculo-striate pathway? Clin. Vision Sci. 1:107-111.

**Barbur JL, Thomson WD. (1987)** Pupil response as an objective measure of visual acuity. Ophthalmol. Physiol. Opt. 7:425-429.

**Beagley HA, Sheldrake JB. (1978)** Differences in brainstem response latency with age and sex. Br. J. Audiol. 12:69-77.

**Beaumont SM, Harris JP, Leendertz JA, Phillipson OT. (1987)** The pupillary light reflex in mild Parkinson's disease. Clin. Vision Sci. 2:123-129.

**Bender WRG. (1933)** The effect of pain and emotional stimuli and alcohol upon pupillary reflex activity. Psychol. Monogr. 44:1-32.

**Bird TD, Reenan AM, Pfeifer M. (1984)** Autonomic nervous system function in genetic neuromuscular disorders: Hereditary motor-sensory neuropathy and myotonic dystrophy. Arch. Neurol. 41:43-46.

**Blumen SC, Feiler-Ofry V, Korczyn AD. (1986)** The pupil cycle time in Horner's syndrome. J. Clin. Neuro-ophthalmol. 6:232-234.

**Borgmann H. (1972a)** Grundlagen für eine klinische Pupillographie. III. Abhängigkeit der Parameter der Lichtreaktion vom Lebensalter. Albr. v. Gr. Arch. klin. exp. Ophthal. 185:1-10.

**Borgmann H. (1972b)** Grundlagen für eine klinische Pupillographie. IV. Abhängigkeit der Pupillenweite und der Lichtreaktion vom Geschlecht, Irisfarbe und Refraction. Albr. v. Gr. Arch. klin. exp. Ophthal. 185:11-21.

**Bos JE. (1988)** Clinical quantitative pupillometry: the latency of pupillary contstriction as measured with the IRIS system (preliminary results). Neuro-ophthalmology 8:299-305.

**Bos JE, Fock JM, Reulen JPH, Lith GHM van. (1990)** Pupil constriction and visual evoked potential latency in optic neuritis. Neuro-ophthalmology 10:187-196.

**Bos JE. (1991)** Detection of the pupil constriction latency. Med. Biol. Eng. Comp. 29, In press.

**Bouma H. (1962)** Size of the static pupil as a function of wavelength and luminosity of the light incident on the human eye. Nature 193:690-691.

**Bradley WG, Whitty CWM. (1967)** Acute optic neuritis: its clinical features and their relation to prognosis for recovery of vision. J. Neurol. Neurosurg. Psychiatry 30:531-538.

**Bramwell E, Addis WR. (1913)** Myotonia Atrophica. Edinburgh Med. J. 11:21-44.

**Brill MH, Waxman SG, Moore JW, Joyner RW. (1977)** Conduction velocity and spike configuration in myelinated fibres: computed dependence on internode distance. J. Neurol. Neurosurg. Psychiatry 40:769-774.

**Broca A. (1924)** Un pupilometre. Rev. d'Optique 3:493-496.

**Brunn W von, Falk R, Matthes H, Matthes K. (1941)** Untersuchungen über die Pupillenreflexe beim Menschen. Pflügers Arch. 244:644-658.

**Buck SL, Makous W. (1982)** Calibrating Maxwellian-view optical systems. J. Opt. Soc. Am. 72:960-962.

**Campbell FW, Whiteside TCD. (1950)** Induced pupillary oscillations. Br. J. Ophthalmol. 34:180-189.

**Cibis GW, Campos EC, Aulhorn E. (1977)** Pupillomotor latent period. Vis. Res. 17:737-738.

**Cirillo D, Gonfiantini E, De Grandis D, Bongiovanni L, Robert JJ, Dinelli L. (1984)** Visual evoked potentials in diabetic children and adolescents. Diabetes Care 7:273-275.

**Clynes M. (1961)** Unidirectional rate sensitivity: a biocybernetical law of reflex and humoral systems as physiologic channels of control communication. Ann. Acad. Sci. 92:946-969.

**Cohen MM, Lessel S, Wolf PA. (1979)** A prospective study of the risk of developing multiple sclerosis in uncomplicated optic neuritis. Neurology 29:208-213.

**Collins Harvey J, Sherboune DH, Siegel CI. (1965)** Smooth muscle involvement in myotonic dystrophy. J. Am. Med. Ass. 39:81-90.

**Crosby EC, Humphrey T, Lauwer EW. (1962)** Correlative anatomy of the nervous system. MacMillan, New York.

**Cüppers C. (1951)** Eine neue Methode zur stetigen Registrierung der konsensuellen Pupillenreaktion. Klin. Monatsbl. Augenheilkd. 119:411-417.

**Dekaban AS, Sadowsky D. (1978)** Changes in brain weights during the span of human life: relation of brain weights to body heights and body weights. Ann. Neurol. 4:345-356.

**Dolenek A. (1960)** Beitrag zur Pupillographie. Ophthalmologica 139:77-83.

**Donders FC. (1866)** Reflexiebewegingen der beide pupillen, bij het invallen van licht aan eene zijde. Ned. Arch. Genees- en Natuurk. 2:106-108.

**Dongen MMMM van. (1991)** Neuro-ophthalmic markers in clinical trials for multiple sclerosis. Ph.D. thesis, Vrije Universiteit Amsterdam.

**Dorfman LJ, Bosley TM. (1979)** Age-related changes in peripheral and central nerve conduction in man. Neurology 29:38-44.

**Drischel H. (1957)** Untersuchungen über die Dynamik des Lichtreflexes der menschlichen Pupille. I. Der normale Reflexablauf nach kurzdauernder Belichtung und seine Variabilität. Pflügers Arch. ges. Physiol. 264:145-168.

**Ellis CJK. (1979)** The afferent pupillary defect in acute optic neuritis. J. Neurol. Neurosurg. Psychiatry 42:1008-1017.

**Ellis CJK. (1981)** The pupillary light reflex in normal subjects. Br. J. Ophthalmol. 65:754-759.

**Feinberg R, Podolak E. (1965)** Latency of pupillary reflex to light stimulation and its relationship to aging. In: Behavior, Aging and the Nervous System. Welford AT, Birren JE, Thomas CC (eds), Springfield, Ill. :326-339.

**Frankenhaeuser B, Huxley AF. (1964)** The action potential in the myelinated nerve fibre of xenopus laevis as computed on the basis of voltage clamp data. J. Physiol. 171:302-315.

**Friedmann SA, Feinberg R, Podolak E, Bedell RHS. (1967)** Pupillary abnormalities in diabetic neuropathy. Ann. Intern. Med. 67:977-983.

**Gadoth N, Schlaen N, Maschkowski D, Bechar M. (1983)** The pupil cycle time in familial dysautonomia. Further evidence for denervation hypersensitivity. Metab. Pediatr. System Ophthalmol. 7:131-134.

**Garten S. (1897)** Beitrage zur Kenntniss des zeitlichen Ablaufes der Pupillarreaction nach Verdunklung. Pflügers Arch. ges. Physiol. 68:68-96.

**Glass L, Mackey MC. (1979)** Pathological conditions resulting from instabilities in physiological control systems. Ann. N.Y. Acad. Sci. 316:214-235.

