THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF MEDICINE (RESEARCH)

ENTITLED:

‘THE PUPIL IN GLAUCOMA’

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DECLARATIONS

Chapter 7

Procyon P3000 pupillometer – the construction of the instrument was performed by the Procyon Instruments Ltd, UK.

Chapter 8

The calibration of the instrument was performed with the help of, and the modification of the instrument was undertaken, by Dr Dan Taylor, Research and Development director of Procyon Instruments Ltd, UK.

Chapter 11

The calibration factor equation was formulated by Dr Dan Taylor, Research and Development director of Procyon Instruments Ltd, UK. The excerpts of this chapter have been published as Shwe-Tin A, Smith GT, Checketts D, Murdoch IE, Taylor D. Evaluation and Calibration of a binocular infrared pupillometer for measuring relative afferent pupillary defect. *Journal of Neuro-ophthalmology* 2012;32(2):111-115.

Link chapter comment

Procyon's proprietary formula was used for estimating pupillographic relative afferent pupillary defect.

Chapter 14

The excerpt of this chapter was presented at the Association for Research in Vison and Ophthalmology (ARVO) 2009 annual conference at Fort Lauderdale, Florida.

I, Audrey Shwe-Tin, confirm that the work in this thesis is my own.
Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

I, Audrey Shwe-Tin, confirm that the study was partly funded by Procyon Instruments Ltd and I have no commercial interest.

ABSTRACT

Glaucoma is the most common preventable cause of blind-registration in elderly Western populations. Case-finding is crucial for the prevention of blindness. There is no single test that can reliably diagnose glaucoma, especially early cases. The relative afferent pupillary detect (RAPD) is known to be sensitive in the detection of optic nerve pathology. The clinical swinging flash light test is well used for this purpose. However, the test requires skill and careful interpretation, and the sensitivity of the test is limited to ≥0.3 log units of relative pupillomotor deficit. Some of the newly-built commercially available pupillometers measure the pupil parameters with accuracy. These instruments have mainly been used in the area of refractive surgery. This thesis considers the applicability of the commercially available pupillometer P3000 to the diagnosis of glaucoma.

In this thesis a pupillometer (P3000) was calibrated before the stimulus parameters were tested for their best suitability for the RAPD test. The stimulus and outcome parameters were optimised. The chosen stimulus configuration (0.4s-1.6s on-off combination) produced repeatable results. The eyes were dark adapted only for 30 seconds before each test sequence for practical use in clinics. The pupillographic RAPD was calculated from the pupil constriction amplitudes calibrated in response to 3 levels of light stimulus. Data was collected on normal and glaucomatous subjects. There was no significant diurnal variation in the RAPD noted for both cohorts and the immediate repeatability was high.

The final test was used in a methods comparison study to detect glaucoma against the gold standard of clinical diagnosis. The area under the Receiver Operating Characteristic curve for the detection of all grades of unilateral or bilateral glaucoma was in the region of 0.81 for the cohort of 101 normal and 117 glaucoma patients. Pupillometry may be helpful as an adjunctive test in the detection of glaucoma.

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The pupillometer was first introduced to me by Mr Guy Smith. We used this instrument to measure the pupil diameters in the investigation of oculo-sympathetic paresis. His keen interest in research has inspired me to take on this thesis. I thank him for being my advisor and committed devotee of this project.

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GLOSSARY

In this thesis the following words are interchangeably used.

- AGIS = advanced glaucoma intervention study
- AION = anterior ischaemic optic neuropathy
- ANS = autonomic nervous system
- CDR = cup disc ratio
- CIGTS = collaborative initial glaucoma treatment study
- CNS = central nervous system
- CNTG = collaborative normal tension glaucoma study
- CONS = Cons = consensual response
- CSFLT = clinical swinging flash light test
- CSLO = confocal scanning laser ophthalmoscopy
- D = dioptre (dioptric power of a lens)
- DA = dark adaptation
- DDLS = disk damage likelihood scale
- DIR = Dir = direct response
- EMGT = Early manifest glaucoma trial
- FDP = frequency doubling perimetry
- GHT = glaucoma hemifield test
- HM = high mesopic stimulus = 4 lux
- HRT = Heidelberg retina tomograph
- ipRGC = intrinsically photosensitive retinal ganglion cells
- IOP = intraocular pressure
- ISI = inter stimulus interval
- LD = left eye direct light response
- LC = left eye consensual light response
- LCL = lower confidence level
- LM = low mesopic stimulus = 0.4 lux
- MD = mean deviation
- NTG = normal tension glaucoma
- OHT = ocular hypertension
- OCT = optical coherence tomography

- ON = stimulus light is on
- OFF = stimulus light is off
- PEX = pigment dispersion glaucoma
- PIPR = post illumination pupil response
- PLR = pupil light response
- PNS = peripheral nervous system
- POAG = primary open angle glaucoma
- pRAPD = pupillographic RAPD
- $pRAPD_{DIR}$ = pupillographic RAPD calculated from direct pupillary responses
- $pRAPD_{CON}$ = pupillographic RAPD calculated from consensual pupillary responses
- RGC = retinal ganglion cells
- PSD = pattern standard deviation
- cPSD = corrected pattern standard deviation
- RAPD = relative afferent pupillary defect
- RC = right eye consensual light response
- RCT = randomised control trial
- RD = right eye direct light response
- RNFL = retinal nerve fibre layer
- SAP = standard automated perimetry
- SC = Sco = scotopic stimulus = 0.04 lux
- SD = standard deviation
- SE = standard error
- SLP = scanning laser polarimetry
- SNR = signal to noise ratio
- Stimulus ON-duration = duration of stimulus
- Stimulus OFF-duration = inters-stimulus interval = darkness pause duration
- Stimulus configuration = stimulus pattern = the alternating stimulus characterised by an X duration of stimulus and Y duration of darkness pause in between stimulus.
- SWAP = short wavelength automated perimetry
- TRV = test re-test variability

- UCL = upper confidence level
- VEP = visual evoke potential

I. INTRODUCTORY CHAPTERS

Chapter 1

Historical Notes of the Pupil Response to Light

"*In the middle of the iris appears a hole which contracts when the light is strong but dilates in obscurity"*

Andreas Vesalius (1514 - 1564), French anatomist, translated this in Latin in a chapter of the medical manual, Libre medicinalis ad Almansorem, written by Rhazes (850-932), Abu-Bakr Muhammed ibn Zakariyaal Razi, of Baghdad for his Persian prince.[1]

The change in size of pupil from darkness to light is not too difficult to be noticed by any observant person. It must have been general knowledge during this time. But the first pupillary reflex test to light ever documented in the ophthalmic literature is dated back to even earlier than Rhazes time. Claudius Galen[2] from Pergamum (129 AD) which is now in western Turkey, in the 2nd century, couched cataracts. As an indicator to predict the outcome of his surgery, he determined the visual potential of his patients by observing the pupil size to light individually. He noticed that in a patient with good vision in both eyes facing to the window (which was his light source), when he put his hand in front of one of the eyes, the pupil of the other eye dilated. Galen explained that there was a "breath of vision" or "pneuma" that came from the brain to the eye through "pneumatic cannel" to control pupil size and vision. When one of the eyes was covered, the pneuma was no longer needed for that eye and went to the other uncovered eye incidentally causing it to dilate more. He thought of the iris as an elastic circular ring. When inflated by "pneuma" the inner margin was stretched and the pupil dilated.[3] He did not think of pupillary movement as a response to light. He would just cover the cataractous eye and observe the other eye to see if it dilated. If it dilated he deduced that there was a visual potential in the cataractous eye that he just covered and scheduled the patient for cataract surgery.[4] Set aside his philosophical speculation Galen had contributed a great deal to the ophthalmic literature. His observation of the pupil reaction to light was the objective sign, or *a reflex*, that could not be confounded by the patient and it made a statement about the part of the visual pathway that was otherwise invisible to the physician. However, he did not test or elicit the direct light response which was documented in Rhazes's report later on in the 9th century. In the 7th century, Paul of Aegina noted the association of large pupil to an eye with bad vision.[5]

Renaissance of medicine began during the 16th century, when Galen's work was translated into Latin and published by Aldine Press in 1525.[5] The Swiss barber-surgeon Pierro Franco (1504 – 1578) was then a cataract coucher. Like Galen he had certain criteria in assessing the potential outcome of his surgery: the colour of the cataract (pearly white meant good prognosis), the degree of visual loss (the more severe it was the better the prognosis), and the pupillary mobility (it should be normal for a good surgical outcome).[5;6] Similarly, Felix Platter (born in 1536) and Ambroise Paré (a French barber)[6] used pupil movement as a sign of visual potential. They closed both of the patient's eyes and pressed and massaged the eye balls before opening to check the pupil light response. Not knowingly, the practice would have been good enough to check the globe and gave some dark adaptation before the pupil light response was tested.[5] Since then for the next 300 years, doctors were taught to look at the pupillary movement as a direct light response before cataract surgery.[7]

The pupil signs were better described and studied only at the turn of the 18th century. Charles de Saint-Yves (1667-1733) wrote a book, *New Treatise on the Diseases of Eyes*, (1722) and commented that without talking to the patient about the visual problem he had been able to make a fairly good estimation of the quality of the patient's vision based only in his examination of the pupil movements.[5;8]Al brecht von Haller, (1743-1753), believed that iris was a tissue which was "sensible" or "irritable" to stimulus, and contracted upon slight touch but on violent touch it contracted only little. He thought that the capacity to contract originated from the iris muscle fibres themselves and not dependent upon the nervous system[3]. Not long after Haller's theories were declared, Robert Whytt (1714-1766), professor of theory of medicine from Edinburgh, demonstrated the presence of the afferent and efferent pathways involved in the pupil light movement and described a direct, a consensual and an accommodative pupillary responses.[9] His astounding work concerned the neuronal mechanism of the peripheral and central neuraxis in the animals and human. He demonstrated the unconscious reflexes, and the pupil dilation, brought upon by putting pressure on the optic thalamus. He called this phenomenon of reflex arc "*sympathy*".[9] Whytt had proved to his colleague that the pupillary response was not due to a direct effect of light on the uvea or pupillary muscles as Haller had suggested or to the flow of vital spirit as Galen had speculated, but involved light acting on the retina, leading to an activation of the

neuraxis through the optic nerve and pupillary constriction via the activity of the nerves to the pupillary muscles. *Whytt's reflex*, along with "*dropsy of the brain*" what it is known today as tuberous meningitis were the remarkable breakthroughs of the medical literature of the late 18th and the early 19th century.[10]

In the 19th century, there was more elaborate understanding and comments by the ophthalmologists regarding various pupillary signs in the patients. William Mackenzie (1791-1868) of Glasgow pointed out to his fellow ophthalmologist in his book, *The Physiology of Vision*, the mobility of pupil in the blind eye.[11]Albrecht von Graefe (1855) made a very important note in regards to assessing vision by means of the pupillary light reaction. He expressly warned against hasty dilation of pupils before pupillary signs were elicited. He stated that pupillary reactivity was only a confirmatory sign of good vision in the tested eye but could not depend on pupil reactivity of light for the diagnosis of organic visual pathology. He also understood that in cases of cortical blindness pupil reactions to and from the midbrain might be normal.[5] Francois Pourfour du Petit (1664-1741) demonstrated pupillary constriction when he cut the sympathetic nerve on the side of the neck and also showed the division of cervical sympathetic nerve in dogs.[12] Without knowing Petit's work, Edward Hare (1812-1838), a house surgeon to the Stafford County General Infirmary in England reported ipsilateral small pupil in a man with a small tumour in the inferior triangle space at the side of the neck.[12] In 1851, Claude Bernard, French physician, repeated Petit's experiment to demonstrate miosis as well as ptosis and enophthalmos in similar cases. It was much later in 1869, Johann Friedrich Horner, Swiss ophthalmologist, additionally described anhydrosis in a woman with a tumour invading sympathetic nerve in the neck. A complete clinical syndrome is now known as Horner's syndrome, Bernard-Horner's syndrome or oculosympathetic paresis.[12-14] Douglas Moray Cooper Lamb Argyll Robertson (1837-1909) Scottish Ophthalmologist from Edinburgh described the effects of spinal disease in causing miosis 1868, and the characteristics of the pupillary reaction in patients with neuro-syphilis as the pupil responds only to accommodation but not to the light. This is later on known as *Argyll Robertson pupils.*[15] Julius Hirschberg (1884) published a case report in German of a 17-year old girl with unilateral visual loss and un-reactive pupil and he had managed to exclude the non-organic causes and diagnosed her as having neuritis retrobulbaris.[5;16] Many other ophthalmologists spoke of the importance of careful pupil

examination in different languages in cases of suspected nonorganic visual causes and in compensation cases.

It can be noted that in the 19th century, ophthalmologists did not suggest very different clinical practice in assessing the pupil light reaction than Ambroise Paré and his contemporaries had done in the 16th century which is to look for the direct light response. Whytt and his colleagues emphasised on testing consensual pupillary response only in the late 19th century. This is probably because when the ophthalmoscope was introduced to the ophthalmologist in 1851 and when anticholinergic medicines were at hand, the wonderful view of the fundus became the primary examination when the pupillary examination was of less interest.[5] The other important reason was that the details of the anatomy and physiology of the pupil light reflex was not well understood at the time. Pupillary signs are not always straight forward signs for the underlying pathology it must have been difficult to incorporate the pupil test routinely then to face with puzzling results. For example, a patient with considerably good vision may have immobile pupil due to anterior segment pathology, and on the other hand a patient with poor vision due to central macular degeneration, deep suppression amblyopia or a non-organic vision loss may have perfectly mobile pupil. In an attempt to explain these perplexing findings which were in contrary to the rules that vision and the pupil responses should go hand in hand, many speculations arose. Some explained that pupillary afferent fibres and the visual afferent fibres were different and responded differently to the injury and disease, some that pupillary fibres were thicker and more resistant to injury, while others that they were in separate fascicles and followed different paths.[17]

During the late 19th century and early 20th century, a breakthrough in the ophthalmic literature was made by a London ophthalmologist and cataract surgeon, Robert Marcus Gunn (1850-1909). He made a remarkable notion about the pupillary response in sustained stimulus. He observed the inability of the eyes with the optic nerve lesions to maintain contraction as much or as long as the fellow good eyes. He then emphasised the importance of this pupillary sign in making a diagnosis of nonorganic visual loss[18] and presented his findings at the British Medical Association meeting in 1902 which was published in Ophthalmic Review in 1904:

"*It is not sufficient to find that it (the pupil of the affected eye) contracts well or fairly well on exposure, the eye must be kept under stimulations of light and the pupil watched as to whether it shows that secondary dilation under continued exposure that is found associated with the amblyopia of retro-ocular neuritis."*[19]

Gunn also noted that this phenomenon of what is now known as *pupillary escape* was evident in both eyes when the affected eye was stimulated, whereas when the good eye was stimulated both pupils remained contracted for the same stimulus and duration. In his later writings there were statements about paradoxical dilation of pupil when the light was shown to the affected eye.[20] Gunn was the first to ever demonstrate the "*pupillary escape*" phenomenon as a sign of inequality in afferent inputs from the two eyes in the ophthalmic literature. There was also evidence that he was performing a form of alternate stimulus testing. Gunn was stimulating one eye at a time with a sustained light, however, the duration, the intensity, the nature of light source, and the timing of light stimulation of either eye were not described.

A neuro-ophthalmologist, Alfred Kestenbaum (1946)[21] modified the Marcus Gunn pupillary sign to demonstrate the asymmetry of pupillomotor input and named it as "***modified Marcus Gunn pupillary sign***". He simply covered one eye when patient was in diffuse bright light and measured the final pupil diameter using a small pupil gauge or a ruler and repeated the measurement on the other eye when the first eye was covered, the wider one signifying the eye with retro-bulbar optic neuritis. This asymmetry of pupil diameter on light exposure was described as "**pseudo-anisocoria sign.**" To quantify the differences in pupil constrictions he subtracted the two pupil diameters, and named them "***Kestenbaum's pupil numbers***" described in millimetres. He was the first to offer a simple way to quantify the differences in afferent pupillomotor input of the two eyes.[21] However, like Marcus Gunn, the method was still unstructured and the lighting conditions were not standardised. The comparison at two different times also means the method is subject to physiological variability, such as hippus and supra-nuclear influences.

Later on in 1959, Paul Levatin[22] made a significant contribution in structuring the Marcus Gunn and Kestenbaum methods of assessing the afferent asymmetry. Instead of

covering and uncovering the eye, he simply used a *stimulus light* (a pocket flash light) in the environment where the lights were dimmed. He also emphasized that the patients should fix their gaze on a spot on the opposite wall to minimise the effect of accommodation. In his method, the light is quickly moved from one eye to the other in alternating manner while the examiner observes the movement of the pupil in the eye that is illuminated. Levatin was trying to appreciate the net result of the afferent input of the two eyes.[23] If the driving force for the pupillary constriction is not as strong as the driving force for the pupil dilation in the second eye (by removing the light back to the first eye), then the net result will be the dilation of the second pupil, "pupillary escape." This modification eliminates most of the problems encountered with Kestenbaum's method. It makes the comparison of the two pupils by the observer easier by simply looking at the net results of the two instead of measuring the individual responses and comparing them. Also, observing the pupillary movement is in effect easier than quantifying the pupil diameter. Levatin called the test a "**swinging flash light test.**" Both Marcus Gunn and Levatin looked at the pupillary escape while Kestenbaum measured direct light responses. The alternating test was indeed found to be more sensitive and specific than Marcus Gus test in detecting the unilateral optic neuropathies.[24]

One of the landmarks during the 20th century medical physics was the introduction of the pupillographic records of the pupil reaction to light. Otto Lowenstein[25] (1885-1965), a German psychiatrist and a pioneer in the pupil study, was one of the lucky scientists to escape Nazi persecution and migrate to America in 1939. He continued his work at the New York University and Columbia Presbyterian University. In 1957, with Irene Loewenfeld, he built the electronic pupillometer and used the infrared technology to accurately measure and analyse the pupil diameter and the pupil motion with different stimuli. His studies concerned the pupil behaviour during specific emotional and psychological states, and during the period of fatigues and alertness from the point of neuro-psychiatrist. He conducted many studies of the central autonomic innervations of the pupil reflex to light using pupillography.[25] Irene Loewenfeld[26] was the physiologist of the pupil, whose interest in pupil began in 1940 when she went to work as a technician in the pupillography laboratory of Professor Otto Lowenstein at New York University. She had a devoted, long and vigorous professional life to the research, the

study and understanding of the workings of the pupil and conducted many animal studies including birds, cats, dogs, and monkeys. From these scientific studies using her pupillometer, she had lifted our understanding of physiology of the pupil light response to a next new level. Among her many contributions, Loewenfeld provided rigorous observations about Adie tonic pupil, anisocoria in optic tract lesions, Argyll Robertson pupil, oculomotor paresis with cyclic spasms, and innovations in electronic recordings of pupil movement, pupillographic behaviour in the optic nerve lesion and many other pharmacological pupillographic studies.[26] One of their new findings was the characteristics in the recordings of the pupil light response in the eyes with optic nerve diseases. Lowenstein and Loewenfeld noted that in a patient with unilateral optic nerve disease, the light response of the pupil of the eye with the optic nerve disease is smaller and slower than that of the fellow eye for the same light stimulus. This was described as a *low intensity light reflex*, a reflex characteristic very similar to that of the eye which receives a dimmer stimulus light. These findings shed more light on the clinical test of the optic nerve function and led Kenstenbaum[21] and Levatin to objectively measure the differences in the pupillary reaction to light knowing that the pupil of the diseased eye would respond less than the normal eye. Loewenfeld and Lowenstein together published a larger volume of pupil literature based on their innovative pupillometer which is indeed the forerunner of the recent more complicated pupillometers of the 21st century.

Many pupillometers have been devised since Lowenstein and Loewenfeld's first invention. Most of the pupillometers, however, are research-based and not readily available. The commercially available pupillometers have been used by ophthalmologists but mainly for refractive surgery which require the precise measurement of the pupil size in the light and the dark conditions. With recent advances in technology, increasing awareness of the importance of pupillary signs in the diagnosis of numerous neuro-ophthalmic diseases and glaucoma, as well as increasing use of pupillometer to diagnose sleep and behaviour disorders, neurological lesions, and psychological dysfunctions, there has been a significant amount of new research optimising the pupillometric test paradigms tailoring to different needs.

Chapter 2

Anatomy and Physiology of Pupil Light Reflex Pathway

2.1 PUPIL LIGHT REFLEX CIRCUITRY

2.1.1 Pupil

The pupil is the opening guarded by the two opposing iris muscles, sphincter pupillae and dilator pupillae. It is approximately circular, slightly eccentric towards the nasal side. The average apparent diameter varies from 2.5 to 4 mm. Due to the refraction of the cornea surfaces the image of the pupil is magnified by 1/8 the actual diameter. Pupil size is smaller in infants and elderly than young adults or middle-age individuals. The size of the pupil is also physiologically determined by the level of consciousness (governed by the autonomic nervous system), ambient lights and the accommodation. Several drugs absorbed locally or systematically can also influence the pupil size.

Spasmodic, rhythmic, but irregular contraction and dilation movement of the pupil at all times is described as *hippus*. This dynamicity is attributed to the central influence, hence the contractions of the left and right pupils are synchronised. The hippus increases in light and decreases in darkness. For alert individuals, the diameter of hippus modulates by less than 0.5 mm in light and 0.1 mm in darkness. Pupil shape can be distorted and movement can be disturbed by local iris disease and also in tonic pupils.

***Function*:** Pupil governs the amount of light that reaches the retina.

2.1.2 Pupil Light Reflex

Pupillary light reflex or pupil light response, ***PLR***, refers to pupillary constriction to light whereas the dilation to dim or dark stimuli is the pupil dark response. Reflex movement of the pupil to light allows for visual homeostasis. Reflex constriction of pupil upon receiving light moderates the amount of light that falls on the retina, preventing over-bleaching of the photoreceptors. The amount of light reduction by pupillary constriction alone is limited to about 1.5 log units of brightness. Although this represents a small fraction (12%) of the 12 log units[27;28] range of the light-sensitivity of the retina, it plays an important and immediate contribution to the early stage of light adaptation.[29] In addition, pupil constriction can also improve the image quality by

increasing depth perception.[29] In dim light, a larger pupil allows more photons of light to fall onto retina maximising visual sensitivity.

The pupillary reactions to various conditions and stimuli have been observed over decades, however, the exact anatomical pathway of the pupil light response is yet to be understood. The anatomy and the physiology involved are complex and include various neuronal pathways incorporating the autonomic nervous system that governs the background pupillary tone. Recent observations in the involvement of a group of retinal ganglion cells participating in the pupil reflex response to light have added new lines to the pupil literature.

Figure, 2.1 outlines the *classical* anatomical pathway that underlines pupillary reaction to light. Grossly, the **afferent limb** of the pupillary light reflex begins with the photo-receptors which lie in the retinal layers. The axons of the retinal ganglion cells, organise themselves in the optic nerve. Approximately, 53% of the fibres that serve the nasal retina cross the midline at the optic chiasm.[30-32] The optic tract, therefore, comprises approximately 47% of the fibres from the ipsilateral eye and 53% of the fibres from the fellow eye.[29-32] At the posterior third of the tract the fibres that subserve the pupillary response leave the tract, run along the medial border of the lateral geniculate body and enter the brachium of superior colliculus (SC) before travelling rostral and medial to the pretectal region or tecto-thalamic junction of the upper midbrain. The axons from the pretectal nuclei (main nucleus, Olivary pretectal nucleus), cross the posterior commissure in proximity to central grey matter, and travel around the aqueduct to reach both Edinger-Westphal nuclei (EWN). The axons of the EWN run along with the right and left oculomotor nerve. The **efferent limb** is via the oculomotor nerve. The parasympathetic neurons of the oculomotor nerve synapse in the ciliary ganglion. Post-ganglionic short ciliary nerves leave the ciliary ganglion to synapse at the constrictor muscle of the iris. As we shall see later, there are separate retinohypothalamic pathways from the intrinsically photosensitive retinal ganglion cells, melanopsin associated biologic regulations, the higher centre controls (cortico-thalamo-hypothalamic influences, visual cortex - area 18 and 19 - influences, psycho-sensory influences, and tonic inhibitory inputs from the cerebral cortex to the EWN) as well as the sympathetic system, all contribute to the pupil size, movement, and reaction at any point of time.

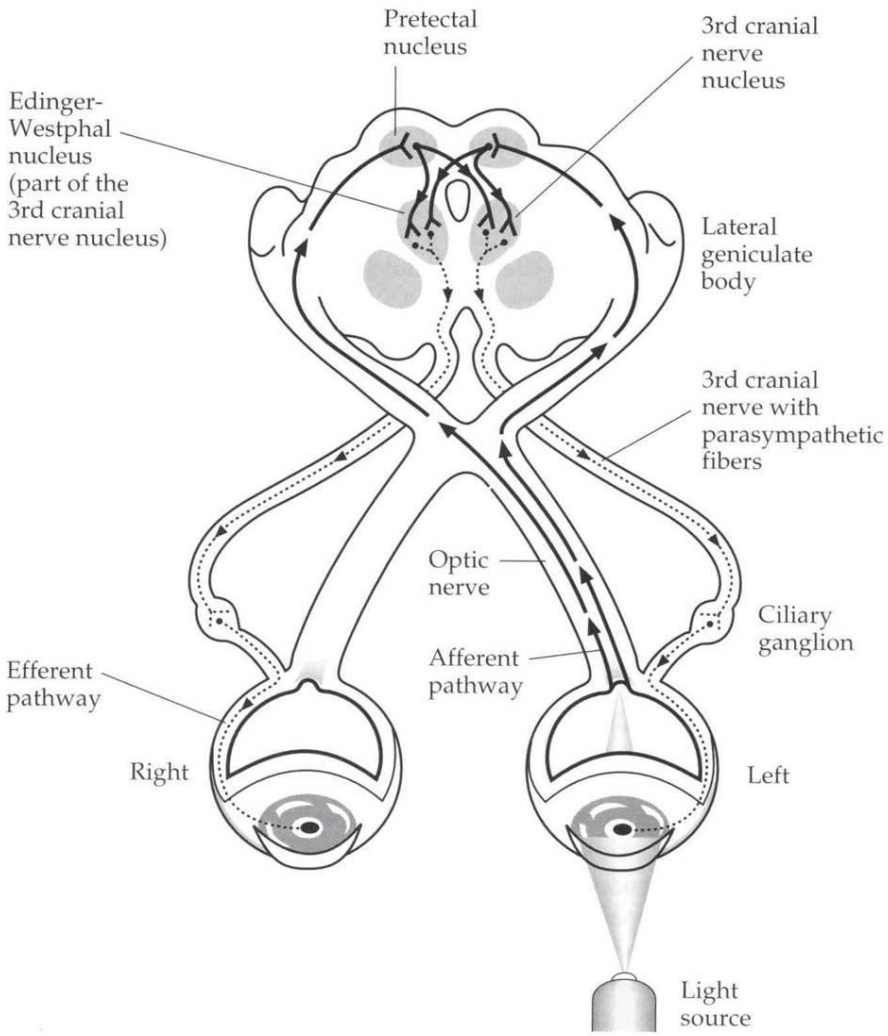


Figure 2.1. Pupil light reflex pathway. Diagram adapted from Gray's Anatomy of the Human Body, 2000.[33]

2.2 AFFERENT PATHWAYS

2.2.1 Photoreceptors

Rods and cones

Rods and cones, the visual cells, are the neuro-epithelium or receptive elements of the retina, figure 2.2. They contain photosensitive substances, and are capable of transforming physical energy into nerve impulses which are transmitted through synapses to the bipolar cells in the external nuclear zone. Melanopsin is now considered the fifth human retinal photo pigment, the other 4 being 3 opsins from cones and 1 from rods.

The approximate ratio of cone to rod is 1:20.[34]The cells are arranged in palisade fashion across the external limiting membrane. Both rods and cones have inner and outer segments, figure 2.2. The outer segment contains membranous discs of photosensitive pigments (rhodopsin for rod and 3 types of iodopsins for cones) and lie close to the RPE cells. The inner segments contain cellular nucleus and give rise to synaptic terminals to the bipolar or horizontal cells. [35]

The density distribution of rods and cones is different. At the fovea, there are no rods but there are about 147,300 cones per mm^2. The density of cones reduces as they come away from the fovea. Fifty percent of the cones are located within the central 30 degree of the visual field.[34]The total number of rods is about 92 to 125 million and cones 4.6 to 6.8 million per human retina.[34;36;37]

Rods and Cones: Visual functions

The basic visual functional differences between rods and cones are tabulated in table, 2.1. The rod pathway produces images with lower spatial resolution than the cone pathway. Therefore rods are associated with poorer visual acuity whereas cones give good visual acuity because they are able to perceive finer details and more rapid changes in image. However, the light sensitivity of rods is better than that of cones. This accounts for their activation in the scotopic vision. In addition to their contribution to good acuity, cones also give good colour discrimination due to their possession of 3 different types of photopsins for different wavelengths of light,[38]figure 2.3.

The range of illuminations over which the rods and cones work is described in figure 2.4. In *mesopic* conditions, both rods and cones are activated. At very low light level (<-4 log cd/m^{-2} of luminous levels), only rods are activated.[40] Under very bright light levels *(photopic* conditions), all the membrane channels of rod close with the resultant stabilisation of the membrane potential and therefore no photo-transduction takes place for rods. In photopic light level, only cones contribute to the vision.[35]

Rods and cones: Non-Visual functions

The entry of light into the visual system is governed by the pupil size. In addition to their contribution to vision, rods and cones take part in the pupillary reaction to light,

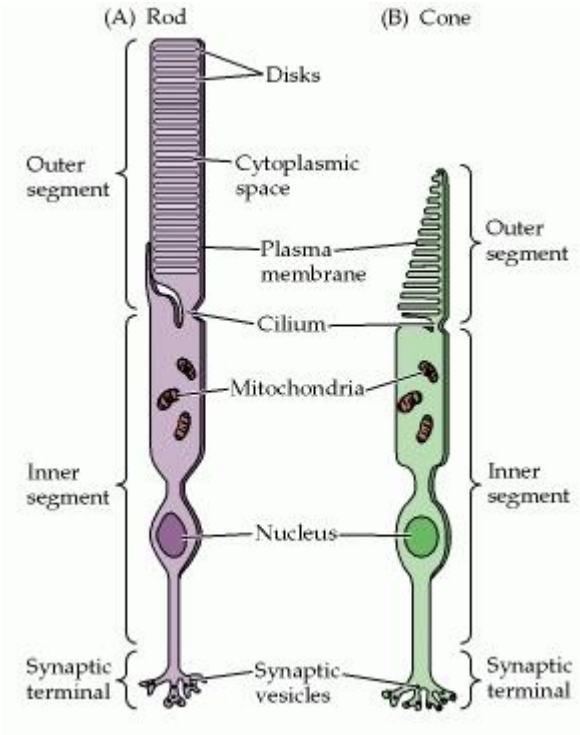


Figure 2.2. Structural differences between rod and cone. Adapted from Neuroscience, 2001.[35]

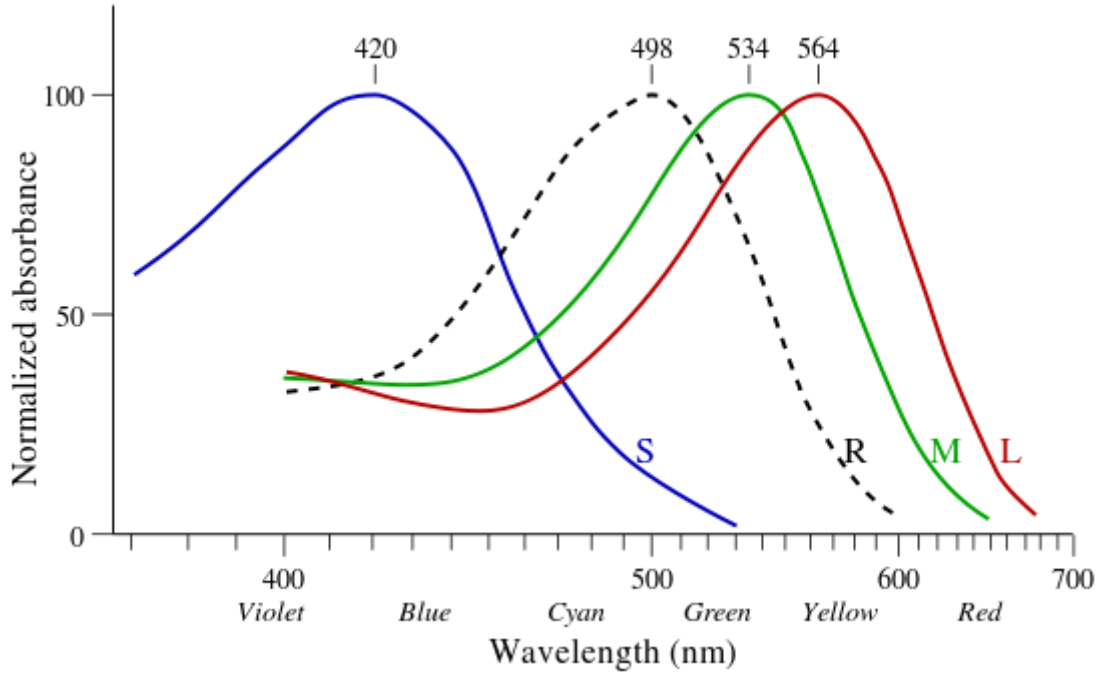


Figure 2.3. Wavelength responsiveness of rods compared to that of 3 types of cones. R, rod; S, short-wavelength cone; M, median-wavelength cone; L, long-wavelength cone. Adapted from Bowmaker et al. 1980.[39]

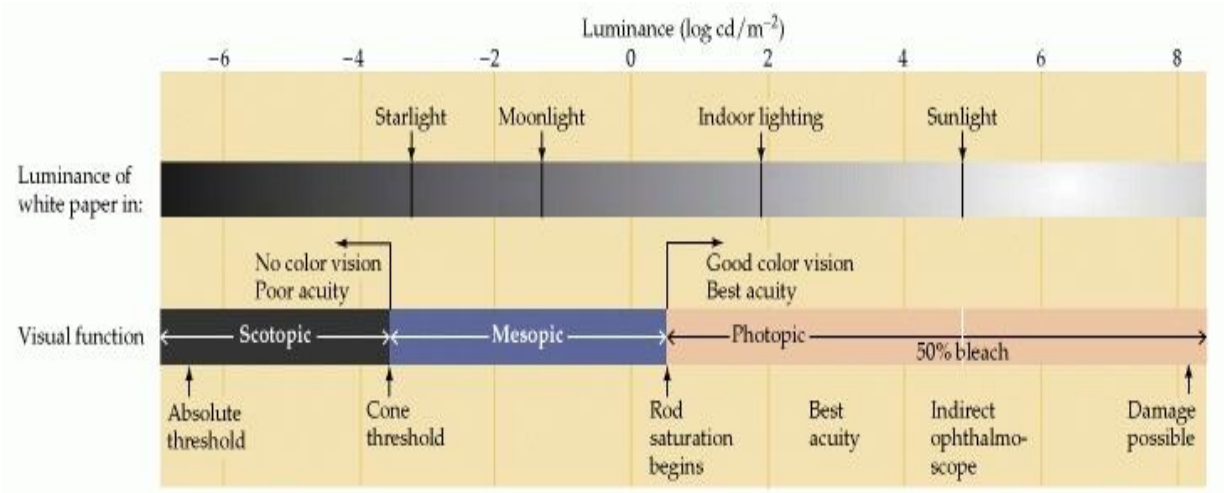


Figure 2.4. Working range of illuminations of rods and cones. Adapted from Neuroscience.[35]

table 2.1.The pupil physiologist, Loewenfeld,[41] in 1999, described rods and cones as the initial receptors in the afferent limb of the pupil light reaction just like they are for the vision.[41] The author stated that for almost all modifications and measurements of stimulus conditions that produce a difference in visual perception, the pupillary response to light parallel to those of visual perception.[29;41] These include change in retinal dark/light adaptation, wavelength, intensity, size, and duration of stimulus light. For example, the wavelength sensitivity of pupil threshold as the stimulus light changes from blue to red, exactly parallels the same wavelength sensitivity of visual perception.[29] Likewise, for Purkinje shift (change from light adaptation to dark adaptation), the shift in sensitivity for the vision threshold and the pupil thresholds are also proportional.[29;41]

Visual Functions	**Rods**	**Cones**
Spatial resolution	low	high
Visual acuity	less acuity perception	good acuity function
Light sensitivity	very sensitive	relatively insensitive
Colour perception	-	good
Very low light level	activated	inactivated
Scotopic vision	only rod-mediated	-
Star light level	activated	beginning of activation
Normal room light	less distribution from rod	major contributor of vision
Photopic vision	-	only cone-mediated

Pupillary functions	Rods	Cones
Pupillary threshold sensitivity	low	high
Pupillary amplitude reaction	small at the suprathreshold level	large at the suprathreshold level
Scotopic condition	activated	-
Mesopic condition	activated	activated
Photopic condition	-	activated

Table 2.1. Functional differences of rods and cones

Retinal ganglion cells (RGC)

Retinal ganglion cell is a type of neurone which receives information from photoreceptor via bipolar cells and amacrine cells and transmits it to the brain via its axon. Retinal ganglion cells vary in size of the cell bodies, shape, axonal thickness, dendritic connections, as well as physiological responses to various visual stimulations. Consequently their different sensitivity thresholds, discharge patterns, latent periods and conduction speed render them selectively suited to respond to particular kinds of light stimuli.[41] RGCs have long axons that form optic nerve, optic chiasm and optic tract. Their central process passes to the thalamus (lateral geniculate body) for relay by the third sensory neuron to the cortex while others synapse at the pretectal area.