**Goldman L, Albus JS. (1968)** Computation of impulse conduction in myelinated fibres; theoretical basis of the velocity-diameter relation. Biophys. J. 8:596-607.

**Gradle H. (1932)** Die Reaktionszeit der normalen Pupille. Klin. Monatsbl. Augenheilk. 89:684.

**Gradle HS, Ackerman W. (1932)** The reaction time of the normal pupil. J. Am. Med. Ass. (Trans. Sect. Ophthalmol.) 99:1334-1336.

**Gradle HS, Eisendrath EB. (1923)** Die Reaktionszeit der normalen Pupille. Klin. Monatsbl. Augenheilk. 71:311-313.

**Grünberger J. (1987)** Faktoranalytische Untersuchung und Rehibilitätsbestimmung der statischen und dynamischen Pupillometrie bei Normalpersonen und psychopathologischen Gruppen. Wien. Med. Wochenschr. 137:135-139.

**Grünberger J, Linzmayer L, Gathmann P, Saletu B. (1985)** Computerassistierte "statische" und lichtevozierte "dynamische" Pupillometrie bei psychosomatischen Patienten. Wien. klin. Wochenschr. 97:775-781.

**Gundlach HR. (1934)** The speed of pupillary contraction in response to light in pigeons, cats and humans. J. genet. Psychol. 44:250-253.

**Guttman E, Stokes AB. (1939)** Effect of drugs in myotonia. Lancet 237:879-881.

**Halliday AM, McDonald WI. (1977)** Pathophysiology of demyelinating disease. Br. Med. Bull. 33:21-27.

**Halliday AM, McDonald WI, Mushin J. (1972)** Delayed visual evoked responses in optic neuritis. Lancet 1:982-985.

**Halliday AM, McDonald WI, Mushin J. (1973a)** Visual evoked responses in diagnosis of multiple sclerosis. Br. Med. J. 4:661-664.

**Halliday AM, McDonald WI, Mushin J. (1973b)** Delayed pattern-evoked responses in optic neuritis in relation to visual acuity. Trans. Ophthalmol. Soc. U.K. 93:315-324.

**Hamilton W, Drewry RDjr. (1983)** Edge-light pupil cycle time and optic nerve disease. Ann. Ophthalmol. 15:714-721.

**Hayashi M, Ishikawa S. (1979)** Pharmacology of pupillary responses in diabetics. -Correlative study of the responses and grade of retinopathy-. Jpn. J. Ophthalmol. 23:65-72.

**Heiden U an der. (1983)** Periodic, aperiodic and stochastic behaviour of differential difference equations. Modeling biological and economical processes. In: Differential-difference equations. Collatz L, Meinardus G, Wetterling W (eds), Birkhauser-Verlag, Boston :91-108.

**Heiden U an der, Mackey MC. (1982)** The dynamics of production and destruction: Analytic insight into complex behaviour. J. Math. Biol. 16:75-101.

**Heijer JC den, Dijk JG van, Bollen E, Bos JE. (1991)** Assessment of autonomic function in myotonic dystrophy. J. Neurol. Neurosurg. Psychiatry 54:531-534.

**Hodgkin AL, Huxley AF. (1952)** A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. 117:500-544.

**Hofstadter DR. (1981)** Metamagical themas. Strange attractors: mathematical patterns delicately poised between order and chaos. Sci. Amer. 245:22-43.

**Hreidarsson AB, Gundersen HJG. (1985)** The pupillary response to light in type 1 (insulin-dependent) diabetes. Diabetologia 28:815-821.

**Hutchinson NA, Koles ZJ, Smith RS. (1970)** Conduction velocity in myelinated nerve fibres of xenopus laevis. J. Physiol. 203:279-289.

**Jakobsen J, Pedersen E. (1985)** Pupil abnormalities in multiple sclerosis (relationship to stage of disease). J. Neurol. suppl. 1 232:67.

**Kehrer F. (1923)** Zur Pathologie der Pupillen. Ztschr. ges. Neurol. u. Psychiat. 81:345-408.

**Kimura J. (1987)** Variability in nerve conduction measurement. In: Electrodiagnosis in diseases of nerve and muscle: principles and practice. F.A. Davis, Philadelphia :97-100.

**Koles ZJ, Rasminsky M. (1972)** A computer simulation of conduction in myelinated nerve fibre. J. Physiol. 227:351-364.

**Kollarits CR, Kollarits FJ, Scheutte WH, Whitehouse WC, Gunkel RD. (1983)** The pupil dark response in normal volunteers. Current Eye Res. 2:255-259.

**Kraats J van der, Smit EP, Slooter JH. (1977)** Objective perimetric measurements by the pupil balance method. Doc. Ophthalmol. 14:213-219.

**Kristek A. (1966)** Die klinische Infrarotpupillographie. Acta Univ. Palackianae Olomucencis 40:389-445.

**Kumnick LS. (1956)** Aging and the latency and duration of pupil constriction in response to light and sound stimuli. J. Gerontology 11:391-396.

**Künkel H. (1961)** Beitrag zur einer regeltheoretischen Analyse der Pupillenreflexdynamik. I. Mitteilung. Experimentelle Ergebnisse. Kybernetik 1:69-75.

**Künkel H. (1963)** Die regeltheoretische Interpretation der Pupillenreflex-Dynamik als Beitrag zur Untersuchung vegetativer Regulationen. Ber. physik. -med. Ges. Würzburg, N.F. 71:74-93.

**Kyrieleis W. (1958)** Myotonie der Pupillenmuskulatur. München med. Wchnschr. 100:619-621.

**Lang HA, Puusa A, Hynninen P, Kuusela V, Jantti V, Sillanpaa M. (1985)** Evolution of nerve conduction velocity in later childhood and adolescence. Muscle & Nerve 8:38-43.

**Lanting P, Bos JE, Aartsen J, Schuman L, Reichert-Thoen JWM, Heimans JJ. (1990)** Assessment of pupillary light reflex latency and darkness adapted pupil size in control subjects and in diabetic patients with and without cardiovascular autonomic neuropathy. J. Neurol. Neurosurg. Psychiatry 53:912-914.

**Lanting P, Heimans JJ, Reulen JPH, Nauta J, Veen EA van der. (1988)** Pupillary light reflex and quantitative sensory and motor neural function tests in diabetic patients. J. Neurol. 235:245-247.

**Lanting P, Strijers RLM, Bos JE, Faes TJC, Heimans JJ. (1991)** The cause of increased pupillary light reflex latencies in diabetic patients: the relationship between pupillary light reflex and visual evoked potential latencies. Electroencephal. Clin. Neurophysiol. 78:111-115.

**Lee RE, Cohen GH, Boynton RM. (1969)** Latency variation in human pupil contraction due to stimulus luminance and/or adaptation level. J. Opt. Soc. Am. 59:97-103.

**Levatin P. (1959)** Pupillary escape in disease of the retina or optic nerve. Arch. Ophthalmol. 62:768-779.

**Link N, Stark L. (1988)** Latency of the pupillary response. IEEE trans. Biom. Eng. 35:214-218.

**Loewenfeld IE. (1966)** Pupillary movements associated with light and near vision: an experimental review of the literature. In: Recent developments in vision research. Whitcomb MA (ed), National Research Council Publicat, Washington :17-105.