There are about 1.5 million RGCs in the human retina.[42] Retinal ganglion cells are densely populated within the central area of the retina. Cone and ganglion cell numbers are not correlated (cone: RGC range from 1:2.9 to 1:7.5 in different eyes)[43].[42] A lower convergence of cones into a ganglion cells means a higher resolution at a later stage of neuronal processing. Within the central area, 0.4-2.0 mm from the foveal centre, ganglion cell densities reach 32,000-38,000 cells/mm^2.[42] In the peripheral retina, densities in nasal retina exceed those at corresponding eccentricities in temporal retina by more than 300%; superior exceeds inferior by 60%.[42]

Various types of retinal ganglion cells have been described in the literature some of which were identified from animal studies. The generally recognised and accepted groups to date are:

(1) Parasol cell (M cells aka Alpha (α) cells, aka Y cells[41]) – projects to magnocellular layers of LGN **(M** pathway)

(2) Midget cell (P cells, aka Beta (β) cells, aka X cells[41]) - project to parvocellular layers of LGN **P** pathway)
(3) Bistratified cell (**K** pathway) – projects to koniocellular (as small as dust) layers of LGN
(4) Intrinsically photosensitive Retinal Ganglion Cells (**ipRGC**) – recent finding
(5) Other ganglion cells projecting to the superior colliculus for eye movements (saccades)

Among other functions, M cells are known to contribute to pupillary light reflex.[41] P cells also take a minor role in transferring pupillomotor signal to the pupillomotor centre.[41] Recent findings suggest that the newly identified melanopsin containing intrinsically photosensitive retinal ganglion cells (ipRGCs) are mainly responsible among other ganglion cells in receiving and transferring pupillary signals. These ganglion cells contain photosensitive melanopsin.

One interesting thing also of note is that, this group of cells are not entirely alien to the neuro-ophthalmologists. There were **γ** cells that were already identified in addition to α and β cells described above. The γ cells were also named as **W** cells.[41] In 1999 Loewenfeld stated that "The oldest group W or gamma cells form about 40% of the total population of the retinal ganglion cells, they are located in all areas of the retina and respond both physically and tonically to relatively slow changes in light intensity. They project exclusively to the midbrain and carry visual as well as oculomotor impulses in lower species. In higher species they transmit the bulk of pupillary afferent stimuli to the pretectum and messages for reflex eye movements to the colliculi."[41] The main afferent pupillary input, therefore, was thought to come from the W cells and some from X and Y cells. However, their possession of melanopsin, and their ability to phototransduct without rods and cones were not known until recently. Recent studies estimated that ipRGCs comprise 0.2% of approximately 1.5 million retinal ganglion cells in the human eye.[44] It is possible that perhaps a small proportion of what was described by Loewenfeld represents those now termed ipRGC. Photosensitivity allows depolarisation to light stimulation in the absence of synaptic input from rods and cones and therefore, ipRGC cells act as independent photoreceptor.

Intrinsically photosensitive RGCs have the largest dendrites and dendritic field among all known RGCs. The cell bodies of ipRGCs are relatively small and thin despite their large dendritic field, and their axons are slowly conducting.[29] They respond primarily to incremental changes in the light intensity but are relatively insensitive to movement[29] unlike other GCs.

The diameter of the dendritic fields is compared with that of Midget, small Bi-stratified and parasol ganglion cells in the table below. Their diameter increases with increase in the distance from the fovea. IpRGCs are absent in the fovea, however dendrites encircle the fovea pit, table 2.2.

Dendritic field	ipRGC	Midget	Parasol	Bistratified
Diameter	350- 1200 μm[45]	4-180 μm[46]	20-400 μm[46]	30- 400 μm[47]

Table 2.2. Dendritic fields of the RGCs.

The spectral sensitivity curve for the ipRGC falls between S-cone and Rod. The peak spectral sensitivity for the S-cone, ipRGC, rod, M-cone and L-cone were 440 nm, 482 nm, 507 nm, 543 nm and 566 nm respectively, figure 2.5.

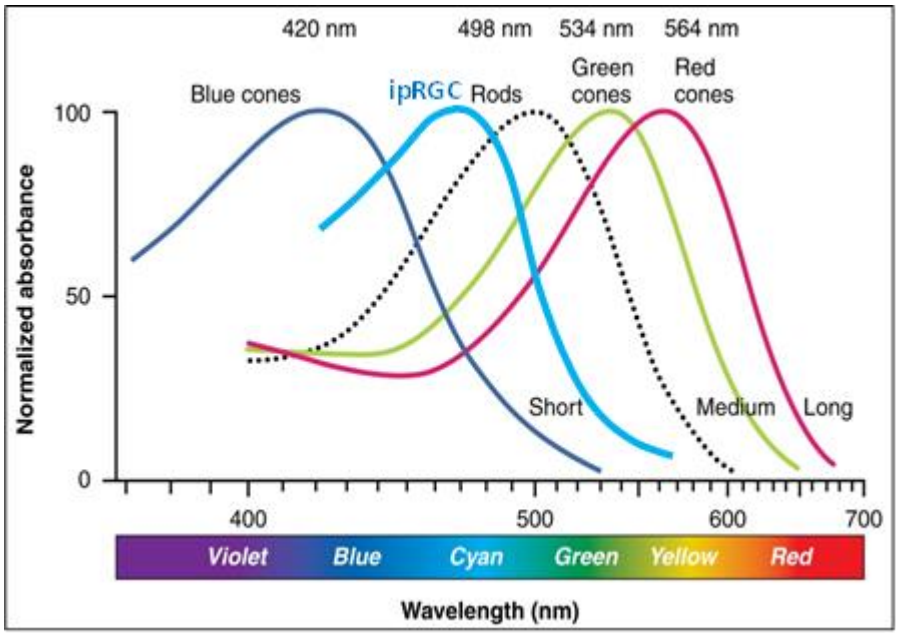


Figure 2.5. Spectrum sensitivities of photoreceptors. Melanopsin's spectral sensitivity peaks at about 482 nm (465 to 485 nm blue light bandwidth).

Intrinsically photosensitive RCGs also receive input from rod and cone photoreceptors. The projections of ipRGC, figure 2.6, include:

(a) 70-95%[48;49] of projections to supra-chiasmic nucleus (SCN) via the retino-hypothalamic tract for setting and maintaining circadian rhythms[50],

(b) projections to other central sites that also modulate the SCN including inter-geniculate division of the lateral geniculate nucleus (phase shifting of circadian rhythm),[34] the ventral supraventricular zone of the hypothalamus, and the ventro-lateral preoptic nucleus.[51]

(c) pre-tectal nuclei: to the Edinger-Westphal nucleus (EW) and to the Olivary pretectal nucleus forming the afferent limb of the pupillary light reflex.[52]

(d) projection to LGN: some evidence in mice[53;54] and primates.[44]

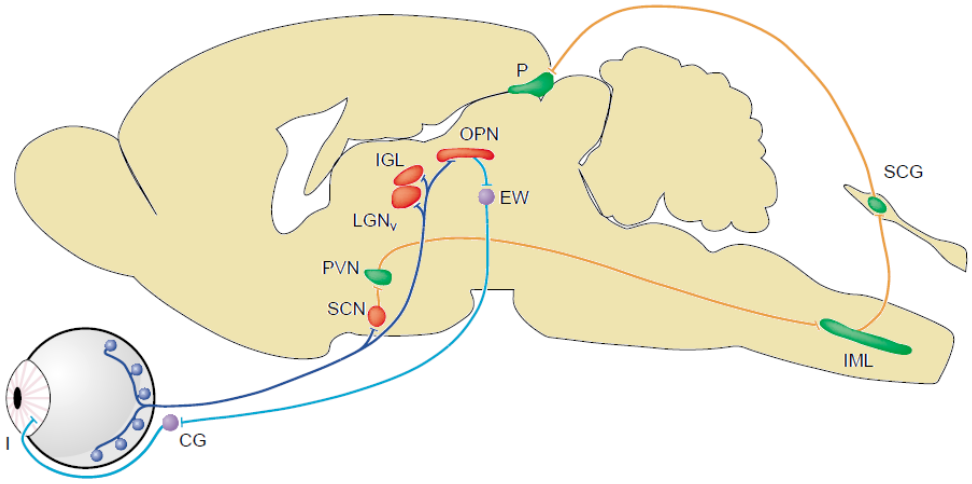


Figure 2.6. Projections of ipRGCs. Diagram adapted and modified from Berson et al. 2003.[50] IpRGCs and axons are in dark blue, their principal targets are in red. Connections to SCN (Supra Chiasmatic nucleus), two divisions of LGN (Lateral Geniculate Nucleus): LGN$_v$ (ventral division of LGN) and IGL (Inter Geniculate leaflet) shown and OPN (Olivary Pretectal Nucleus). Orange pathway with green nuclei shows the polysynaptic circuit that regulates melatonin release, PVN (ParaVentricular Nucleus), IML (Intermedio Lateral Nucleus), SCG (Cervical Sympathetic Ganglion), P(Pineal gland). The blue circuit with purple nuclei represent the pupil light reflex from OPN and Edinger-Westphal Nuclei (EW) to Ciliary Ganglion (CG) and the target organ, Iris (I).

Functions of RGCs

Retinal ganglion cells encode spatial and temporal information before projecting to the visual cortex via the LGN.[55] The non-image forming ipRGC connect hypothalamus via retinohypothalamic tracts and contribute to circadian rhythm, pupillary light reflex and

the resizing of the pupil. The functions of ipRGCs currently described in the literature are summarised below.

(1) Irradiance detection: detection of environmental brightness at subconscious level, providing primary input of environmental light to the Supra-Chiasmatic Nucleus (SCN). This function permits the adjustment of biological rhythm to the solar day[56] and circadian photo-entrainment.

(2) Melatonin secretion by the pineal gland.[52;56;57]

(3) Light induced activity of locomotor activity in rodents (negative masking).[58]

(4) Photoreceptor: photosensitivity allowing depolarisation to light stimulation in the absence of synaptic input from rods and cones. Therefore, ipRGC cells act as independent photoreceptor.

(5) Visual input: IpRGCs project to the visual cortex via the LGN[55;59] Researchers have concluded so far that the melanopsin-expressing RGCs may contribute to conscious visual perception; however, they do not appear to have the functional properties for the direct image formation like those of rods and cones.[60]

(6) Light dark adaptation: Intrinsically photosensitive retinal ganglion cells display both light and dark adaptation. The response amplitude and latency vary with prior adaptation level.[61;62]Light adaptation reduces[61] and dark adaptation increases the intrinsic sensitivity in rats.[62]

(7) Pupillary light reflex: maintenance of pupil diameter, recovery, post- illumination pupil constriction of PLR.[61;63] The ipRGCs have slow onset and sustained depolarisation that is maintained for up to 30 seconds[44] after the light is withdrawn. This character unique to ipRGC is termed post-illumination pupillary response (PIPR) or sustained pupillary response[63].

Table 2.3 summarises the basic characters of ipRGCs, rod and cone photoreceptor cells.

Photoreceptors	**ipRGCs**	**Rods**	**L,M,S Cones**
Location	inner retina	outer retina	outer retina
Number in retina	s1000-3000[64;65] (~ 1%, Kardon et al)[29]	~92 million[64-66]	~ 4.6 million[64;65]
Peak cell density	20-25 cells/mm^2 at 2º eccentricity[44]	176,200 cells cells/mm^2 at 21º eccentricity.[66]	~ 150-200,000 cells cells/mm^2 at fovea[66] (L, M cones) 2600 cells/mm^2 at 0.6º eccentricity (S-cones)[67]

Cell bodies location	40% INL, 60% GCL[44;64]	ONL [64]	ONL[64]
Dendrite stratification	extreme outer and inner IPL[44;65]	OPL[65]	OPL[65]
Receptive field	very large, photo receptive net[64]	very small[64]	very small[64]
Input	intrinsically photosensitive,[44] rod and cone input[65; 44]	intrinsically photosensitive[65]	intrinsically photosensitive[65]
Peak λ sensitivity	broad band, most sensitive to 482 nm λ[44;64]	most sensitive to 507 nm λ[64;68]	all visible wavelengths, most sensitive to 440, 543, 566 nm λ[64;69]
Photo-pigments	melanopsin[64;70]	rodopsin[64;65]	cyanolabe[71] chlorolabe[72] erythrolabe[72]
Synapses	DB6 bipolar cells amacrine cells[65]	rod-cone gap junctions.[65;73] rod ON bipolar cells[74]	cone midget, parasol and bi-stratified bipolar cells[65] horizontal cells[75]
Properties	temporal integration of ambient light[64]	adaptation fine spatial resolution[64]	adaptation, fine spatial resolution,[64] S cone – spatial acuity[67]
Function	circadian clock, pupillary light reflex[64]	image formation, pupillary light reflex[64]	image formation, pupillary light reflex[64]

Table 2.3. The ipRGCs and rod and cone photoreceptors, INL = inner nuclear layer, GCL = ganglion cell layer, ONL = outer nuclear layer, OPL = outer plexiform layer, IPL = inner plexiform layer.

2.2.1.1 Photoreceptor contributions to the pupil light response

IpRGCs complement rods and cones to drive the full range of mammalian vision, operating at high light levels. A single photo-response of ipRGC is larger than rod and cone photoreceptors.[76] Their intrinsic photosensitivity operates over a long duration of time[77] lasting nearly 10 s (or ~20 fold longer than rods and 100 fold longer than cones).[78] However, they are less sensitive than cones.[54;79;80] This is associated with much less photo pigment melanopsin per cell membrane surface area, ~ 3 molecules[76] μm,$^{-2}$ in contrast to rods and cones which express photo pigment density of ~25,000 molecules μm^{-2}. Low melanopsin expression somewhat prevents interference with photo capture by rods and cones which give quicker response to light. In response to light, rods and cones undergo rapid and transient depolarisation, with subsequent bleaching and adaptation on continued exposure to light. The IpRGC, however, shows

slow-onset depolarisation, that is evident even when it is detached from the retina.[44;81] Because the action potential is large and long lasting it sustains for a period of time and hovers around the threshold, it may fire even after the stimulus light is removed.[77] This action is described as post-illumination pupil response.

While cone reactions are transient and continue with adaptation, and rods are deactivated in the bright light, it is the ipRGCs in the retina that maintain the pupil in the sustained constricted tonic position.

Pupil constriction response to a longer duration (5-10 seconds) of *bright* white light in a normal human can be described as having two components: transient state and sustained state of the constriction for the duration of stimulus.[64] At the light onset, there is a rapid-onset high velocity pupil constriction (latency of which depends on the stimulus intensity and the pre-adapted state and often is often very short for a high intensity stimulus) until it reaches the maximum constriction amplitude. This is rapidly followed by the pupillary redilation (escape) and to a more sustained state of pupil constriction that continues for the remainder of the light stimulus, figure 2.7.[64] The early transient constriction under photopic condition represents predominantly cone driven response and the sustained pupil constriction represents a summation of the adapted cone response as well as the steady-state intrinsic retinal ganglion cell activation.[64;82] The pupil continues to contract upon the offset of light for a duration of time. This post-illumination pupil response (PIPR) is predominantly contributed by the ipRGCs as described above.[82] The sustained reaction of both cone and ipRGC is accentuated by the intensity of light,[64] figure 2.8.

It is explained above the contribution of cone and ipRGC to the pupil light reflex brought about by a bright stimulus. The rods also contribute to the pupillary reactions but in the scotopic and mesopic settings when the stimulus light exceeds rod threshold. The pupil can be set to motion by the stimulation of rods alone especially when large stimulus fields are used and these reflexes are thought to be extremely sensitive.[83] However, rods are not so efficient in generating pupillomotor impulses as compared to cones, and require cones input to produce optimal reflexes. The pupillomotor impulses

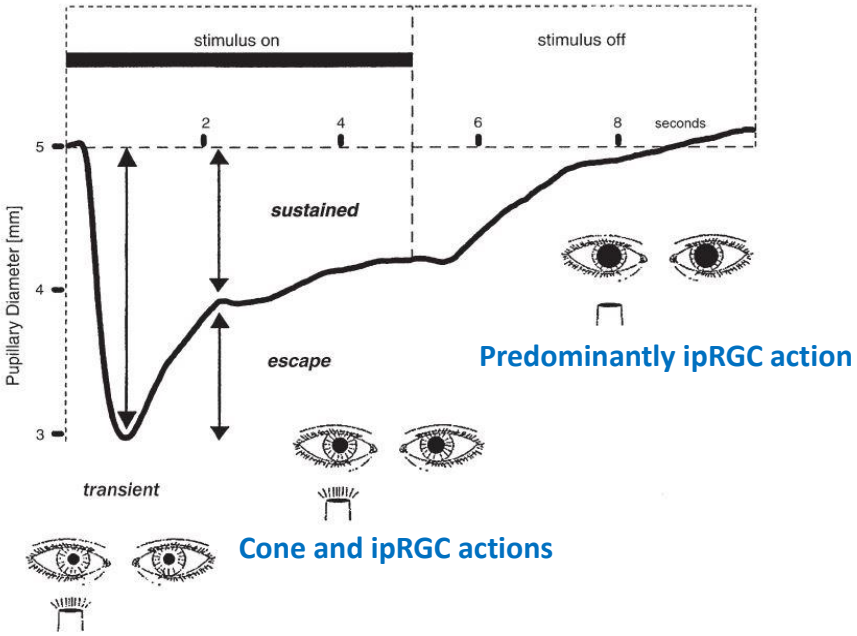


Figure 2.7. A pupillographic recording of 5 second bright white light in a normal human subject. Transient phase is characterised by a short latency, high velocity maximal changes in pupil size followed by pupil redilation (escape). A sustained phase is where pupil partially constricts within the duration of stimulus immediately after the escape. Adopted from Kawasaki and Kardon 2007.[64]

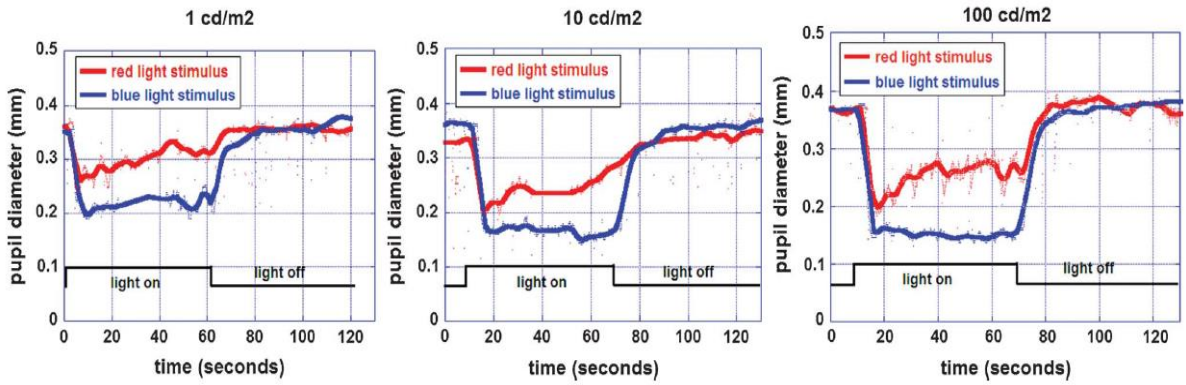


Figure 2.8. Pupillographic recordings to equiluminant chromatic red (600-620 nm) and blue (465-485nm) lights for 60 seconds using 3 sets of stimulus intensities on human subjects. Experiment of Kawasaki and Kardon 2007.[64] It can be seen that the pupillary constriction to the blue light stimulus is larger than that of red light stimulus. Reaction of blue light is contributed by the actions of both ipRGC and cone photoreceptors while that of red light is mostly contributed by red cone photoreceptors. For the stimulus duration of 60 seconds, with higher intensities, sustained reactions are more pronounced with little adaptation.[64]The blue line in the right most diagram (highest intensities) remains flat with little signs of adaptation.

generated by rods and cones are additive and together they produce a much more extensive pupillary reflexes at a given light intensity than do the cones alone in the light adapted eyes.[83] Cones, on the other hand, are effective generator of pupillomotor impulses compared to rods but they are relatively insensitive. In the light adapted eye, the pupillary threshold for cone is just above the cone visual threshold.[83] With near-threshold intensity stimuli, the pupillary reactions of cones are very similar to rods but the moment the light intensity gets brighter, more extensive reflexes are produced which can be seen in the intensity response curve as a sharp rise in the slope of the pupil constriction.[83]

Pupil light reactions are often studied with flash light of shorter duration in many experiments as it will be in this thesis. When the phasic pupillary constrictions are studied with a brief (0.4 seconds) white light stimuli, it is almost entirely a cone response. Although it is conveyed via ipRGCs, as well as RGCs, melanopsin intrinsic sensitivity plays almost no role as these ipRGCs are driven only synaptically by cone but not photically by light.