**Longtin A, Milton JG. (1988)** Complex oscillations in the human pupil light reflex with "mixed" and delayed feedback. Math. Biosci. 90:183-199.

**Longtin A, Milton JG. (1989a)** Insight into the transfer function, gain, and oscillation onset for the pupil light reflex using nonlinear delay-differential equations. Biol. Cybern. 61:51-58.

**Longtin A, Milton JG. (1989b)** Modelling autonomous oscillations in the human pupil light reflex using non-linear delay-differential equations. Bull. Mathem. Biol. 51:605-624.

**Longtin A, Milton JG, Bos JE, Mackey MC. (1990)** Noise and critical behaviour of the pupil light reflex at oscillation onset. Phys. Rev. A 41:6992-7005.

**Lowenstein O. (1949)** Die zentralnervosen Faktoren in der Genese des acuten Glaucoma simplex. Ergebnisse experimentell pupillographischer Studien. Mschr. f. Psychiatr. u Neurol. 117:294-306.

**Lowenstein O. (1954)** Clinical pupillary symptoms in lesions of the optic nerve, optic chiasm, optic tract. Arch. Ophthalmol. 52:385-403.

**Lowenstein O, Friedman ED. (1942)** Pupillographic studies. I. Present state of pupillography; its method and diagnostic significance. Arch. Ophthalmol. 27:969-993.

**Lowenstein O, Kawabata H, Loewenfeld IE. (1964)** The pupil as indicator of retinal activity. Am. J. Ophthalmol. 57:569-596.

**Lowenstein O, Loewenfeld IE. (1951)** Types of central autonomic innervation and fatigue. Arch. Neurol. 66:580-599.

**Lowenstein O, Loewenfeld IE. (1952a)** Disintegration of central autonomic regulation during fatigue

and its reintegration by psychosensory controlling mechanisms. I. Disintegration. Pupillographic studies. J. Nerv. & Ment. Dis. 115:1-21.

**Lowenstein O, Loewenfeld IE. (1952b)** Disintegration of central autonomic regulation during fatigue and its reintegration by psycosensory controlling mechanisms. II. Reintegration; Pupillographic studies. J. Nerv. & Ment. Dis. 115:121-145.

**Lowenstein O, Loewenfeld IE. (1958)** Electronic pupillography: A new instrument and some clinical applications. A.M.A. Arch. Ophthalmol. 59:352-363.

**Lowenstein O, Loewenfeld IE. (1959a)** Scotopic and photopic thresholds of the pupillary light reflex in normal man. Am. J. Ophthalmol. 48:87-98.

**Lowenstein O, Loewenfeld IE. (1959b)** Influence of retinal adaptation upon the pupillary reflex to light in normal man: Part I: Effect of adaptation to bright light on the pupillary threshold. Am. J. Ophthalmol. 48:536-550.

**Lowenstein O, Loewenfeld IE. (1962)** The pupil. In: The eye, Ch 9. Davson H (ed), Ac. Press, London :231-267.

**Lowenstein O, Loewenfeld IE. (1969)** Effect of various light stimuli. In: Davson H (ed), Academic Press, New York :274.

**Lowenstein O, Westphal A. (1933)** Experimentelle und klinische Studien zur Physiologie und Pathologie der Pupillenbewegungen (mit besonderer Berücksichtigung der Schizophrenie). Abhdl. aus d. Neurol., Psychiatr., Psychol. u. ihren Grenzgeb. Berlin, 1933 70.

**Lusted LB, Keats TE. (1972)** Atlas of roentgenographic measurement. Year book Medical Publ. Inc., Chicago, 3-rd ed.

**Maas O, Paterson AS. (1947)** Dystrophia myotonica as a generalized disease. Monatsschr. Psychiatr. u. Neurol. 113:79-99.

**Maas O, Zondek H. (1920)** Untersuchungsbefund an einem Fall von Dystrophia myotonica. Zeitschr. ges. Neurol. u. Psychiat. 59:322-331.

**Machemer H. (1935)** Beitrage zur Physiologie und Pathologie der Pupille. II Mitteilung: Ueber den Ablauf des normalen Lichtreflexes. Klin. Monatsbl. Augenheilk. 94:305-319.

**Machemer H. (1941)** Beitrage zur Physiologie und Pathologie der Pupille. III Mitteilung: Die Latenzzeit des Lichtreflexes der normalen menschlichen Pupille. Klin. Monatsbl. Augenheilk. 106:385-405.

**Mackey MC, Glass L. (1977)** Oscillation and chaos in physiological control systems. Science 197:287-289.

**Mackey MC, Milton JG. (1987)** Dynamical diseases. Ann. N.Y. Acad. Sci. 504:16-32.

**Manor RS, Yassur Y, Ben-Sira I. (1982a)** Pupil cycle time in space occupying lesions of anterior optic pathways. Ann. Ophthalmol. 14:1030-1032.

**Manor RS, Yassur Y, Ben-Sira I. (1982b)** Pupil cycle time in noncompressive optic neuropathy. Ann. Ophthalmol. 14:546-550.

**Manor RS, Yassur Y, Siegal R, Ben-Sira I. (1981)** The pupil cycle time test: age variations in normal subjects. Br. J. Ophthalmol. 65:750-753.

**Martyn CN, Ewing DJ. (1984)** The pupil light reflex - a simple way of detecting diabetic autonomic neuropathy. Diabetes Med. 1:157.

**Martyn CN, Ewing DJ. (1986)** Pupil cycle time: a simple way of measuring an autonomic reflex. J. Neurol. Neurosurg. Psychiatry. 49:771-774.

**Matthes K. (1941)** Über die Registrierung von Bewegungsvorgängen mit dem lichtelectrischen Reflexionsmesser. Klin. Wochenschr. 20:295-297.

**McCrary JA III. (1977)** Light reflex anatomy and the afferent pupil defect. Tr. Am. Acad. Ophth. & Otol. 83:OP820-OP826.

**McDonald WI. (1977)** Acute optic neuritis. Br. J. Hosp. Med. 18:42-48.

**McDonald WI, Sears TA. (1970)** The effects of experimental demyelination on conduction in the central nervous system. Brain 93:583-598.

**Mechler F, Mastaglia FL. (1981)** Vascular adrenergic receptor responses in skeletal muscle in myotonic dystrophy. Ann. Neurol. 9:157-162.

**Miller SD, Thompson HS. (1978a)** Edge-light pupil cycle time. Br. J. Ophthalmol. 62:495-500.

**Miller SD, Thompson HS. (1978b)** Pupil cycle time in optic neuritis. Am. J. Ophthalmol. 85:635-642.

**Milton JG, Longtin A, Beuter A, Mackey MC, Glass L. (1989)** Complex dynamics and bifurcations in neurology. J. theor. Biol. 138:129-147.

**Milton JG, Longtin A, Kirkham TH, Francis GS. (1988)** Irregular pupil cycling as a characteristic

abnormality in patients with demyelinative optic neuropathy. Am. J. Ophthalmol. 105:402-407.

**Morone G. (1948)** Ricerche pupillografiche nella distrofia miotonica. Boll. d'oculist. 27:481-506.

**Moses RA. (1975)** The iris and the pupil. In: Adler's Physiology of the eye, Ch 12. Moses RA (ed), :320-352.

**Müller-Jensen A. (1978)** Untersuchungen zur Pupillen-Lichtreflex-Dynamik mittels Infrarot-Reflexpupillographie. (Pupillary reflex dynamics studied with infrared pupillography). Fortschr. Med. 96:27-31.

**Müller-Jensen A, Hagenah R. (1976)** Untersuchungen zur Variabilität des phasischen Pupillenlichtreflexes. J. Neurol. 212:123-132.

**Müller-Jensen A, Zschocke S. (1979)** Possibilities of simultaneous analysis of phasic pupillary light reflex and visual evoked potentials. Electroencephal. Clin. Neurophysiol. 47:239-242.

**Namba K, Utsumi T, Kitazawa A. (1980)** Diabetes mellitus and the pupil. (A preliminary report). Nippon Ganka Gakkai Zasshi 84:398-405.

**Nygaard RW, Frumkes TE. (1982)** Calibration of the retinal illuminamce provided by maxwellian views. Vis. Res. 22:433-434.

**Ohba N, Alpern M. (1972)** Adaptation of the pupil light reflex. Vis. Res. 12:953-967.

**Parinaud H. (1884)** Trouble oculaires de la sclérose en placques. Progr. Med., Paris 12:641.

**Petersen P. (1956)** Die Pupillographie und das Pupillogramm. Eine methodische Studie. Acta Physiol. Scand. 37, suppl. 125:1-141.

**Pfeifer MA, Cook C, Brodsky J, Tice D, Parrish D, Reeman A, Halter JB, Porte D. (1982)** Quantitative evaluation of sympathetic and parasympathetic control of iris function. Diabetes Care 5:518-529.

**Pfeifer MA, Weinberg CR, Cook D, Best JD, Reenan A, Halter JB. (1983)** Differential changes of autonomic nervous system function with age in man. Am. J. Med. 75:249-258.

**Pfeifer MA, Weinberg CR, Cook DL, Reenan A, Halter JB, Ensinck JW, Porte D. (1984)** Autonomic neural dysfunction in recently diagnosed diabetic subjects. Diabetes Care 7:447-453.

**Piltz J. (1904)** Ein neuer Apparat zum Photographieren der Pupillenbewegungen. Neurol. Centralbl. 23:801-853.

**Poel JC van der. (1985)** Visual function in optic neuritis in relation to multiple sclerosis. M.D. thesis, Erasmus Universiteit Rotterdam.

**Pozzessere G, Rizzo PA, Valle E, Mollica MA, Meccia A, Morano S, Di Mario U, Andreani D, Morocutti C. (1988)** Early detection of neurological involvement in IDDM and NIDDM. Multimodal evoked potentials versus metabolic control. Diabetes Care 6:473-480.

**Puvanendran K, Devathasan G, Wong PK. (1983)** Visual evoked responses in diabetes. J. Neurol. Neurosurg. Psychiatry 46:643-647.

**Rasminsky M, Sears TA. (1972)** Internodal conduction in undissected demyelinated nerve fibres. J. Physiol. 227:323-250.

**Reeves P. (1920)** The response of the average pupil to various intensities of light. J. Opt. Soc. Am. 4:35-43.

**Regan D. (1989)** Human Brain Electrophysiology. Evoked potentials and evoked magnetic fields in science and medicine. Elsevier, New York.

**Reulen JPH, Marcus JT, Gilst MJ van, Koops D, Bos JE, Tiesinga G, Vries FR de, Boshuizen K. (1988)** Stimulation and recording of dynamic pupillary reflex: the IRIS technique. Part 2. Med. Biol. Eng. Comp. 26:27-32.

**Riemslag FCC. (1986)** Retinal and occipital pattern evoked potentials. Ph.D. thesis, Universiteit van Amsterdam.

**Riemslag FCC, Ringo JL, Spekreyse H, Verduyn Lunel HF. (1985)** The luminance origin of the pattern electroretinogram in man. J. Physiol. 363:191-209.

**Saenger A. (1902)** Ueber myotonische Pupillenbewegung. Neurol. Zentralbl. 21:837-839.

**Safran AB, Walser A, Roth A, Gauthier G. (1981a)** Influence of central depressant drugs on pupil function: an evaluation with the pupil cycle induction test. 183:214-219.

**Safran AB, Walser A, Roth A, Gauthier G. (1981b)** Pupil cycle inducion test: a way of evaluating the pupillary light reflex. Ophthalmologica 183:205-213.

**Sandberg A, Stark LW. (1968)** Wiener G-function analysis as an approach to nonlinear characteristics of human pupil light reflex. Brain Res. 11:194-211.

**Scharnke, Full. (1920)** Innere Sekretion und Myotonia dystrophia. Ztschr. ges. Neurol. u. Psychiat. 61:146-173.

**Schauf CL, Davis FA. (1974)** Impulse conduction in multiple sclerosis: a theoretical basis for modification by temperature and pharmacological agents. J. Neurol. Neurosurg. Psychiatry 37:152-161.

**Semmlow JL, Hansmann D, Stark L. (1975)** Variation in pupillomotor responsiveness with mean pupil size. Vis. Res. 15:85-90.

**Semmlow JL, Stark LW. (1973)** Pupil movements to light and accomodative stimulation: A comparative study. Vis. Res. 13:1087-1100.

**Shimizu Y, Stark L. (1977)** Pupillary escape. 10th Symp. on the Pupil, New York.

**Slooter J, Norren D van. (1980)** Visual acuity measured with pupil responses to checkerboard stimuli. Invest. Ophthalmol. Vis. Sci. 19:105-108.

**Slooter JH. (1981)** Clinical use of visual acuity measured with pupil responses. Doc. Ophthalmol. 50:389-399.

**Slooter JH. (1985)** The pupil, mirror of visual acuity. M.D. thesis, Katholieke Universiteit Nijmegen.

**Smith JD, Masek GA, Ichinose LY, Watanabe T, Stark LW. (1970)** Single neuron activity in the pupillary system. Brain Res. 24:219-234.

**Smith SA, Smith SE. (1983)** Reduced pupillary light reflexes in diabetic autonomic neuropathy. Diabetologia 24:330-332.

**Smith SE, Smith SA, Brown PM, Fox C, Sonksen PH. (1978)** Pupillary signs in diabetic autonomic neuropathy. Br. Med. J. 2:924-927.

**Sobel I, Stark LW. (1962)** Re-evaluation of the pupil system. Quart. Progs. Rep. Res. Lab. Electr. MIT 66:412-419.

**Sood AK, Mithal S, Elhence A, Maini A. (1985)** Pupil cycle time. Indian J. Ophthalmol. 33:41-43.

**Spaans F. (1981)** Het onderzoek van de zenuwgeleiding. In: Klinische electromyografie. Notermans SLH (ed), Stafleu's Wetensch. Uitg. B.V., Alphen a/d Rijn :108-149.

**Spekrijse H, Estevez O, Tweel LH van der. (1973)** Luminance response to pattern reversal. Doc. Ophthalmol. Proceedings series 10:205-211.