2.2.1.2 Photoreceptor contributions in light bleach and dark adaptation

Photoreceptor transduction can be modulated by changes in the light level.[10] In the process of adaptation, the body maintains working range of transduction cascades within the physiological useful region of light intensities.[84] When rods saturate quickly and become refractory, cones remain sensitive to light, maintaining the transduction so that we can see. This process of adaptation is also described as **background adaptation** and is dependent upon the amount of free calcium whose intracellular free concentration decreases with illumination.[84]

Another adaptation called **beaching adaptation or light adaptation**, occurs as the sensitivity of the photoreceptor is reduced by precedent exposure to light bright enough to bleach a substantial portion of the photo-pigments (depolarisation of photoreceptors).[84] This is perceived as a dazzle in response to bright light. The process reduces the available visual pigments in the photoreceptors as well as the quantum catch (physical quantity of signals generated in the photoreceptors), producing proportional decline in the light sensitivity. During this adaptation, cone function is favoured against

rod function. Within about a minute, the cones are sufficiently excited by the bright light to take over as visual acuity and colour vision continue to improve over the next few minutes.

The process of recovery from bleaching adaptation is called **dark adaptation**.[84] This happens in exposure to darker stimulus with precedent exposure to the brighter stimulus. The initial blackness seen from moving from bright stimulus to dark stimulus is because cones cease functioning in the dark environment. Once saturated, rods (which work in the dark environment) have a long refractory time to recover (may take up to an hour to completely recover). Cones quickly recover within a few minutes and help in the process of dark adaptation and allow us to continue to see in a quickly changing light environment.

Intrinsic sensitivity of ipRGCs also reduces with light adaptation and increases with dark adaptation suggesting its involvement in the photo adaptation process.[85]

2.2.2 Optic chiasm

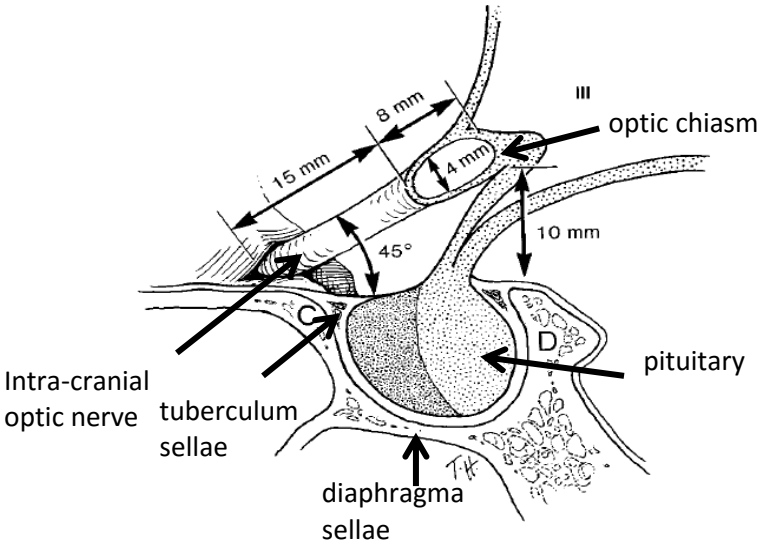


Figure 2.9. Relationship of chiasm, optic nerve and 3rd ventricle (III), C. anterior clinoid, D. dorsum sellae. Redrawn from Walsh & Hoyt. Clinical Neuro-ophthalmology[34]

The axons of the ganglion cells organise themselves into bundles of fibres, in an arcuate pattern specific to the region (papillo-macular, nasal, temporal, superior and inferior), in the nerve fibre layers before turning into the optic nerve.

The optic chiasm, figure 2.9, is a crossroad where the optic nerves of the two eyes cross and segregate before entering the cerebrum. It is about 10-20 mm in traverse diameter and 4-13 mm in anterio-posterior diameter,[34]covered, except where it attaches to the brain, by the pia and arachnoid mater.[34] It is continuous anteriorly with the CSF in the subarachnoid space and posteriorly with the third ventricle[34] as it forms the floor of the antero-inferior midline recess of the third ventricle. In the majority of adults (79 %) it lies less than 10 mm above the diaphragma sellae.[32;86]

The segregation of the optic nerve fibres in the chiasm is dependent on the retinal position of ganglion cells the axons belong to. Ganglion cell axons from the nasal retina cross to the other side whilst those of temporal retina remain ipsilateral in the chiasm. This allows for the bilateral connections that underline normal binocular vision. The proportion of crossed fibres is always larger, typically in the region of 53:47 (crossed: uncrossed).[30] This may reflect both visual and pupillary fibres. The crossed fibres from the dorsal retina project more caudally than those of the ventral fibres.[34] At the anterior aspect of the optic chiasm, the inferior nasal fibres (representing the superior-temporal visual field) travel within or close to contralateral optic nerve.[87;88]

Before reaching the LGN, pupillary fibres, leave the tract medially to synapse in the pretectal area of mesencephalon. A few of them enter the LGN,[34] the role of which is still not entirely clear. The lesions of the optic tract give rise to a homonymous hemianopia as well as contralateral relative afferent pupillary defect.[29]

2.2.3 Pupillary fibres to central processing neurones

Before reaching the lateral geniculate body, fibres that subserve the pupillary light reflex- the majority of axons of the γ ipRGC cells and some from α and β RGCs[29]- branch off the optic tract via the *brachium* of superior colliculus,[89] synapsing in the pretectal nuclei of the mesencephalon or midbrain.

As described earlier, nasal pupil fibres (left nasal, right nasal) which receive the visual information from the temporal field cross at the chiasm to join the temporal fibres (right temporal, left temporal), which receive the visual information from the nasal visual field, from the contralateral side. This means to say that, for pupillary pathway also,

ganglion cells serving homonymous portions of the same image distribute to the same pretectal nucleus, contralateral to the image, like those of the visual fibres (figure 2.10).[29] Wernicke's pupil reaction - whereby the pupils fail to constrict when hemifield stimulations are placed in the blind homonymous hemifield of patients with contralateral hemianopsia due to optic tract lesions – supports the crossing of the pupil fibres at the chiasma like that of visual fibres.[29;31;90;91] Axons serving the right side of the visual field serve the left pretectal nucleus for pupillary response and those of the left side of the visual field the right pretectal nucleus, figure 2.11.

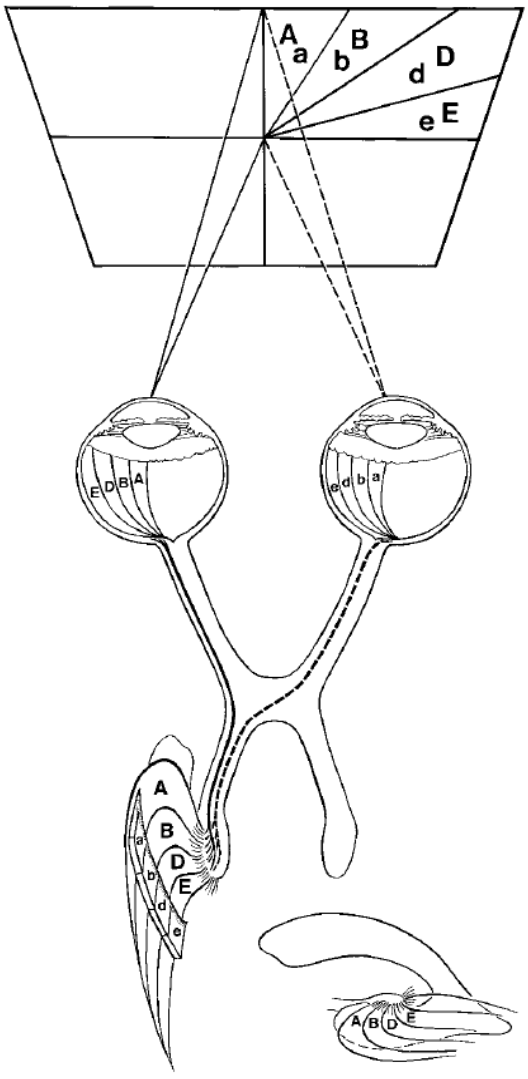


Figure 2.10. The organisation of optic radiation and occipital mapping, representing inferior retina quadrants in temporal lobe portion of human. Macular fibres lie mesial to those of peripheral retina fibres. Adapted from Walsh and Hoyt's 2005.[34]

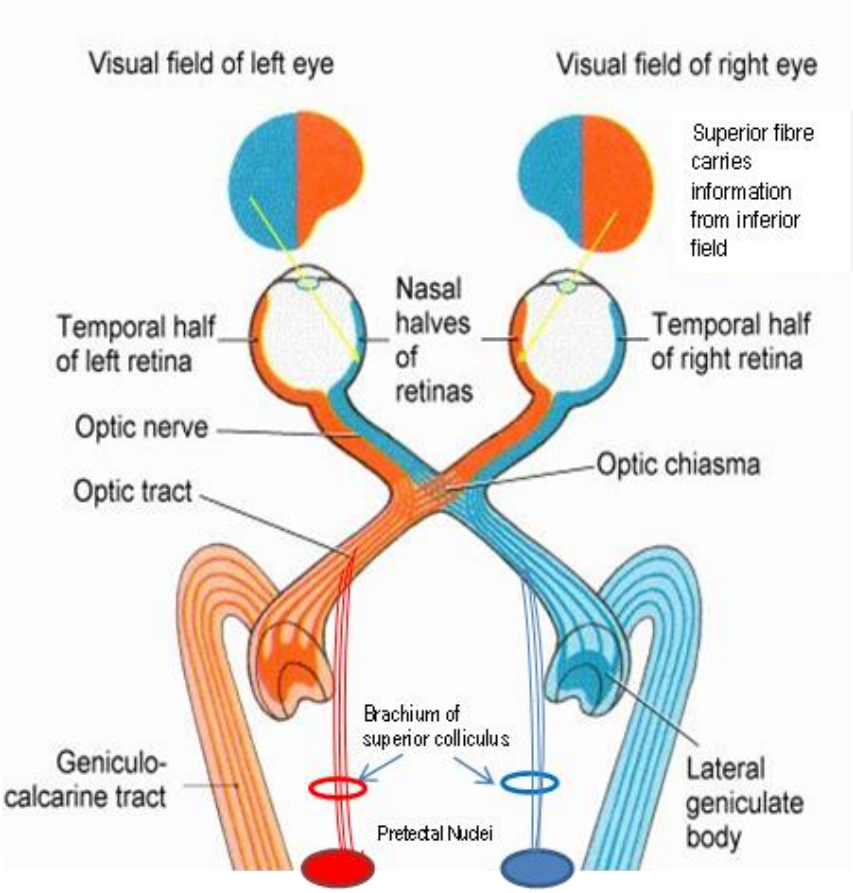


Figure 2.11. Schematic drawing of hemifield organisation of pupillary fibres travelling to the Pretectal Nuclei via the brachium of colliculus, in comparison to visual fibres which enter LGN. Diagram altered and redrawn from www.ivline.org for illustration.

The pretectal region of the mesencephalon is located at the level of the superior commissure, rostral to the superior colliculus and contains many nuclei among which those subserve the pupillary responses are the sublentiform nucleus, nucleus of the optic tract, nucleus of the pretectal area and Olivary Pretectal Nucleus (OPN).[92-94] Although the tracer studies were mainly done on primates, it is agreed that the OPN is the main neuronal centre that mediates and processes the pupillary information in humans.[29] The pretectal neurones have temporal summation with intensity-dependent time delay properties different from other neurones involved in the visual system. This makes it possible to study pupil traces in order to measure retinal and optic nerve lesions.[29] There are 2 sub-populations of pretectal neurons evident in the physiological studies: one with centre-weighted receptive field (those that respond with greater frequency to central retina stimuli than peripheral retina stimuli) and the other which give lower steady responses to stimuli anywhere in the retina.[29] The exact anatomy of which type of

ganglion cells from which part of the retina connect to a particular type pretectal neurone is yet to be understood.

At the pretectal nuclei, many ganglion cell axons connect with a much smaller number of dendritic processes of OPN – *convergence*.[29] The hemifield organisation of the pupillary light reflex and the amount of chiasmal and pretectal decussation is species specific.[29] In humans, the integrated pupillary signal is distributed almost equally to the right and the left *Edinger-Westphal* nuclei.[29] This duality of the pupillary pathways accounts for the anatomic basis of the consensual response to light. If not the same, very similar amount of pupillary constriction is expected for each eye.

2.2.4 Central neurones

Excitatory central occipital cortex neurones

The occipital lobes (mainly area 18 and 19) as well as other suprageniculate (post LGN) neurones give excitatory input to the pretectal and visceral oculomotor neorones.[29] This is supported by the findings of many investigators who noted pupillary hemi-akinesia or pupillary hemi-hypokinesia in response to stimuli placed in the blind hemifield of patients with acute suprageniculate lesions before any potential trans-synaptic degeneration ensued.[95-97]

These neurones follow the same centrifugal pathway as those of the occipital motor pathways to somatic components of the oculomotor complex.[29] Supranuclear pathways that mediate the accommodative pupil reactions appear to lie more ventro-laterally in the upper midbrain justifying their resistance to many pretectal lesions that impair the light response but not the accommodative response.[29]

Inhibitory central neurones

(a) Supranuclear inhibition

The sympathetic supranuclear input to the Edinger-Westphal nucleus is inhibitory. The darkness reaction is whereby the dilation of the pupil occurs in response to removing the stimulated light from the light adapted eye. In cases of interrupted sympathetic innervation the darkness reaction continues to occur in light adapted eyes of otherwise healthy subjects. This is due to the supranuclear input.[29;98]

(b) Cortical and hypothalamic inhibition.

In awake patients, pupillary impulses travel via cortico-thalamo-hypothalamic pathways or cortico-limbic pathways to give inhibitory influences on the parasympathetic outflow.[29] In support of this, there are cases where pupil dilation was achieved by (a) stimulation of the diencephalon or cortex of sympathectomised cats and monkeys,[98] and (b) stimulation of sensorimotor areas of the brain, hypothalamus and frontal lobe in subjects with intact sympathetic innervation.[29;99] In a normal physiological state, these inhibitory influences may involve psycho sensory input from higher centre neurones modulating the pupil responses. During sleep, the inhibitory influences of the cortex, hypothalamus, and reticular activating system are reduced, with a subsequent reduction in pupil size. But upon waking up psycho-sensory reinstitution occurs.[100]

(c) Brain-stem and spinal cord inhibition

There are 2 ascending spino-reticular pathways in the brain stem identified by Loewy and colleagues[101] (1973) which are associated with pupillary dilation independent from the sympathetic pathways. In 1975, Kerr FWL and colleagues traced some of these fibres from the spinal cord to the visual oculomotor nuclei in Macaca Mulatta macaque monkeys.[102] They were located in the periaqueductal gray area of the spinal cord connecting to the visceral neurones of the Edinger-Westphal nucleus.[102]

2.3 ANATOMY OF EFFERENT PATHWAYS

There are two important pathways that carry the efferent impulses to the iris muscles, the parasympathetic outflow pathway and the sympathetic pathway.

2.3.1 Parasympathetic outflow pathway

As mentioned above the pretectal nuclei (most importantly Olivary pretectal nucleus) passes the processed pupillary information equally to both visceral oculomotor nuclei.[29]

The **visceral oculomotor nuclei** are believed to contain cell bodies of preganglionic parasympathetic neurons that project to the ciliary ganglion and subserve pupillary constriction and accommodation responses. Although these nuclei are often collectively addressed as Edinger-Westphal nuclei (EWN) by most authors, they contain EWN

themselves (in dorsal ventral visceral cell column) as well as the anterior median nuclei and the nucleus of Pelia.[29]

The **pupillomotor fibres** are located in the oculomotor fasciculus in the area of EWN in the brain stem. They run in the trunk of the oculomotor III nerve through the anterior part of the cavernous sinus and superior orbital fissure. Between the brain stem and the middle of cavernous sinus, the fibres are concentrated around the medial superior aspect of the oculomotor third nerve.[103] They run medially and inferiorly in the sinus. In the anterior part of the sinus and in the orbit they are located among the somatic fibres of the anterior division of the oculomotor nerve.[29] The fibres lie very superficially immediately beneath the epineurium throughout.[103]

Pre-ganglionic parasympathetic fibres synapse at the **ciliary ganglion** located 1 cm anterior to the medial end of the superior orbital fissure and the annulus of Zinn, and 1.5 to 2 cm behind the globe. It lies on the temporal side of the ophthalmic artery between the optic nerve and the lateral rectus, close to inferior division of the oculomotor nerve.[29]

Only 3% of post ganglionic parasympathetic fibres supply iris (94% supply the ciliary body).[104] About 8-20 **short ciliary nerves** carry 3 types of fibres (a) postganglionic para sympathetic fibres which pierce the sclera to the iris muscles, (b) post ganglionic sympathetic vasomotor fibres and (c) afferent sensory fibres of the trigeminal nerve.[29]

The remaining fibres including those sub-serving the convergence reflexes are relayed in the accessory ganglion of Axenfeld, whose anterior cell station may be located in the ciliary body or iris. (Duke-Elder 1971)

2.3.2 Sympathetic pathway

The sympathetic pathway serves alongside the parasympathetic pathway for the physiological pupillary movements and reflex reactions. The path begins in hypothalamus and ends in iris and involves a 3-neuron reflex arc, figure 2.12.

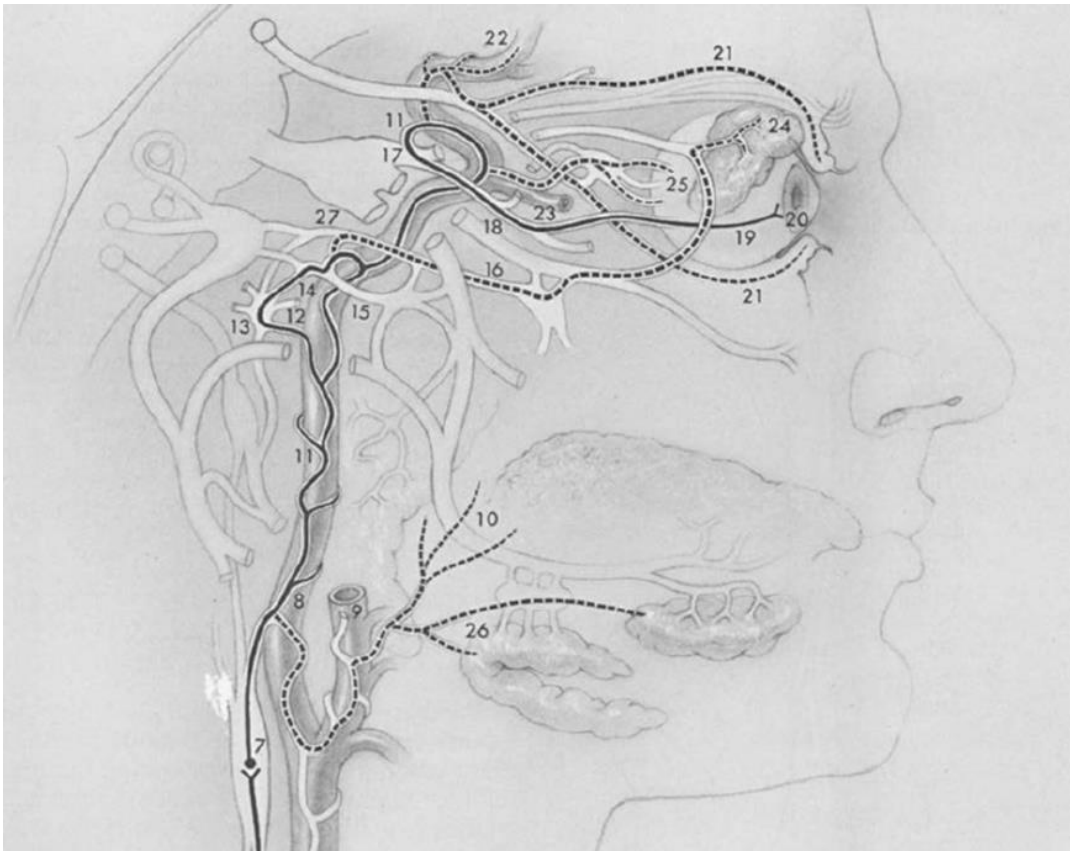


Figure.2.12. Sympathetic pathway to the face and eye. Adopted from Walsh and Hoyt's clinical neuro-ophthalmology 2005.[29] Solid line represents the pupillary dilator fibres. 7, superior cervical ganglion; 8, internal carotid artery; 9. external carotid artery; 10, pseudomotor fibres to face; 11, carotid plexus; 12, carotico-tympanic nerve; 13, tympanic plexus; 14, deep petrosal nerve; 15, lesser superficial petrosal nerve; 16, sympathetic contribution to vidian nerve; 17, ophthalmic division of the trigeminal nerve; 18, naso-ciliary nerve; 19, long ciliary nerve; 20, ciliary muscle and iris dilator muscle; 21, probable pathway of sympathetic contribution to retractor muscles of the iris; 22, vasomotor and pseudomotor fibres; 23, ophthalmic artery; 24, lacrimal gland; 25, short ciliary nerve; 26, sympathetic contribution to salivary glands; 27, greater superficial petrosal nerve.

The sympathetic neurones, 1st order neurons

They lie in the posterio-lateral region of the hypothalamus and subserve the pupillary reaction. Destruction of these neurons results in miosis, ptosis and reduction of pupillary reflex dilation.[29] As descending fibres travel through the brain stem they are mainly uncrossed (although there is a possibility of a few fibres crossing over in the decussation of Forel).[29] The fibres travel beneath the grey matter around the central canal in the pons. In the inferior cerebellar peduncle the fibres shift ventrally and laterally towards the lateral spino-thalamic tract. In the medulla, the fibres run through

the intermediate or lateral part of ventral reticular formation and down the antero-lateral column of upper cervical cord.

The sympathetic 2nd order neurons

The fibres synapse at the *cilio-spinal centre of Budge and Waller* at the intermedio-lateral tract of the grey matter of spinal cord, 2nd order neuron. Until they leave the intermedio-lateral cell column, the sympathetic fibres lie adjacent to the vasomotor fibres, thus stimulation of the cervical cord in the intermedio-lateral cell column causes pupillary dilation as well as vasomotor responses.[29]

The pre-ganglionic sympathetic fibres leave the cord by the anterior/ventral spinal root between C8- T4 (mainly T1) to travel the paravertebral sympathetic chains, without synapsing through thoracic and stellate ganglion close to the pleura at the apex of the lung. They traverse the inferior cervical ganglion and the anterior loop of the annulus of Vieussens (ansa subclavia).

Sympathetic 3rd order neurons

The fibres ascend in the superior cervical ganglion, the largest sympathetic ganglion of 2-3 cm long, and located below the base of the skull, at the level of bifurcation of common carotid artery (C3-C4). Shortly after emerging from the ganglion, the postganglionic sudomotor and vasomotor fibres branch off to travel along the external carotid artery to supply the sweat glands and the blood vessels of the face. The pupillomotor fibres accompany the internal carotid artery to enter the skull and form the carotid plexus surrounding the artery on lateral surface. They travel through carotid canal, foramen lacerum to travel over the gasserian ganglion and cavernous sinus. Within the sinus, the oculosympathetic fibres fuse with the abducent (VI) nerve for a short distance before joining the ophthalmic nerve. Most of the pupillary sympathetic nerves join the ophthalmic division of trigeminal (V) nerve and enter the orbit through the superior orbital fissure. The fibres travel with the nasociliary nerve, bypassing the ciliary ganglion to reach the eye as long cilliary nerves. Along the course some sympathetic fibres travel toward the eye by the sympathetic plexuses associated with the vertebral and basilar arteries, some relay at the ciliary ganglion and others in the sympathetic elements scattered throughout the uveal tract.

The long ciliary nerve has a specific role in controlling the iris movement. It blends with the short ciliary nerves, which also contain sympathetic fibres, in the choroid and sclera to form a rich plexus to supply iris dilator muscles, ciliary muscles and vessel wall.

Sympathetic and parasympathetic nerves intermingle in the ground plexus of the iris.

2.3.3 Iris

The shape of the pupil is determined by the position of the iris. The iris is a component of the uveal tract and delineates the anterior and posterior chamber of the eye. The muscle of the iris has 4 distinctive layers histologically: (a) the anterior border layer, (b) the stroma and the sphincter, (c) the dilator muscle layer and (e) the posterior epithelium,[3;29;36] figure 2.13.

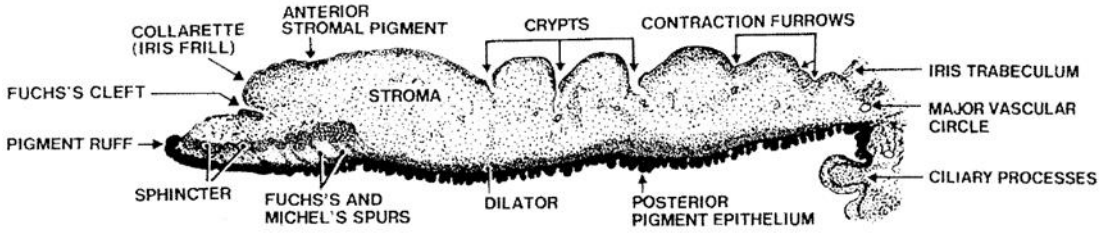


Figure 2.13 Sagittal section of the iris. Adapted from Loewenfeld IE.[3]

The anterior border layer has dense pigmented or non-pigmented cells. The absence of cells produces the "crypts" in the border layer.[36]

The stroma is made up of fibro-collagenous tissue: spindle shaped fibrobalsts, blood vessels, nerves, and macrophages (clump cells of Koganei).[33;36] At the pupillary margin, there are spindle shaped, circumferentially arranged, ribbon-like, meridionally orientated involuntary smooth muscle cells (about 1 mm in diameter and 0.1mm thickness)[29;36] which make the sphincter muscles of the iris (***sphincter pupillae***). The muscle cells in the sphincter muscle bundle are connected to each other by tight junctions and gap junctions, working together as a unit. Only one cell requires nerve innervation as the junctions allow depolarisation to spread throughout. [29]At the pupillary margin they are separated from the pigment epithelium by collagen strands

and posteriorly they are firmly attached to the collagen tissue containing vessels and nerves.[29]

The posterior boundary of the iris stroma peripheral to the sphincter muscle is a layer of elastic muscle layer (***dilator pupillae***).[33;36] The dilator layer measures about 12.5 μm in thickness and has a muscular basal portion that projects into the iris stoma and an epithelial apical portion that lies adjacent to the posterior epithelial layer of the iris.[29] The spindle shaped myoepithelial processes are in the muscular part that lies radially at the periphery.[29] There are tight and gap junctions between the muscle fibres allowing them to work as a unit. When dilator muscles contract, the iris is drawn into folds and the pupil dilates.

The posterior epithelium layer consists of 2 layers of densely pigmented cells; the anterior layer of which is continuous with the fibres of the dilator muscle.

The circular sphincter muscles are supplied by the short ciliary nerve, a branch of the oculomotor (3rd) cranial nerve which carries parasympathetic fibres along its efferent arm.[36] Other structures are supplied by the long ciliary nerve of the sympathetic nervous system as they run through the choroidal coat of the eye ball. Along the margin of the iris, these nerve fibres form a network and work their way into the iris in a radial fashion resulting in a triangular shape loop (apex of the triangle pointing towards the pupil) the borders of which coincide with the blood vessels.[36]

***Function*:** Innervated reciprocally by both sympathetic and parasympathetic autonomic fibres, the two systems work together. Reflex contraction of the sphincter pupillae is brought on by parasympathetic contraction of the sphincter via cholinergic receptor, as well as inhibition of the dilator muscle through cholinergic receptors on the dilator muscles. This results in the constriction of the pupil and reduces the amount of light entry into the eye. Similarly, sympathetic dilation of the pupil is brought on by adrenergic contraction of the dilator pupillae and adrenergic inhibition of the sphincter muscle via adrenergic receptors.[3;29] The brighter the light the more contracted are the pupil and vice versa. The posterior pigmented epithelial layer prevents light entering the eye ball. The arrangement of the iris muscles limits the pupillary movement at its extreme sizes.

Chapter 3

Relative Afferent Pupillary Defect – Clinical Approach

3.1 CLINICAL TECHNIQUES FOR DETECTING AND QUANTIFYING THE RELATIVE AFFERENT PUPILLARY DEFECT

3.1.1 The swinging flash light test – methods and grading

The methods of performing clinical swinging flash light (SFLT) have been extensively described in the literature. Since Marcus Gunn introduced the concept of pupillary escape and Kestenbaum and Levatin structured the clinical swinging test (chapter 1), the test was further formalised by the neuro-ophthalmologist, Stanley Thompson. He noted that a "barn door" afferent defect is easily picked up by the swinging flash light test; however, subtle defects can easily be missed if the observer is purely looking for the pupillary escape as stated by Levatin. Thompson pointed out that:

"*An eye with a small afferent defect may show a respectable initial constriction but if you wait and hold the light on the eye just a little longer, a pupillary dilation will be seen which was not present when the other eye was stimulated. If there is a clear pupillary escape in* ***each eye****, then look for small differences in the amount of redilation in the two eyes. In the relative afferent pupil defect, the pupil will consistently escape to a wider diameter.*" [23]

Since Levatin's and Thompson's publications, there have been numerous modifications and refinements to better detect and define the RAPD. Cox has also characterised the defective pupillary constrictions in afferent pupillary defects pupillographically in 1986.[105] To date, there is still a variation in practice between the clinicians but some basic rules need to be followed.

- As for any medical examination, it is important to take a relevant medical history and initial observation before the pupillary reflex test is performed.

- The structure of the pupils is then examined to exclude any local or mechanical factors that may confound the test results. Examination of a pupil includes the notation of pupil colour, size and shape in the ambient room light to appreciate whether there is heterochromia, anisocoria, irregularity due to iris atrophy, posterior synechiae, trauma, surgery, or neuro-syphilis, etc. Slit lamp examination of the local structures (iris atrophy, vermiform movement, posterior synechiae, ciliary body

tumour, angle closure glaucoma, pupil block syndrome, features of previous trauma or surgery, etc.) is needed if there is an abnormality suspected.

- Anisocoria can confound the RAPD testing because of the differences in the pupil size affecting on pupillary movement as well as the unequal retinal bleaching. If present the pupils are examined in the light and in the dark to appreciate whether the anisocoria is larger in the dark or in the light, whether smaller pupil or the larger pupil is abnormal.

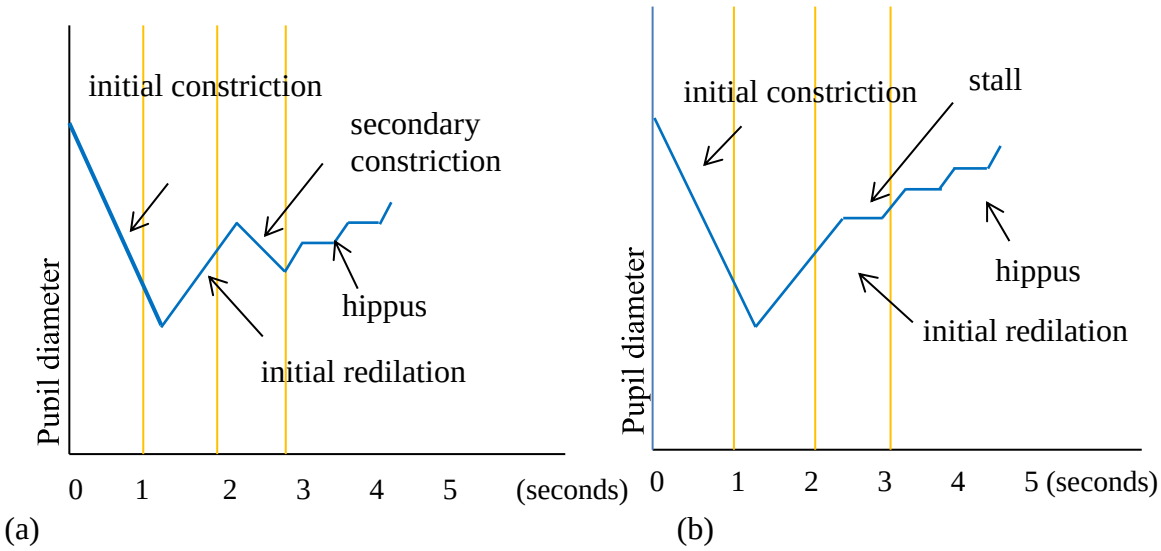
- If there is no significant anisocoria (<2mm), direct and consensual pupillary reactions are tested.[106] If one pupil is immobile due to efferent pathway causes, RAPD can still be tested provided pupil sizes are not too different. But in cases when neither pupil reacts to light, no further testing can be done.

- The evaluation of the pupil is performed in the dimly illuminated room[23] which allows for the dilation of the pupil and therefore provides more capacity for pupillary constriction. Dim light, instead of darkness, allows observation of the consensual pupillary response. The subject is asked to fixate on a distant object to relax accommodation.

- The light is held at about 30 cm from the patient's eye.[107] It is introduced either 45 degree below the level of the line of sight or tangentially to the optical axis of the eye[23;107] so that the light source would not interfere with the patient's fixation to distant object and also the examiner can observe the pupil without difficulty. For small pupils, off axis illumination also keeps the corneal reflex out of the way making the observation easier.[23]

- The optimum intensity of light chosen (not too weak to produce little pupil reaction but not too strong to bleach the retina excessively) is then applied to one of the eyes in the manner described above for about 2 to 3 seconds and is shifted quickly to the contralateral eye. This sequence is repeated for 2- 3 times. The quickness of the swing is important so that the net effect from both eyes can be tested. The examiner can either count slowly to 3 or look for the phases of the pupillary reactions that happen in the first 3 seconds of light illumination. These are (1) initial pupil constriction, (2) initial redilation (3) secondary constriction which in some patients may be a stall instead of constriction, figure 3.1. It is, however, important that the

duration of light presented on each eye is equal for both eyes to prevent unequal retinal bleaching. Also if the duration is longer than 3 seconds, hippus (section 2.1.1) can confound the measurements.[108] The non-linear range of pupillary reaction begins with pupil diameter of 3.5- 4 mm and therefore theoretically the best results of pupillary reaction should be obtained with a pupil not constricting below 3 mm.[107]



Figures 3.1. Schematic drawing of the pupil reaction to light as seen in the clinical SFLT with the stimulus duration of 3 seconds. (a) secondary constriction after initial dilation (b) pupil stalls after initial redilation. Diagram re-drawn from Bell RA 1993.[108]

- At the end of the sequence of alternating light and observing the pupil repeated for a few times, the conclusions are made. Repeating the swings a few times lessen the confounding effect of hippus (section 2.1.1).

- No RAPD is seen if both pupils constrict equally without an evidence of (a) pupillary re-dilation during the swinging flashlight test or (b) differences in the amount or speed of initial pupillary constriction, and (c) amount or speed of initial pupillary redilation.

Grading of RAPD by the clinical method

When there is a RAPD, the severity of the RAPD can be graded clinically, figure 3.2:[108]

- Grade 1 (Mild RAPD) - The affected pupil shows a weak initial constriction, followed by dilation to a greater size.
- Grade 2 (Moderate RAPD) - The affected pupil shows a stable or unchanged level of constriction (initial stall), followed by dilation to a greater size.