**Stakenburg M. (1990)** On the absence of pupillary constriction during accommodation. Ph.D. thesis, Rijksuniversiteit Groningen.

**Stark LW. (1959)** Stability, oscillation and noise in the human pupil servomechanism. Proc. IRE 47:1925-1939.

**Stark LW. (1962a)** Biological rhythms, noise, and asymmetry in the pupil-retinal control system. Ann. N.Y. Acad. Sci. 98:1096-1108.

**Stark LW. (1962b)** Environmental clamping of biological systems: Pupil servomechanism. J. Opt. Soc. Am. 52:925-930.

**Stark L. (1987)** Neurological oscillations: formulation of mathematical control models and application to clinical syndromes. In: Temporal disorders in human oscillatory systems. Rensing L, Heiden U an der, Mackey MC (eds), Springer-Verlag, Berlin :83-101.

**Stark LW, Cornsweet TN. (1958)** Testing a servoanalytic hypothests for pupil oscillations. Science 127:588.

**Stark LW, Shermann PM. (1957)** A servoanalytic study of consensual pupil reflex to light. J. Neurophysiol. 20:17-26.

**Stern HJ. (1944)** A simple method for the early diagnosis of abnormality of the pupillary reaction. Br. J. Ophthalmol. 28:275-276.

**Stockard JJ, Hughes JF, Sharbrough FW. (1979)** Visually evoked potentials to electronic pattern reversal: latency variations with gender, age and technical factors. Am. J. EEG Technol. 19:171-204.

**Stockard JJ, Stockard JE, Sharbrough FW. (1978)** Nonpathologic factors influencing brainstem auditory evoked potentials. Am. J. EEG Technol. 18:177-209.

**Sun F, Krenz WC, Stark LW. (1983a)** A systems model for the pupil size effect: I Transient data. Biol. Cybern. 48:101-108.

**Sun F, Liu H, Liu Y. (1981)** Dynamic pupillary response to positive differential of light stimulus. Sci. Sin. 24:872-884.

**Sun F, Stark L. (1983)** Pupillary escape intensified by large pupillary size. Vis. Res. 23:611-615.

**Sun F, Tauchi P, Stark L. (1983b)** Dynamic pupillary response controlled bij the pupil size effect. Exp. Neurol. 82:313-324.

**Sun F, Tauchi P, Stark L. (1983c)** Binocular alternating pulse stimuli: experimental and modeling studies of the pupil reflex to light. Math. Biosci. 67:225-245.

**Talbot SA. (1938a)** Cinematography of the pupil. Am. J. Physiol. 123:200.

**Talbot SA. (1938b)** Pupillography and the pupillary transient. Ph.D. thesis, Harvard Univiversity.

**Taylor RE. (1963)** Cable theory. In: Physical techniques in biological research. Electrophysiological methods Part B. Nastuk WL (ed), Acad. Press, New York :219-262.

**Thivierge J, Cote R. (1987)** Brainstem auditory evoked response (BAER): normative study in children and adults. Electoencephal. Clin. Neurophysiol. 68:479-484.

**Thompson HS. (1966)** Afferent pupillary defects. Am. J. Ophthalmol. 62:860-873.

**Thompson HS, Allen MW van, Noorden GK von. (1964)** The pupil in myotonic dystrophy. Invest. Ophthalmol. Vis. Sci. 3:325-338.

**Thompson HS, Franceschetti AT, Thompson PM. (1971)** Hippus: semantic and historic considerations of the word. Am. J. Ophthalmol. 71:1116-1120.

**Thompson HS, Jiang M. (1987)** Letter to the editor on an article by Browning & Tiedeman, Ophthalmology 94:53-55 (1987), Ophthalmology 94:1360-1362.

**Tiedt N. (1963)** Die 24Stunden-Rhytmik der Kinetik des Lichtreflexes der menschlichen Pupille. Pflügers Arch. ges. Physiol. 277:458-472.

**Troelstra A. (1968)** Detection of time-varying light signals as measure by the pupillary response. J. Opt. Soc. Am. 58:685-690.

**Tschirren B. (1947)** Die fortlaufende Registrierung des Pupillen-reflexes. Helv. Physiol. Pharmacol. Acta. 5:C57-C58.

**Tweel LH van der, Denier van der Gon JJ. (1959)** The light reflex of the normal pupil of man. Acta Physiol. Pharmacol. Neerlandica 8:52-88.

**Ukai K. (1985)** Spatial pattern as a stimulus to the pupillary system. J. Opt. Soc. Am. 2:1094-1100.

**Ukai K, Higashi JT, Ishikawa S. (1980)** Edge-light pupil oscillation of optic neuritis. Neuro-ophthalmology 1:33-43.

**Usui S. (1974)** Functional organization of the human pupillary light reflex system. Ph.D. thesis, UC Berkeley :86.

**Usui S, Stark LW. (1982)** A model for nonlinear stochastic behavior of the pupil. Biol. Cybern. 45:13-22.

**Utsumi T, Ishikawa S, Kimura T. (1976)** Diurnal variation of the pupillary movements observed by binocular infrared pupillogram. Adv. Neurol. Sci. 20:977-989.

**Utsumi T, Onishi Y, Hashimoto T. (1978)** Pupillary diurnal variation under open-loop condition (Differences between open-loop and closed-loop condition). Adv. Neurol. Sci. (Tokyo) 22:615-623.

**Varju D. (1964)** Der Einfluss sinusformiger Leuchtdichteänderungen auf die mittlere Pupillenwite und auf die subjectieve Helligkeit. Kybernetic 2:33-43.

**Veringa F, Collewijn H, Hof MW van, Brink G van de. (1978)** Het gezicht. In: Leerboek der neurofysiologie, Ch 13. Voorhoeve PE (ed), Elsevier :328-446.

**Vintschgau M von. (1881)** Zeitbestimmungen der Bewegungen der eigenen Iris. Arch. f. Physiol. 26:324-390.

**Walraven J, Enroth-Cugell C, Hood DC, MacLeod DIA, Schnapf JL. (1990)** The control of visual sensitivity (receptoral and postreceptoral processes). In: Visual Perception: The Neurophysiological Foundations, Ch. 5. Academic Press, Inc. :54-101.

**Walsh FB, Hoyt WF. (1985)** Disorders of pupillary function, accommodation, and lacrimation. In: Clinical Neuro-ophthalmology, Ch 31. Miller NR (ed), Williams & Wilkins, Baltimore :469-556.

**Waxman SG. (1978)** Prerequisites for conduction in demyelinated fibres. Neurology 28:27-33.

**Webster JG. (1969)** Critical duration for the pupillary light reflex. J. Opt. Soc. Am. 59:1473-1478.

**Webster JG. (1971)** Pupillary light reflex: development of teaching models. IEEE Trans. 18:187-194.

**Webster JG, Cohen GH, Boynton RM. (1968)** Optimizing the use of the criterion response for the pupil light reflex. J. Opt. Soc. Am. 58:419-424.

**Weiler K. (1910)** Untersuchungen der Pupille und der Irisbewegungen beim Menschen. Zeitschr. Ges. Neurol. Psychiat. II:101-274.