- Grade 3 (Severe RAPD) - The affected pupil shows an immediate dilation to a greater size.
- When the RAPD is regarded as a grade 3, further grading can be performed by shinning the light to the better eye for about 6 seconds. This is to artificially induce bleaching of the retina of the better eye to increase the pupillary threshold sensitivity to the next level and then re-comparing the two eyes for further grading.[108]
- Grade 4 – Immediate pupillary dilation with secondary constriction.[108]
- Grade 5 – Immediate pupillary dilation with no secondary constriction.[108]

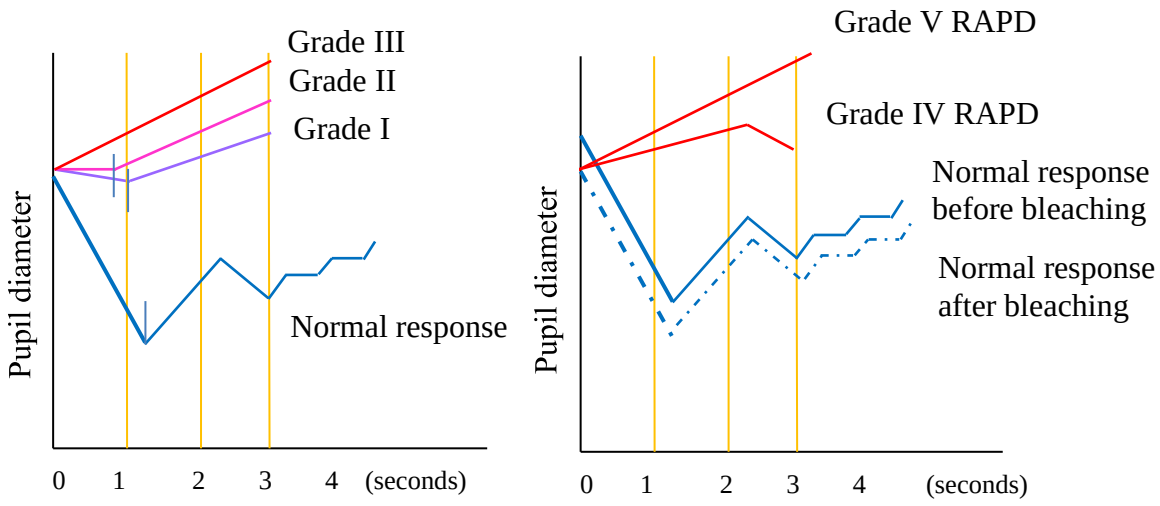


Figure 3.2. Schematic drawing of the grades of RAPD by clinical methods. The diagram modified from Bell's et al.[108]

In the clinical method, the abnormality here is the weakness of the pupil response to light or reduced pupillary reaction to light. Both amplitude (amount) of constriction and the velocity (rate) of constriction are reduced in parallel.[109] If however, the velocity of pupillary constriction is reduced out of proportion to the reduction in the amount of constriction (sluggish pupillary response)[109] then a different pathology is suspected.

What is the optimum intensity of light for the clinical swinging flash light test to detect a RAPD?

No set level of intensity can be defined for the optimum pupillary reaction to test the RAPD clinically because the intensity-dependent results also depend on numerous other factors including the level of retinal adaptation, the light level in the test environment,

and the type of the stimulus light used; all of which cannot be standardised in the clinical testing. Most authors suggested a strong steady light from the halogen indirect ophthalmoscope or halogen bulb flash light or a wall-mounted Finhoff trans-illuminator.[23] If the light level is too low, the pupillary reaction is small in both amplitude and duration. A bright light is used for a stronger pupil constriction for both duration and amplitude so that the afferent defect is more easily seen. It is said that *the amount of RAPD* is also larger with the increase in the intensity of the stimulus light but asymptotically at higher levels. In the experiment performed by Johnson LN, the steady state level of RAPD was reached at the stimulus illumination of about 430 lux when tested in the dark room.[110] The author proposed that as the level of illumination is increased, more retinal ganglion cells contribute to the pupillary response to elicit the relative difference between the two eyes.[110] If the light stimulus is too strong, however, it causes the retinal bleaching so much so that it produces an afterimage which is bright enough to keep the pupil from redilating, thus keeping the pupil in spasm.[108] What is more important for a clinician is to use is the light level bright enough to elicit different phases of pupillary reaction in the dimly lit room, but bright enough for the clinician to see the pupils, and to use the same setup and the same light setting each time for a better comparison at each visit.

Do the duration of stimulus and the swing time matter?

The duration should not be longer than about 3 seconds due to potential hippus with longer durations. In severe cases, the RAPD can be detected clinically with the duration of stimulus as short as one second or less but for more subtle RAPDs the duration of light should be long enough to separate the phases of the pupil reactions for a comparison. The important point here is to make sure that the duration of stimulus presented is the same for both eyes. If the light is presented longer in on eye than the other it causes unequal retinal bleaching of the retina which confounds the results. In regards to duration of darkness or the swing time, it is important that the light is swung as quickly as possible between the eyes so that the net result of the pupillomotor driving force of one eye and the pupillary dilation of the other can be more accurately estimated.

Using the direct and the consensual response for the clinical swinging flash light test

Another variant of the clinical method is to observe one eye while comparing the direct and consensual responses.[107] For example, the light is shown to one eye with the manner described above and the pupil reaction is observed over 1-3 seconds. The light is then quickly swung to the other eye for the same duration while the examiner carries on looking at the same eye for the pupillary reaction. The differences in the amplitude or the speed of constriction between the direct and the consensual responses (or the pupillary escapes) are then compared mentally over the swings to determine if there is a weaker response with the direct (the eye observed is weaker) or consensual reaction (the fellow eye is weaker). This method requires that the direct and the consensual reactions are "identical" for a person to make a sound comparison of afferent pupillomotor input of the two eyes. Comparison of the direct and consensual responses during clinical swinging flash light test is considered slightly less accurate than comparing direct responses.[107] This is because of the potential issues of less constriction in consensual then the direct response (contraction anisocoria) which can be present in up to 85% of normal individuals.[111] Clinicians only use this method for situations where there is only one working pupil, for example: unilateral globe contusion, unilateral efferent pathologies such as synechiae or pupillo-static drops.[23] In these situations, the ambient room light should be adjusted to make the pre-stimulus pupil diameter of the two eyes as similar as possible before the start of the swinging flash light test to avoid pupil size effect on the pupillary movement and unequal retinal bleaching.

Other modifications

The clinical swinging flash light test may be modified by the following means -

(1) The slit lamp microscope to detect the relative afferent pupillary defect – using the slit lamp to view the direct and consensual responses of the same eye when light is swung from side to side.[112]

(2) The magnifier assisted swinging flash light test – a 20+ lens is used. The Area under the receiver operating characteristic curve (AUC) of 0.86 vs 0.61 with the traditional clinical SFLT was reported in assessing asymmetry in glaucoma patients.[112;113]

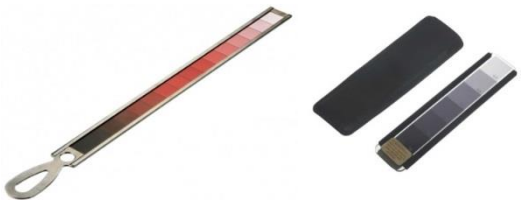
(3) The ophthalmoscope swinging flash light test - the direct ophthalmoscope, set at +10 D lens, and held at approximately 1 foot in front of the face.[113]

Absolute pupillary light test vs relative pupillary light test

The absolute pupillary light test, whereby the individual pupillary reaction is measured to directly compare within different subjects, is not a feasible option for various reasons. The test condition and the stimulus intensity are variable among different clinicians for a swinging flash light test. The amount of pupillary constriction varies with starting size of the pupil;[23] and there is extreme inter-individual variability of the pupil reaction to light due to moment to moment variability of the higher centre inhibitory influence on the EWN within mental activity of an individual.[23;107] The relative test compares the afferent pupillomotor input of the two eyes. The presence of RAPD thus means that one eye is relatively better or worse than the other eye. The absence of RAPD does not necessarily mean that both pupils are normal, as they might be equally impaired.[107]

Quantification of RAPD with the swinging flash light test

The quantification of RAPD is important for serial evaluation of patients, and for comparing the results of the RAPD with other tests both for clinical and research purposes. This is traditionally performed by the neutral density filter. Some authors attempted to use the easily available **Sbisa bar or bagolini filter** bar. Others use **double polarised filters** as this is easy to use on paediatric patients), figure 3.3.



(a) (b)

Figures 3.3. (a) Sbisa (bagolini filter bar) , (b) neutral density filter bar

The neutral density filters (NDF) are photographic density filters that attenuate the intensity of all wavelengths of light that pass through them. The neutral density filter (NDF) acts as a surrogate for afferent pathology in the better eye. NDF of 0.3 log units increment are placed in front of the good eye and the swinging flash light test is repeated until no RAPD is seen. The density of the NDFs recorded in log units estimate the size of relative difference between the two eyes. It is also a good practice to overshoot the end point until the RAPD is obtained in the good eye.[23] Then removing

the 0.3 filter away from the good eye, the balance can be reached with certainty, figure 3.4.

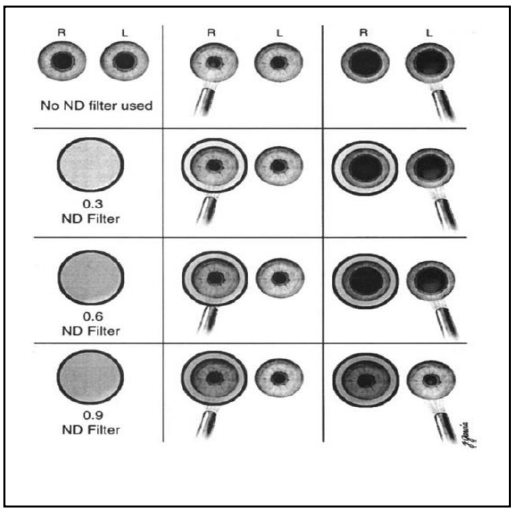


Figure 3.4. RAPD quantified by the neutral density filter. In this case the RAPD is balanced with the 0.9 log units density filter. Diagram adopted from Walsh and Hoyt's Clinical Neuro-Ophthalmology (2005).[114]

When a very subtle RAPD is suspected, a 0.3 log units filter is placed in front of the suspected eye.[23] If the filter is placed in front of the eye with subtle RAPD, the RAPD will be accentuated (figure 3.5 bottom) but if the eye under the filter is the better eye of the two, no RAPD will be detected (figure 5.3 middle). If the RAPD moves with the filter, on the other hand, there is no real RAPD.[107]

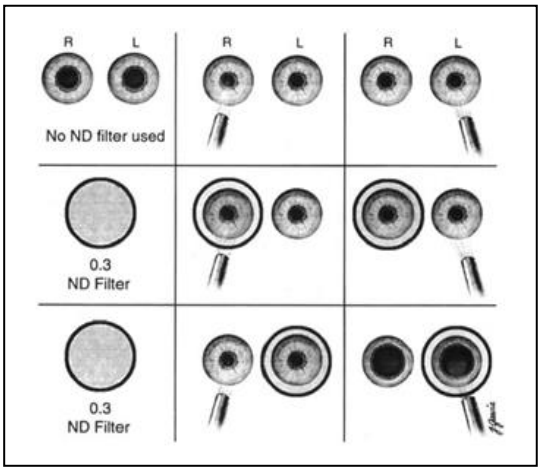


Figure 3.5. The use of NDF to enhance the subtle RAPD. Diagram adopted from Walsh & Hoyt's Clinical Neuro-ophthalmology (2005).[114]

While using the NDFs it is important that the followings points are observed:

(1) the duration of exposure to light is the same for each eye,

(2) the filters are kept close to the nose to avoid light leaking to the other eye. If there are other facilities to control the light leakage, the filters can be placed in front of the light source instead because it gives a better visibility of the pupil under examination.[107] (3) not more than 2-3 swings of light should be used for each test to minimise the asymmetric bleaching of the retina by the NDF itself.[23] NDFs, especially of high log units, are known to cause retinal dark adaptation. If more than 3 swings are required, the NDF s should be removed from the good eye, and the bright light is exposed to both eyes to equalise the retinal sensitivity before the NDF is placed again in front of the good eye.[23;115]

3.2 CLINICAL APPLICATIONS OF RELATIVE AFFERENT PUPILLARY DEFECT

A relative afferent pupillary defect can be estimated by various methods described above, but what does the presence of RAPD mean clinically? How does it relate to other clinical tests? Before the pathological aspects are looked at it is worth noting that the RAPD can be present in normal subjects and that RAPD can be induced by merely occluding one eye.

3.2.1 Physiological RAPD

Normal subjects can have a small degree of afferent pupillomotor asymmetry[107;116;117] proven by the evidence of small differences in the number of ganglion cells (section 2.2), asymmetric neuronal afferent input, asymmetric higher centre input and asymmetrical innervation of the pupillary muscles;[116] but this is typically small and not apparent clinically. Asymmetric hippus may also have effects on physiological RAPD. The RAPD may sometimes change side when tested at different times on different days. The physiological range of RAPD is up to about 0.3 log units using the clinical method (which is the lowest grade of neutral density filters) but in a more accurate pupillometric study, by Wilhelm et al (2007), the RAPD as high as 0.39 log units has been recorded in normal subjects who represent the outliers of the distribution (52% have <0.07 log units, 42% 0.08 – 0.22 log units, and only 6% 0.23 to 0.39 log units).[118] Physiological asymmetry is also evident in perimetry testing – the inter-eye mean deviation differences for subjects with normal visual functions are between 0 and 0.3 dB.[119]

Therefore, it is important to note that a small RAPD, if detected, may not necessarily imply pathologic condition especially when clinical signs or symptoms are absent. In these situations, it is necessary to exclude other confounders; for example, asymmetric dense cataract may contribute to a RAPD in the less affected eye[120].

3.2.2 RAPD induced by occlusion

RAPD can be induced by occlusion of one eye. The eye which is occluded undergoes retinal dark adaptation and becomes more sensitive to light. This causes contralateral RAPD. This effect is most marked during the first 3 to 5 minutes after removal of the occlusion.[107] RAPD ceases exponentially thereafter and at about 15 minutes after the removal of occlusion, both pupils react equally. This is caused by the differences in the pupillomotor sensitivity. , It is, therefore, important to avoid covering one eye with the NDF longer than the other eye during the SFLT. Similarly, in cases of unilateral periorbital oedema, RAPD should not be tested during the first 15 minutes of lifting the eye lid in the affected eye. When it is not possible to open the eye for long in these situations, both eyes should be closed for 30 minutes before the test of relative afferent pupillary defect is tested to create the same level of retinal adaptation.[107]

3.2.3 RAPD and pathologies

The relative afferent pupillary defect, RAPD, is the manifestation of a number of pathologies that result in asymmetrical afferent input to the pupillomotor centre of the brain, conditions that diminish the effectiveness of a light stimulus in producing pupillary constriction in one eye relative to the other. The list of conditions that lead to a relative afferent pupillary defect is exhausting. Unilateral or bilateral asymmetrical optic neuropathies cause RAPD with or without visual acuity loss. For retinal causes, the amount of RAPD is less compared to that of visual acuity loss.

The RAPD has *not* been associated with refractive errors, media opacity (except for a dense vitreous haemorrhage shadowing the retina), previous eye surgeries unless it involves retina and optic nerve, strabismus, conditions with *efferent* pupillary defect (such as third nerve palsy, Adie's pupil, Horner's syndrome), non-ischaemic vein occlusion, mild macular degeneration, background diabetic retinopathy, and conditions

that are typically bilateral and symmetrical (such as retinitis pigmentosa, bilateral nutritional or metabolic optic neuropathies).

Unilateral or asymmetric lesions of the retina, optic nerve produce unilateral *ipsilateral* RAPD whereas unilateral optic tract lesions, lateral geniculate lesions and partial retro-geniculate lesions very close to the LGN, certain unilateral thalamic or midbrain lesions produce *contralateral* RAPD in spite of having symmetrical visual field loss. [23;107;119;121-126] This is due to the higher number of optic nerve fibres crossing at the optic chiasm (section 2.2.2). The inherit assumption here is that the number of pupillary fibres are proportional to the number of visual fibres. About 53 to 67 % of the optic nerve fibres cross at the chiasm.[30;31] Therefore, uncrossed fibres only represent 47 to 33 %. In a person who has 53% of crossed fibres, for example, the right optic tract lesion thus involves 53% of nasal pupillomotor fibres from the left side and 47% of uncrossed pupillomotor fibres from the right temporal retina, producing left RAPD, figure 3.6. Although the sidedness of the RAPD for the post-chiasm lesions is always contralateral, the amount of RAPD does not always correspond to the number of estimated crossed and uncrossed fibres.[31] This may be because of the variability in the measurement process, differences between nasal and temporal retinal sensitivity or the area illuminated between the nasal and temporal side, and the percentage of involvement of the fibres at the site of lesion.

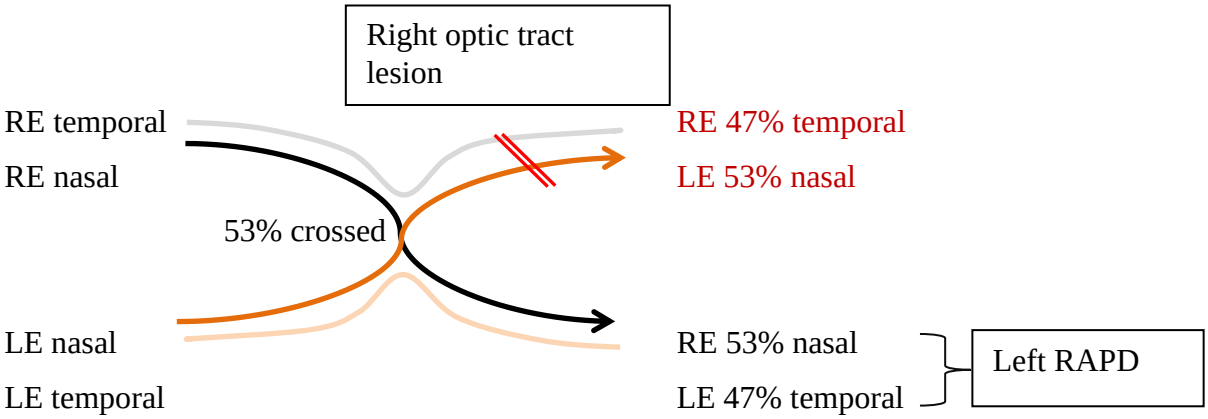


Figure 3.6. A schematic presentation of the involvement of optic nerve fibres in a post-chiasm lesion.