**Weinstein JM, Gilder JC van, Thompson HS. (1980)** Pupil cycle time in optic nerve compression. Am. J. Ophthalmol. 89:263-267.

**West RW. (1988)** Explanation of Broca's pupillometer. Am. J. Optom. Physiol. Opt. 65:506-511.
**Westheimer G. (1966)** The Maxwellian view. Vis. Res. 6:669-682.
**Wildt GJ van der, Bouman MA. (1974)** Dependence of the dynamic behaviour of the the human pupil system on the input signal. Optica Acta 21:59-74.
**Wybar KC. (1952)** The ocular manifestations of disseminated sclerosis. Proc. R. Soc. Med. 45:315-320.
**Young FA, Biersdorf WR. (1954)** Pupillary contraction and dilation in light and darkness. J. comp. Physiol. Psychol. 47:264-268.

## SUMMARY

This thesis deals with the pupil light reflex: the narrowing (constriction) and widening (dilatation) of the pupil when the amount of light, incident on the eye, is changed. Constriction and dilatation both are active processes controlled entirely by the autonomic nervous system (ANS). Pupil constriction is regulated by the parasympathetic part of the ANS, dilatation by the sympathetic part. Pupil behaviour under changing light conditions is, however, characterized primarily by parasympathetic functioning. The constriction is therefore of major importance in pupil dynamics. The theme that runs through this thesis concerns the temporal aspects of pupil constriction.

Investigations on these temporal aspects were facilitated by a newly developed pupillometer, the IRIS system. It satisfies the present interest and meets the ensuing requirements of accurate measurement of the pupil reflex. Both eyes can be measured simultaneously, facilitating the investigation of interocular differences.

A so-called Maxwellian view stimulator is incorporated in the pupillometer. This device projects the top of a convergent beam of light within the boundaries of the pupil, and stimulates a large retinal area. Iris control of retinal illumination is thus eliminated, and the pupil light reflex can be stimulated directly where it originates, using well-defined stimuli under open-loop conditions. A fixation target, projected onto the retina from within the same stimulator, optimally suppresses eye movements and accommodation, and this unravels the control due to the near triad. A calibration procedure that accounts for the average retinal illuminance is introduced.

The most prominent pupil reflex is caused by a change in the total amount of light presented to the eye. Besides, the pupil responds to stimuli with a changing spatial structure, even if the total amount of light striking the retina remains unchanged. This reflex is, again, characterized by constrictions, of which the literature only mentions the contrast origin. This thesis proves that the constrictions are not caused by the changing contrasts, but by the changing local-luminance distribution accompanying the changes in contrast. The local-luminance origin is explained by means of a model.

Because contrast is essential for visual perception, the pupil reflex cannot be utilized for testing of the visual acuity as suggested in the

literature. Furthermore, application of spatial structures in stimuli for studying pupil dynamics is of no additional use.

An intriguing aspect of pupil behaviour are artificially induced oscillations. Two system parameters are of particular importance for inducing them: gain and delay. Here, gain is related to the ratio between the change in retinal light flux, due to a change in pupil size, and the change in flux, due to a change in stimulus intensity causing the reflex. The delay in the pupil system originates from the retinal translation of light into neural activity, the conduction of neural pulses by the ANS, and the conversion of neural activity into a changing pupil size in the iris neuro-muscular junction. To incite the pupil system to produce oscillations by destabilizing it, at least one of the parameters should be increased beyond a certain threshold, for the pupil system is stable under normal conditions. The parameters are manipulated by environmental clamping, a technique in which the reflex loop is opened by applying Maxwellian view, and it is reclosed by an external device regulating stimulus intensity depending on measured pupil size. High-gain induced oscillations have been described in the literature, extended-delay induced oscillations, however, have not. Nevertheless, it was assumed in that literature that abnormally prolonged oscillation periods are due to abnormally prolonged delays.

Oscillatory behaviour, predicted in this thesis with the help of a first-order model, and behaviour observed in experiments, are shown to be alike. Special attention is paid to the pupil cycle time. An extremely high correlation is now demonstrated between both the predicted and the observed oscillation periods and delay, independent of the system's gain. The oscillation period equals roughly twice the delay, apart from a certain offset. A foundation is thus laid for research on these oscillations, where additional studies are needed on the exact dependency of oscillation period on delay, to allow application for delay determination.

Whereas the delay cannot yet be determined by using high-gain and extended-delay induced oscillations, it can be determined by using instant increases in stimulus-light intensity. The delay, as described above, is then generally referred to as latency, and can be determined on the basis of the time-lapse between stimulus and constriction onset (constriction latency, CL). Another important parameter constitutes the moment of peak constriction-velocity (PCVL).

The importance and the way of objective quantification of these parameters are described. Depending on the sample rate, one of two possibilities is to be used. If data are digitized at a high rate (≈200 Hz), the observation of the first sample in a series with velocities consecutively less than zero, gives the constriction onset with an accuracy of about 5 ms. This accuracy is satisfactory in view of latency differences to be assessed. Another method should be used if data are sampled at lower rates, as by applying TV pupillometers (≈25 Hz). Then some model has to be adopted to predict the moment of constriction onset, since this event may occur somewhere in between two samples. A second-order model, preceeded by a linear trend, to allow accurate determination of this onset, is shown. Both methods yield latencies independent of signal amplitude, and differences in latencies determined by both of them are less than 5 ms on the average. The IRIS pupillometer therefore reliably enables latency determination by either of these methods. Because of its simplicity, the first method is preferred.

Furthermore, averaging of individual constriction responses, to eliminate physiological and machine noise, should be avoided preparatory to determining the latency. The onset moment of such an averaged response is advanced with respect to the average onset of the individual responses, and thus averaging yields too small latencies.

Normative data were gathered by applying the methods described. The equality of the latencies of the direct and consensual reflex, irrespective which eye is stimulated, is confirmed. Thus no further restrictions have to be imposed on a model for locating possible defects.

The CL was already known to decrease with increasing stimulus intensity. Under the current conditions, the CL depends roughly on the difference between the stimulus intensity and half the background intensity. Deviations are encountered, however, especially with regard to differences in backgrounds. This advises a comparison between different groups of subjects by parameters measured under identical conditions. A slight increase in latency is found with age (≈0.5 ms/year on the average), and female subjects have shorter latencies than male subjects.

These findings are now claimed to be true for the PCVL as well. The PCVL is linearly related to the CL with a slope approximating 1, independent of any other parameter involved.

It is argued that the CL, determined under photopic, wide-angle stimulation, is affected predominantly by parasympathetic neural functioning, while the difference between PCVL and CL reflects muscular functioning only.

Finally, this thesis describes the use of constriction latencies for differentiating between neural unilateral, bilateral, afferent and efferent, and muscular defects. A selection of diseases, thought to attain these ends, comprise optic neuritis (ON) and multiple sclerosis (MS), diabetes mellitus (DM) and myotonic dystrophy (MyD).

When the impaired eyes of ON/MS patients are stimulated, the average latency differs from that when the healthy fellow-eyes are stimulated. The direct and consensual reflexes do not differ with respect to the CL. Pupillometry can therefore reveal relative afferent defects in patients suffering from an inflammation of the optic nerve, as in ON and MS. Because of the equality of the first neural trajectory, leading to both pupil constrictions and visual evoked potentials (VEP), pupil data are compared to latencies related to the VEP (VEPL). Just after the acute phase of the inflammation, a high correlation is demonstrated between CLs and VEPLs. After a few months, however, VEPLs return to normal again, while CLs remain prolonged. Despite the inapplicability of the CL for individual diagnosis of ON, due to the short average prolongation observed with respect to the normal CL variability, CL prolongation is of fundamental interest.

In DM patients, in contrast to those of the ON/MS group, the CL shows a long prolongation in all cases (direct and consensual reflex, no matter which eye is stimulated). VEPLs are normal in a far higher percentage of the DM patients than of those of the ON/MS group. In DM no correlation is found between the CL and VEPL. It is therefore concluded that CL prolongation in DM is caused by an efferent defect, and that CL assessment can contribute to the individual diagnosis of DM.

In both the ON/MS and the DM group, no prolongation of the difference between PCVL and CL is found. However, in the patients suffering from MyD, the time to peak-velocity of contraction is significantly longer, while the average CL is not altered as compared to normal. This implies that abnormal pupil behaviour in MyD is not caused by slow-down of nervous pulse transmission, but by muscle stiffness only.

The CL, determined under photopic, wide-angle stimulation, is assumed to be influenced predominantly by neural functioning, whereas the difference between PCVL and CL reflects muscular functioning only. In all investigations described in this thesis, no effects of retinal disfunction are found. The CL and PCVL are therefore useful for the distiction between unilateral, bilateral, neural and muscular defects.

## SAMENVATTING

Dit proefschrift behandelt de pupillichtreflex: het vernauwen (constrictie) en verwijden (dilatatie) van de pupil bij verandering van de hoeveelheid licht die op het oog valt. Constrictie en dilatatie zijn beide actieve processen die volledig door het autonome zenuwstelsel ("autonomic nervous system", ANS) worden gestuurd. Pupilconstrictie wordt geregeld door het parasympathische deel van het ANS, dilatatie door het sympathische deel ervan. Pupilgedrag onder veranderende lichtcondities wordt echter voornamelijk bepaald door de parasympathische functie, en daarom is de constrictie van groot belang in de pupildynamica. De temporele aspecten van pupilconstricties vormen de rode draad die door dit proefschrift loopt.

Onderzoek naar deze temporele aspecten werd vergemakkelijkt door een recent ontwikkelde pupillometer, het IRIS-systeem. Dit apparaat voldoet aan de huidige wensen, vooral wat betreft de nauwkeurigheid waarmee pupilveranderingen geregistreerd kunnen worden. Beide ogen kunnen gelijktijdig bemeten worden, wat het onderzoek naar eventuele verschillen tussen beide ogen vereenvoudigd.

Een zogenaamde "Maxwellian view"-stimulator is onderdeel van de pupillometer. Deze stimulator projecteert de top van een convergerende lichtbundel binnen de grenzen van de pupil en stimuleert zo een groot gedeelte van het netvlies. De invloed van de iris op de belichting van het netvlies wordt zodoende ongedaan gemaakt en de pupilreflex kan op de plaats van oorsprong onder "open-loop"-condities gestimuleerd worden met goed te definiëren stimuli. Een fixatie-object, dat vanuit dezelfde stimulator op het netvlies wordt geprojecteerd, onderdrukt zo goed mogelijk oogbewegingen en accommodatie, hetgeen een scheiding mogelijk maakt naar de aansturing ten gevolge van de nabijheidstrits. Eveneens wordt een calibratieprocedure geïntroduceerd die rekening houdt met de gemiddelde verlichting van het netvlies door deze stimulator.

De meest in het oog vallende pupilreflex wordt veroorzaakt door een verandering in de totale hoeveelheid aan het oog aangeboden licht. Daarnaast reageert de pupil ook op stimuli met een veranderende ruimtelijke structuur, zelfs wanneer de totale hoeveelheid licht gelijk blijft. Ook deze reflex wordt gekenmerkt door de optredende constricties, waarvan de literatuur alleen de contrast-oorsprong vermeldt. Dit proefschrift laat zien dat genoemde constricties niet veroorzaakt worden door veranderingen in het aangeboden contrast,

maar door de veranderingen in locale helderheden die deze contrastveranderingen vergezellen. Aan de hand van een model wordt aangegeven hoe dit mogelijk is.

Pupilonderzoek heeft dan ook geen nut voor het bepalen van de visus, zoals voorgesteld in de literatuur, omdat contrast een essentiële bijdrage levert aan de visuele perceptie. Verder geeft bovengenoemde bevinding aan dat het gebruik van stimuli met een spatiële structuur niet van aanvullende waarde is voor pupildynamisch onderzoek.

Kunstmatig opgewekte oscillaties vormen een intrigerend aspect van pupilgedrag. Versterking en vertraging zijn hierbij twee bijzonder belangrijke systeemparameters. Versterking betreft de verhouding tussen een verandering in retinale lichtflux als gevolg van een veranderde pupilgrootte en de verandering in flux als gevolg van de lichtintensiteitsverandering die de reflex veroorzaakt. De vertraging, inherent aan het pupilsysteem, wordt veroorzaakt door de omzetting van lichtinformatie in electrische activiteit in het netvlies, het transport via het ANS, en de omzetting van electrische activiteit in een veranderende pupilgrootte in de neuro-musculaire overgang in de iris. Onder normale omstandigheden is het pupilsysteem stabiel. Om dit systeem aan te zetten tot het produceren van oscillaties door een destabilisatie, moet tenminste één van beide parameters vergroot worden tot boven een zekere drempelwaarde. Voor de experimenten beschreven in dit proefschrift werden genoemde parameters gemanipuleerd met een uitwendige terugkoppeling. Hierbij wordt het gesloten pupilsysteem eerst geopend door de toepassing van de "Maxwellian view"-stimulator, en buiten het pupilsysteem om weer gesloten door de stimulusintensiteit te laten afhangen van de gemeten pupilgrootte. Oscillaties, aldus veroorzaakt door een vergrote versterking, zijn in de literatuur reeds besproken; die, veroorzaakt door een vergrote vertraging, echter niet. Desondanks wordt in de literatuur wel aangenomen dat een abnormaal verlengde oscillatieperiode het gevolg is van een abnormaal grote vertraging van het pupilsysteem.

Oscillatoir gedrag, zoals berekend aan de hand van een model, en waargenomen oscillatoir gedrag bij experimenteel onderzoek, blijken in dit proefschrift tot op zekere hoogte goed overeen te komen. Hierbij wordt extra aandacht geschonken aan de oscillatieperiode. Zowel theoretisch als experimenteel correleert deze zeer goed met de vertraging, onafhankelijk van de versterking. Op een zekere constante na, is de oscillatie-periode ongeveer twee maal zo groot als de vertraging. Met dit onderzoek wordt zo een basis gelegd voor verder onderzoek naar deze oscillaties, vooral naar de precieze relatie tussen vertraging en oscillatieperiode en de toepasbaarheid van deze relatie.