RAPD testing is sensitive in detecting optic nerve pathologies. Although diseases of the retina can produce RAPD, inferences can be made whether this is due to retinal or optic nerve pathologies based on the amount of RAPD measured and other clinical clues. If

the measured RAPD is far greater than the clinical findings, further investigations are warranted. For example, a patient with a macular disease (macular degeneration or central serous retinopathy) will rarely have a RAPD of more than 0.5 log units.[127;128]

RAPD has been reported in various cases of unilateral and asymmetrical glaucoma.[129;130] The initial lack of description of RAPD in glaucoma may be due to the frequency of miotic treatments of glaucoma in earlier years.[129] RAPD is a very important and sensitive sign of asymmetric retinal ganglion cell and axonal damage. When glaucoma is bilateral it is almost always asymmetrical. An accurate test of RAPD would mean diagnosing glaucoma with high sensitivity and specificity in the suspected populations.

Unilateral rhegmatogenous retinal detachment or macular detachment can contribute up to 0.68 log units of RAPD while each peripheral quadrant to about 0.35 log units.[131]
In regards to the lesions of the optic chiasm, when the defects in the field of vision are symmetrical, a symmetrical pupillary reaction will result and no RAPD will be elicited. But these pupils tend to dilate quicker or more than the normal pupils (bilateral pupillary hemi-hypokinesia). If the bi-temporal hemianopia is complete, the light directed at the blind nasal hemi-retina will produce pupillary reaction that is much reduced than the light shown to the normal temporal hemi-retina (taking into account the light scatter inside the eye). However, if the chiasm lesion causes asymmetric field defects, a better pupillary reaction may be obtained from the eye with a smaller visual field defect. In cases of anterior chiasmal syndrome, involvement of optic chiasm and one of the optic nerves, there will be a RAPD ipsilateral to the involved optic nerve.

3.2.4 Cataract and RAPD

Cataract does not produce a RAPD in the affected eye. However, RAPD has been stated in the contralateral eyes in subjects with unilateral dense cataracts.[120] The visual acuity in the eye with cataract in these cases is of counting finger or worse. The mean RAPD reported is about 0.44 log units.[120] In the article written by Lam and Thompson (1990),[120] the RAPD disappeared in the contralateral eye after removing the cataract. They also reported the lessening of RAPD in an eye with optic neuritis when the cataract developed and got denser in the same eye.[120] This contralateral RAPD is

thought to be due to the test light being scattered directly more onto the macula by the opaque media than in cases of clear media (Ulbricht's bowl effect).[107;132-135] Some other authors postulate that the compensatory mechanism enhances the retinal input in the cataract eyes compared to the normal fellow eyes. This increases the pupillomotor effectiveness of the stimulus light in the cataractous eye making the contralateral eye relatively weaker.[120;136] If RAPD is noted in the eye with a dense cataract, optic nerve pathology is strongly suspected.

3.2.5 Anisocoria and RAPD

Severe disease in one eye leading to an RAPD will not lead to anisocoria. The diseased eye's pupil will appear to be of equal size to the other eye due to the consensual light reaction (unless the iris itself is diseased or unreactive or there are issues with efferent pathway) since both pupils are controlled by the efferent sympathetic pathway. It means to say that if there is an anisocoria as well as decreased or reduced vision in one eye, we are dealing with two pathologies.[107] However, anisocoria can induce an RAPD.

Simple anisocoria represents a small amount of anisocoria in 40-50% of normal population often in the region of 0.4 mm or more. It does not vary with time of the day and is not influenced by age, sex, iris colour or surrounding light level.[137;138]

In cases of significant anisocoria, less light enters the smaller pupil during SFLT giving rise to an unequal retinal bleaching[23] and an apparent RAPD. Another reason also is the mechanical factor - the larger pupil constricts more than the smaller pupil due to the peculiar arrangement of the iris muscles (section 2.3.3) that accommodates the larger pupil with more room to constrict than the smaller pupil,[139;140] For small anisocorias of up to about 2 mm retinal adaptation may be able to balance the difference in light reaching the retina.[23;141] The light entering through the smaller pupil can still scatter especially if there is a refractive media such as lens media and reach the entire retina as far as the intensity of light and the ocular media allow.

Approximately 1 dB (0.1 log unit) of RAPD is expected with 1 mm of anisocoria.[141] Since the RAPD up to 0.3 log units can be present in normal eyes, anisocoria of more than 2 mm is required to produce a clinically significant RAPD.[141] Thompson HS[23] and Cox TA[105] suggested the use of neutral density filter on the larger pupil at a rate of

approximately 0.1 log unit for every millimetre of anisocoria to nullify the effect of anisocoria for RAPD measurement.[23;105]

3.2.6 Strabismus and RAPD

In cases of neuro-muscular strabismus or mechanical ocular misalignment such as in thyroid orbitopathy, the clinical swinging flash light can be performed so long as the stimulating light is presented at the same angle for each eye. As such, it is possible to perform the swinging flash light test for small strabismus but larger ones impose technical difficulties.

3.2.7 Amblyopia and RAPD

A relative afferent pupillary defect can be detected in an amblyopic eye that has an identifiable developmental hypoplasia of retina and/or optic nerve. The aetiology and pathophysiology of amblyopia are diverse, and the proportion of amblyopia that shows RAPD varied considerably in published cases,[142-146]This variability can be due to the true variability in the pupil involvement in the amblyopia or due to the differences in techniques employed in the examination and quantification of pupillary defect by different authors- clinical SFLT vs pupillometric methods, full-field stimulation vs segmental stimulation vs stimulation by contrast grating, using contraction amplitude vs latency as an outcome measures, and comparison of the affected eye and presumed normal fellow eye (amblyopia as a person) vs comparison among normal eyes of non-amblyopic subjects (amblyopia as an eye).

In one study conducted by Barbur JL and colleagues (1994).[143] It was found that the normal fellow eyes of amblyopic patients have statistically reduced amplitude and latency of both pupil light reaction and pupil grating response compared to those of normal subjects.[143] It is in this regard the pathophysiology of RAPD in amblyopia is complicated.

Nonetheless, there are a few key features that are commonly agreed in relation to the pupillary defect in amblyopic patients:

- The efferent pupil light reflex pathway is not involved in amblyopia and the pathology is suspected to be in the retina or optic nerve (afferent pathway) either primarily or secondary to the cortical abnormalities.[146]
- RAPD is mainly detected in cases with recognisable afferent pathologies.
- The RAPD in amblyopia normally manifests as a subtle defect.[142] It may be a challenge to confirm amblyopia when there is a concurrent physiological RAPD because both may be in the region of 0.3 log units.[119]
- When amblyopia is suspected as a cause of a visual defect, an RAPD of 0.6 log units or more is very unusual and it warrants a critical re-appraisal of the diagnosis.[106]
- If there is an RAPD it is always found in the amblyopic eye.[143]
- There is no apparent relation between the amount of RAPD and the amount of visual suppression.[142;145;146]
- The pupillary defect is not correlated with the cause of amblyopia, the defect in the visual evoked potential (VEP) or the colour vision defects.[142]
- For those with pupillary deficits, vision may still be improved with occlusion therapy.[146]

3.2.8 Relationship of RAPD and other visual function tests

(i) RAPD and visual acuity

Not all patients with RAPD will have reduction in visual acuity. Some conditions will lead to a marked reduction of visual acuity with an RAPD, while others spare the central vision. Often an extensive loss of peripheral vision or loss of central vision correlates with an RAPD. For example a patient with glaucoma will have no acuity loss when the central fields are preserved although he may have a significant RAPD. Similarly, a patient with an altitudinal visual field defect associated with anterior ischaemic optic neuropathy, may have no other optic nerve function signs but the RAPD.[147] Therefore, RAPD and the visual acuity have poor correlation.

(ii) RAPD and perimetry

There is a degree of correlation between RAPD and the inter-eye differences in the parameters of both static and kinetic perimetric analysis for patients with optic

neuropathies.[148-152] The correlation does not improve when visual field loss outside of 30 degrees are considered.[150] The correlation coefficient (r) in the linear regression analysis is often less than 0.7 for non-compressive lesions but up to about 0.84 for compressive optic neuropathies in the published literature.[150-152] The correlation seems to be stronger with larger RAPD than with smaller RAPD. RAPD of < 1.2 log units do not have strong correlation with inter-eye perimetric differences in the published literature.[148] The correlation between functional RAPD test and inter-eye mean deviation differences are poorest for optic neuritis patients compared to patients with anterior ischaemic optic neuropathy, intracranial hypertension or compressive optic neuropathy.[150] Compressive lesions also give a larger RAPD than non-compressive optic neuritis.[150] The range of correlation for patients with optic neuropathies is about 0.58 to 0.69.[150-152]

There are some points to consider between the RAPD and inter-eye differences in perimetric test results –

(1) The pre-geniculate afferent pathway pathologies do not necessarily affect visual threshold as tested by perimetry (threshold test) and by pupillary light reflex test (a supra-threshold test) in the same way.[150]

(2) In perimetric tests, a weighted average of light thresholds is determined from many small focal stimuli, whereas in the RAPD test a large global light stimulus is applied.

(3) The sensitivity profile across the visual field and the pupil field are not the same.[95;153] A study, by Bremner and co-workers, which compared the pupil perimetry and the visual perimetry on patients with Leber's hereditary optic neuropathy, confirmed that visual deficits exceed pupil deficits by an average 7.5 dB at all retinal locations.

(4) The photoreceptors involved in visual and pupil testing are the same (rods and cones) but the signals are conveyed to the brain by different populations of ganglion cells which may not be equally affected by any given pathology.

Whilst the visual field defect may exist prior to the detection of a RAPD, a majority of reports find the RAPD before the visual field defect[125;129;151] in early disease conditions. RAPD is considered to be an important predictor of optic nerve pathologies. About 25%-35% of the ganglion cells can be damaged before a clinically significant visual

field defect is demonstrated in glaucoma patients.[126;154] But for the RAPD test, the absolute inter-ocular differences of as small as 6 % (estimated nasal vs temporal cross fibres = 53% vs 47%) or the 13% relative difference ((0.53/0.47)/100%)[155] in the amount of optic nerve fibres in afferent pathway can produce a RAPD. This is demonstrated in patients with post chiasmal lesions.[31] Thus, RAPD often presents before recordable VF defects are obtained. A difference in Humphrey perimetric mean deviation of more than 8.7 dB (in the absence of ptosis, other ocular media opacification or other confounders) implies functional loss if there is no detectable RAPD.[151]

In patients with glaucoma, the RAPD correlates with the perimetric inter-eye sensitivity difference (Octopus perimeter) but not with cup-to-disc ratio asymmetry in the study conducted by Brown et al (1987).[149] According to the authors all patients with a RAPD have inter-eye perimetric sensitivity difference of $\geq$ 13%.[149]

(iii) RAPD and IOP

Optic disc damage is the end point in the process of glaucoma. The RAPD test is potentially useful for diagnosis as well as detection of progression of glaucoma. The correlation between the amount of IOP rise and that of the development of RAPD is time dependent.[156] An acute rise of IOP, a maximal IOP or the initial IOP at presentation is not always associated with a large RAPD at presentation. A significant correlation is reported between the inter-eye IOP difference and the change in the level of RAPD. The higher the difference of IOP between the eyes, the more likely that the RAPD will increase in the worse eye.[156]

(iv) RAPD and retinal nerve fibre layer (RNFL) thickness reduction

The log scale RAPD has an inverse correlation with the average RNFL thickness ratio (more affected eye/less affected eye) measured by the optical coherence tomography (OCT) in various cases of optic neuropathies.[157;158] The correlation coefficient of the linear inverse relation of the two (r) is reported to be -0.7 in glaucoma patients when the RAPD of these patients is 0.6 log units or more estimated by the clinical swinging flash light method.[158] Average RNFL thickness asymmetry of 23-27% has been associated with 0.6 log units RAPD in cases of optic neuropathies including glaucoma.

When Rhesus monkeys underwent laser ablation of the macula in one histological study by Kerrison (2001), 0.6 log units of RAPD was detectable when 25-30% of the retinal ganglion calls were histologically lost.[159] Largreze and Kardon[160] attempted to correlate RAPD and the retinal ganglion cell loss estimated from visual field defects using pre-determined templates.[161] The template when superimposed on static or kinetic visual fields, give estimates of the percentage loss of ganglion cells. Their results support that the RAPD correlates well with estimated retinal ganglion cell loss in optic nerve disease. They also stated that the spatial distribution of pupillomotor retinal ganglion cells seems to be proportional to the distribution of light-sensitive ganglion cells projecting to the lateral geniculate nucleus.

RAPD measured with the pupillometric method was compared with the ganglion cells lost as determined by the functional visual field pattern deviation (Humphrey), Kardon and co-workers(1998) reported the correlation coefficient (r) to be 0.7 ($R2 = 0.46$). [160]

There is also a moderate correlation between the RAPD and the inter-eye difference in the neuroretinal rim ($r = 0.67$).[162]

Therefore, the structural studies, histological studies and the functional studies all support the correlation the RAPD with amount of ganglion cells or the NFL thickness loss.

(v) <u>RAPD and visual evoked potential (VEP)</u>

There is evidence of a good correlation between the RAPD and the amplitude of the visual evoked potential in patients with unilateral anterior ischaemic optic neuropathy (AION)[163] or optic neuritis[123] but not its latency. The RAPD testing is more sensitive than VEP latency or the critical flicker frequency in discriminating resolved optic neuritis from the CSR [164] because the patient with resolved CSR has a minimum RAPD while optic neuritis patients have larger RAPD. However, it does not replace VEP in detecting past optic neuritis.[165]

3. 3 ADVANTAGES AND DISADVANTAGES OF USING A RELATIVE PUPILLARY TEST IN CLINICAL PRACTICE

There are important advantages to the test of relative afferent pupillary test:

(1) As a within-person comparative test, RAPD test overcomes the problem of intra-individual variabilities that confound many biological tests;

(2) In view of the fact that both pupils respond together in *almost* identical manner, and the higher centre influences are bilateral, the test has a potential sensitivity in delineating the eye with a weaker pupillomotor drive;

(3) RAPD is a useful clinical test to detect pre-geniculate lesions in the absence of any ophthalmoscopic evidence of such diseases.

(4) RAPD test is helpful in differentiating functional from organic disease.[166] For example; the absence of RAPD with mean deviation difference of > 8.7 dB may imply functional loss.

(5) RAPD is the test of choice for early cases of optic neuropathy. The presence of RAPD may be the only objective sign of retro-bulbar optic neuropathies.[166] It is advocated for screening optic nerve diseases.

(6) Because of the consensual light reactions, only one functioning pupil is needed to determine the presence of an RAPD.

(7) Although the test demands skill, experience and careful interpretation of the findings, it is a quick test to perform.

(8) In experienced hands the diagnostic ability of the CSFLT is substantial as the examiner can modify the test as required. For example, for a sluggish pupil, the examiner may hold the light for a longer time to enhance an initial pupillary constriction; for larger pupils when the asymmetry is difficult to see, he may hold the light on the eye for a shorter time to reduce the pupillary constriction symmetrically; in cases of physiological anisocoria, he may use the differences in the direct and consensual responses of an eye as an endpoint maker, whereas in cases of contraction anisocoria, he may use the direct responses only to estimate the RAPD.

(9) Quantification of RAPD is possible in the serial assessment of chronic optic nerve disorders such as glaucoma, intracranial hypertension, and compressive optic neuropathies.[166] It allows the assessment of the severity of the disease and thus

prognosis. For example, an initial RAPD of > 2.1 log unit has been associated with poor prognosis in patients with traumatic optic neuropathies.[167]

(10) Unlike the visual field test (which relies on a patient's ability to understand and perform the test), RAPD does not require patient participation. This is an advantage in clinical practice especially for paediatric, mentally retarded, or senile patients.

(11) In theory, the RAPD test can compare an afferent input difference of as small as 6% (53-47%).[31]

However, as with any biological test, allowances need to be made for the physiological range since a healthy person can have a small amount of physiological RAPD. The disadvantage of being a relative comparative test also is that its sensitivity declines in cases of bilateral conditions of similar severity. If each eye has severe but *equal* afferent pathway disease, in rare situations, there will be no RAPD. Therefore, the absence of RAPD does not necessarily mean there is no disease. Also RAPD is not the test where the results can be compared directly between different subjects. The clinical methods of detecting the RAPD are variable and demand skills; and the method of quantification is technically difficult. The pupillometric methods can circumvent most of the problems encountered with the clinical method.

Chapter 4

Factors that Affect Parameters of Pupillary Light Reflex

4.1 PUPILLOMETRY

Pupillometry is the measurement of the pupil size whether in a static condition or during induced movement by changing light conditions or by near or distant vision. Pupillometer is a term used to describe an instrument that measures the pupillary distance or an instrument that measures, records and monitors the pupillary response to various stimuli. The pupillometer in this thesis describes a computer based electronic binocular infrared video device that is designed to measure and record the pupil dynamics upon certain stimulus applied. A modern pupillometer incorporates (a) a system for delivering the stimulus to the retina (usually a light source or an accommodative target), (b) a system for recording the pupil dynamics (an infrared light source for recording in the light or dark conditions and a scanning device or a video camera), (c) a system for storing the recorded information in the digital format (usually a laptop) and a system of displaying the recorded data in the chosen format.

The detail of the information obtained by the imaging system is termed the resolution and it describes how close the two points can be to each other and still visibly resolved. A high resolution is an important feature of a pupillometer because it determines the quality of the image obtained and subsequently the accuracy of its measurement of the pupil dynamics. In digital images the resolution can be described in many ways in terms of space (spatial resolution), of time (temporal resolution), of size (pixel resolution), of spectrum (spectral resolution), or of density (radiometric resolution). Spatial resolution is the ability of an imaging system to discriminate between two adjacent high-contrast objects. The Pixels (picture elements) are the smallest units of an image, the higher number of pixels mean higher resolution. The spatial resolution also refers to the number of independent pixel values per unit length. Temporal resolution is used for the recording devices such as high speed camera. It describes a number of frames the device can register per unit time. The resolution provided by the commercially available pupillometers range from 0.01 mm to 0.1 mm for space measure and 5 to 400 Hz for time measure.

There are two types of light delivery optical systems in the pupil literature: those that use Maxwellian open loop optics and those that do not. In Maxwellian system, a

converging lens forms an image in the plane of the entrance pupil of the observer. This optical arrangement makes it possible to choose the point of incidence of the light. When it is set, all of the light from the stimulator passes through the centre of the pupil and potentially the whole of the retina can be illumined.