Waar de vertraging nog niet bepaald kan worden met behulp van oscillaties, opgewekt door een hoge versterking of een verlengde vertraging, kan deze wel bepaald worden door de stimulusintensiteit stapsgewijs te laten veranderen. De vertraging, zoals boven omschreven, wordt dan algemeen "latentie" genoemd, en kan bepaald worden aan de hand van het tijdsinterval tussen stimulusaanvang en het begin van de constrictie (constrictie-latentie, CL). Een andere parameter van belang bij deze constricties wordt gegeven door het moment waarop de constrictiesnelheid maximaal is ("peak constriction-velocity latency", PCVL).

Het belang en de manier van objectieve quantificering van deze parameters worden beschreven. Afhankelijk van de bemonsterfrequentie dient één van twee methoden gebruikt te worden. Bij hoge bemonsterfrequenties (≈200 Hz) volstaat het zoeken naar het eerste punt in een bemonsteringsreeks, waarvan de snelheden opvolgend alle kleiner dan nul zijn. Dit geeft een onnauwkeurigheid in de CL van ongeveer 5 ms. De aldus verkregen nauwkeurigheid is voldoende voor de bepaling van latenties die in het kader van dit onderzoek van belang zijn. Een andere manier dient echter gebruikt te worden wanneer de bemonsterfrequentie laag is, zoals bij toepassing van een TV-pupillometer (≈25 Hz). Wil in dit geval een vergelijkbare nauwkeurigheid gehaald worden, dan moet een model worden aangenomen dat het begin van een constrictie kan voorspellen, ergens tussen twee bemonsteringspunten in. Een tweede-orde-model, voorafgegaan door een lineaire trend, kan dit doel goed dienen. Beide methoden geven latenties onafhankelijk van de signaalamplitude, en verschillen in latenties tussen beide methoden zijn gemiddeld minder dan 5 ms. Het gebruik van de IRIS-pupillometer in combinatie met één van deze twee detectiemethoden maakt daarom een nauwkeurige registratie van latenties mogelijk. Vanwege de eenvoud van de eerste methode heeft het gebruik daarvan echter de voorkeur.

Een veel toegepaste methode om ruis te elimineren is het middelen van individuele responsies. Voor nauwkeurige CL-bepaling dient dit echter achterwege gelaten te worden. Het begin van een constrictie van een gemiddelde respons is vervroegd t.o.v. het gemiddelde begin van de individuele constricties, en middelen geeft dus te kleine latentiewaarden.

Normaalwaarden voor de CL zijn bepaald met de beschreven methoden. De gelijkheid van de latenties voor de directe en consensuele reacties, ongeacht welk oog wordt gestimuleerd, wordt bevestigd. In dit opzicht hoeven er dus geen beperkingen opgelegd te worden aan een model voor het localiseren van eventuele defecten in het pupilsysteem.

Het was reeds bekend dat de CL afneemt met toenemende stimulusintensiteit. Onder de in dit proefschrift beschreven condities blijkt de CL aldus

afhankelijk te zijn van ongeveer het verschil in stapintensiteit en de helft van de achtergrondsintensiteit. Dit is echter niet altijd zo, met name voor verschillen in achtergrondsintensiteiten. Dit maakt een vergelijking tussen patiëntengegevens en normaalwaarden onder identiek gemeten omstandigheden noodzakelijk. De CL blijkt voorts met de leeftijd iets toe te nemen (gemiddeld ≈0.5 ms/jaar), en vrouwen hebben een kortere latentie dan mannen.

Deze bevindingen blijken ook voor de PCVL te gelden. Dit moment van maximale constrictiesnelheid treedt op, onafhankelijk van welke onderzochte parameter dan ook, ongeveer 100 ms nadat de constrictie begonnen is.

Beargumenteerd wordt dat de CL, bepaald onder fotopische stimulatie met een groot stimulusveld, voornamelijk beïnvloed wordt door het parasympathische zenuwstelsel, terwijl het verschil tussen PCVL en CL uitsluitend door spierfunctie bepaald wordt.

Als laatste wordt in dit proefschrift de toepassing van constrictie-latenties beschreven bij het differentiëren naar mogelijke afferente en efferente aandoeningen en naar spieraandoeningen. Ziekten waarbij dit mogelijk is zijn bijvoorbeeld neuritis optica ("optical neuritis", ON), multipele sclerose (MS), suikerziekte (diabetes mellitus, DM) en dystrofia myotonica ("myotonic dystrophy", MyD).

Wanneer de aangetaste ogen van ON/MS-patiënten worden gestimuleerd, verschilt het gemiddelde van deze constrictie-latenties van dat van het gezonde oog. De directe en consensuele reacties vertonen in beide gevallen geen verschil in latenties. Hiermee is aangetoond dat met behulp van pupillometrie een relatief afferent defect in patiënten met een oogzenuwontsteking, zoals in ON en MS, onderkend kan worden. Omdat het eerste neurale traject gelijk is voor zowel de pupilaansturing als het opwekken van visuele electrische corticale potentialen ("visual evoked potentials", VEP), zijn latenties van de pupil vergeleken met die van de VEP (VEPL), gemeten bij dezelfde patienten. Vlak na het begin van de ziekte (in de acute fase) vertonen beide latenties een hoge correlatie. Na een aantal maanden herstellen de VEPL's zich weer, terwijl CL's vertraagd blijven. Ondanks het feit dat, door het relatief kleine verschil tussen normale en abnormale CL's ten opzichte van de normale spreiding in deze latenties, de CL niet diagnostisch toegepast kan worden op individuele patiënten, is het gevonden verschil wel van fundamenteel wetenschappelijk belang.

In patienten met suikerziekte blijkt een veel grotere vertraging in latenties te bestaan dan in de onderzochte ON/MS-groep. Deze vertraging treedt zowel op in de directe als in de consensuele reactie, ongeacht welk oog gestimuleerd wordt. VEPL's zijn veel vaker normaal in DM- dan in ON/MS-patiënten, en

er bestaat ook geen duidelijke correlatie tussen CL's en VEPL's. Op basis van deze bevindingen wordt daarom de conclusie getrokken dat in DM de latentievertraging bij de pupilconstrictie een gevolg is van een efferent defect, en dat pupillometrie een bijdrage kan leveren aan de diagnose van DM.

Zowel in de onderzochte ON/MS-groep als in DM-patienten werd geen verandering gevonden van het verschil tussen PCVL en CL ten opzichte van normaal. In MyD blijkt echter juist dit verschil abnormaal verlengd, terwijl de CL normaal is. Dit betekent dat abnormaal pupilgedrag in MyD niet het gevolg is van een vertraagde zenuwgeleiding, maar van een stijfheid van de irisspieren.

Aannemelijk wordt gemaakt dat de CL, bepaald onder fotopische stimulatie met een breed stimulusveld, voornamelijk beïnvloed wordt door de neurale functie, terwijl het verschil tussen PCVL en CL alleen bepaald wordt door musculaire functie. In geen van de in dit proefschrift beschreven onderzoekingen werd een invloed gevonden van retinale afwijkingen. De CL en PCVL zijn daarom van waarde bij het onderscheiden van unilaterale en bilaterale defecten, en van neurale defecten en spierdefecten.

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