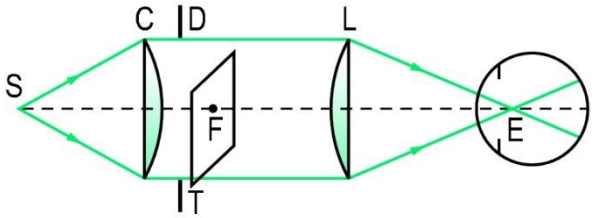


Figure 4.1. Maxwellian view system. S-light source, C-collimator, D-diaphragm, T-target placed in the focal plane of the lens L, F-first focal point of lens L and conjugate of the retina of unaccommodated emmetropic eye, E-centre of the entrance pupil of the eye and conjugate of S. Adapted from Dictionary of Optometry and Visual Science.[168]

This method is intended to overcome the issue of unequal illumination of retina due to differences in pupil sizes between the subjects (old vs young subjects) and within the subjects themselves (physiological anisocoria). Furthermore, the effect of optical aberrations of the eye is minimised. Distance of light source to the eye directly affects the intensity[169] as well as the area illuminated. With the Maxwellian view, it is thus important that the converging optics for both the infrared light source and the light emitting diode lights are kept in the centre of each pupil. In order to achieve this most systems have their optical units moveable in the x, y and z planes by motors often under software control.[31] This makes sure that the reflected image of the pupil and the light stimulus are kept in alignment during the course of the pupil light assessment. With non-Maxwellian view pupillometers, the optical units are fixed in a position so that the intensity and the illuminated area are kept constant.

4.2 Pupillogram

The recorded pupil dynamics are usually displayed in a graphical format. It is typically represented by a scaled record of time against movement (or dimensional changes) of the pupil, where duration is recorded in the abscissa and dimensional changes in the ordinate parts of the graph. This graphical record is called a pupillogram.

When a normal eye is subjected to a gradual change in the intensity of stimulus light, relatively little pupillary constriction results pertaining to the retinal adaptation that makes "pupillary effectiveness of the stimulus" less significant.[83] The pupil responds to the *flash* light, a stimulus that is too brief for the retinal adaptation, by a vigorous constriction and redilates during the withdrawal of the stimulus.[83] For the purposes of this thesis, pupil responses to flash light stimuli are discussed.

4.2.1 Reflex shape

The typical reflex shape of a flash light stimulus in human is of "V" pattern in the pupillogram – constriction followed by dilation.,[83;170;171] figure 4.2. However, the amplitude and the pattern can be variable from one moment to the next due to the constantly shifting background of sympathetic and parasympathetic efferent outflow as well as the higher centre inhibitory influences on the pupillomotor drive at any given time.[83] The reflexes become more shallow and irregular (square, W or attenuated V shapes) when the person is tired. These changes recover as the person becomes awake again. These reflex-shape changes due to physiological autonomic fluctuations are not repeatable as they merely represent the fleeting expression of the subject's momentary physiological state. The segment of the reflex shape that is least affected is the initial steep part of the constriction phase.[83] In pathological conditions, however, the changes in reflex shape are more permanent. In cases of optic neuropathy due to glaucoma the reflex shape obtained is delayed and attenuated due to slower and less pronounced pupillary response compared to the normal eyes.

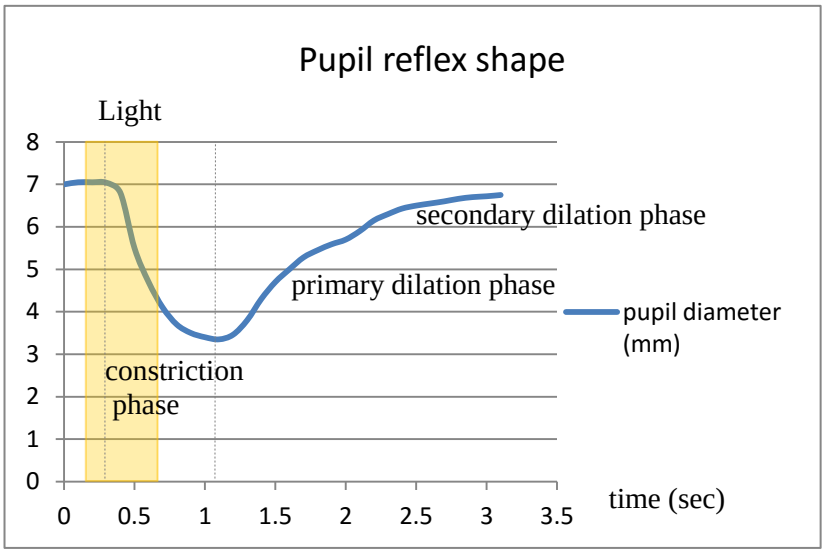


Figure 4.2. Pupil reflex shape depicting constriction and dilation phases.

The phases of a pupillogram can be described as below, figure 4.3.

At light onset,

(1) there is a latent period before it begins to constrict (*Lc*).

(2) during constriction, the velocity increases (*Ac*) and reaches its maximum velocity of contraction (*Mc*). The velocity of constriction reduces (*Dc*), before it achieves its maximal amplitude of constriction (yellow vertical line in the diagram).

At the light offset,

(3) the pupil tends to continue its constriction at the end of stimulus presentation before it dilates. The duration between the end of stimulus and the beginning of dilation is termed latency of dilation (*Ld).*

(4) the pupil then dilates more rapidly in the initial phase (*Ad*) followed by the slower redilation phase (*Dd*) till it reaches its pre-stimulus base line pupil diameter.

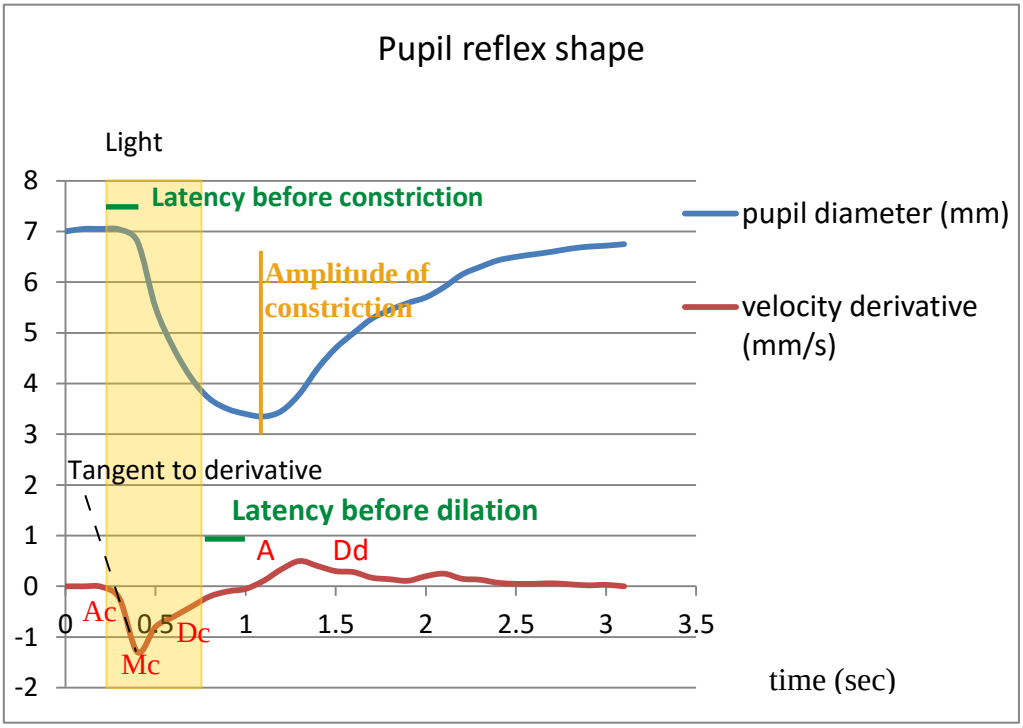


Figure 4.3. Derivative velocity curve as a useful adjunct to the pupillary response curve in interpreting a pupillogram. Ac(acceleration of constriction), Mc(maximal constriction velocity), Dc(deceleration of constriction), Ad(acceleration of dilation), Dd(deceleration of dilation).

The reflex duration is the time period between the beginning of pupil constriction and the end of pupil redilation. With a dim (low intensity) light the pupil may dilate (escape)

almost back to their pre-stimulus size while a brighter (high intensity) light may result in the less complete redilation.[83] Secondary dilation is less extensive than the primary dilation phase, and is extremely variable as to its presence or absence or its extent. Both constriction and dilation are rapid in the first part and slower in their last parts.

As depicted in the diagram, figure 4.3, the segments of the pupillogram are better appreciated using the first derivative of the pupil response curve - the velocity curve (red line in the diagram).[170] The rate of change is reflected in the distance of the curve from the base line. Although it is not shown in the diagram, the second derivative (acceleration mm/s^2) may be used to identify the points where the acceleration is maximum or minimum. The tangent line drawn on the velocity curve reflects the acceleration at the point concerned.

Direct response as well as consensual responses can be plotted on the pupillogram. For binocular pupillometers, both measurements are performed simultaneously. Normal healthy eyes with symmetrical crosses of pupillomotor fibres in their pathways of the light reflex, the direct and consensual reflexes are alike and mostly identical. Any differences or similarities that are present in the pupil dimensions during the light response are easily displayed on the pupillogram.

The commonly used parameters of the light reflex based on the phases described are: amplitude of pupil constriction, mean or maximum constriction velocity and latency of constriction. Mean or percentages of dilation velocity, constriction time, pupil size (pupil area, pupil diameter or pupil radius) are also used in the literature.

4.2.2 Autonomic components of the pupillogram

How do the autonomic components translate to the pupillogram that is recorded by the pupillometers? A wave form of pupil light response can be considered as an expression of the firing of the retinal ganglion cells. The characteristics of the wave are shaped by the sympathetic and parasympathetic outflows that reciprocally deliver the response via the muscles of the iris.

The human iris sphincter is mainly innervated by excitatory cholinergic nerve fibres[172] and sparsely by the adrenergic fibres.[172] The sphincter muscles have alpha and beta adrenoceptors.[172;173] Activation of alpha receptors causes contraction and beta receptors relaxation.[172] The dilator muscles are innervated by the adrenergic excitatory and cholinergic inhibitory fibres.[174] However, it is not known in practice these receptors involve in the pupil physiology. Although it is possible to study the receptors of the iris muscles in vivo or in vitro by using pharmacological agents, it is not easy to identify the specific receptor and the effect contributed solely by the sympathetic and by the parasympathetic system for the different phases of pupil light reflex using pharmacological agents in human. This is due to the inter-locked dependent effects of pupil size on parameters of light reflex, interdependence between the two eyes, dose response relation, and also drugs highly selective to a particular receptor may not be available. The muscles of the iris follow Sherrington's law of reciprocal innervation, whereby the phasic inhibition of the antagonistic outflow coincides with the activation of the agonistic muscle, combination of which bring about the pupillary light contraction and reflex dilation.

In the light reflex, it is commonly agreed that the constriction phases of the reflex are due to the cholinergic parasympathetic activation but modified by central sympathetic disinhibition of the EWN, figure 4.4.[171;175] It is assumed that acetylcholine (Ach) is also released in the antagonistic dilator muscle[176] to inhibit the action of noradrenaline activity to make constriction possible (Sherrington's Law). The primary dilation phase is mainly due to parasympathetic relaxation while the secondary redilation is due to an increase in peripheral sympathetic tone.[175] There is also a suggestion of cholinergic inhibition of the dilator muscle, and decrease in the level of central sympathetic inhibition on the EWN. [171;175] The reflex inhibition of the antagonistic muscles (dilator muscle during constriction and sphincter muscle during dilation) takes place in the central nervous system. This hinders the firing of the preganglionic motor neurones to the antagonistic muscles at a rapid rate.[17]

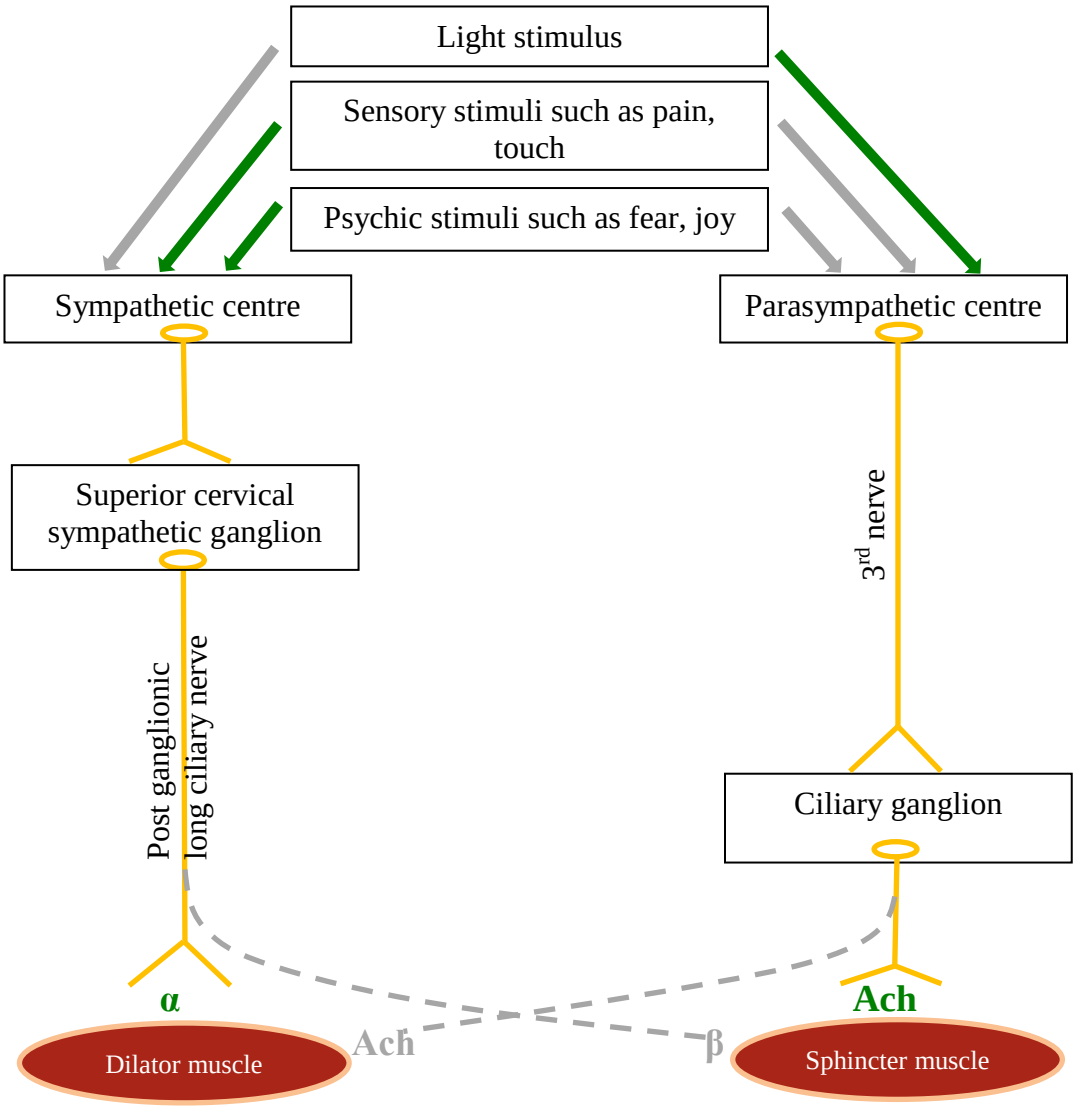


Figure 4.4. Sympathetic and parasympathetic innervations of the iris muscles. Green represents excitation and grey inhibition.

4.3 FACTORS THAT INFLUENCE MEASUREMENTS OF THE PUPIL LIGHT REFLEX

4.3.1 Local factors

4.3.1.1 Iris colour

The results of the histological study show no statistically significant difference between the melanin content of the iris in blue and brown eyes, but melanin pigments are present in higher amount in the ciliary body and retinal pigment epithelium-choroid from brown eyes than in blue eyes.[177] It may be that blue and brown eyes have different mix of eumelanin and pheomelanin subtypes. Blue and brown eyes with higher colour

intensity have more melanin than the corresponding eyes with lesser intensity of colour.[177].

Different studies showed that pupil size is not associated with iris colour.[178;179] However, one recent study on Caucasian population of Basel[179] has associated iris colour and differences in the dynamics of the pupillary light reflex. The authors reported that pupils with brown (grade 4) iris have larger and longer pupillary response compared to pupils with blue (grade 1) iris. According to their data, the pupil light response parameters most affected by the colouration of the iris are the contraction velocity (14% less in blue eyes), redilation velocity (15% less in blue eyes), and the amplitude of contraction (15% less in blue eyes). However, difference in pupil constriction time (the time interval between the onset of pupil contraction to the end of pupil contraction) was not found to be statistically significant in the same study. The initial pupil size and latency time before constriction were not found to be affected by the colour of the iris either.[179] It is not known whether amount and sensitivity of the adrenergic receptor in melanocytes (efferent pathway) or the melanocytes in the retinal pigment epithelial cells (afferent) are responsible for these differences in pupil dynamics of these eyes. Nonetheless, RAPD is a comparative test and little effect of iris colour is expected on the estimation of RAPD.

4.3.1.2 Pupil size and motor ranges of pupil movement

Resting pupil size reflects balance of parasympathetic & sympathetic 'tone' and has many influences, both external (ambient light), peripheral (retinal state, iris muscles) and central (autonomic status, arousal etc.). The effect of pupil size on the PLR is twofold: (a) under non-Maxwellian conditions less light enters the eye through smaller pupil, influencing the *afferent* limb of the reflex, (b) the mechanics of the iris and the length-tension relationships of iris muscles (section 2.3.3) affect the ability of the pupil to constrict to a light stimulus, i.e. an influence on the *efferent* limb of the reflex.

A normal pupil can change its diameter from 1 mm to 10 mm.[180] In extreme miosis sphincter muscles shrink to about 10 % of its original length; in extreme mydriasis, however, the dilator muscle contracts to a very narrow band which almost disappears behind the corneo-scleral band.[180] The linear relationship between the pupil size and the

pupil response is only true for a limited range of pupil diameters. Both pharmacological,[180] and accommodative studies[139;140;181] have shown that the pupillomotor responsiveness is nonlinear and works as a function of the mean pupil size. There is an operating range where the range of pupil responsiveness to the same light stimulus is determined by the range of different pupil sizes (size-dependent working range).

Nonlinear relation of pupil size to light, accommodation and vergence responses

Pupil escape is a description of a situation when the pupil, under a sustained light stimulation, does not maintain its constriction and redilates slowly after initial constriction. *Pupil capture* describes a situation when the pupil continues to constrict for certain period of time at the level of its peak constriction under sustained light stimulation. In photopic condition, the small pupil continues to stay small with tonic contraction (*pupil capture*) to limit more light entering the eye but in mesopic or scotopic conditions the large pupil constricts initially but redilates (*pupil escape*) and allows more light to enter the eye and constricts again. This visual homeostatic physiological reflex response is only possible because pupillary escape is intensified by the large pupil size and pupil capture is intensified by the small pupil size.[140] When pupil sizes are not controlled by light but by accommodation so that there is no effect of retinal bleaching or adaptation, the larger pupil shows phasic contraction with escape to the light stimulus but the smaller pupil responds by a tonic contraction and pupil capture, figure 4.5. This is again true for vergence pupil responses.[139]

In order to find the working range of different pupil sizes, Stark and colleagues tested the pupillomotor response on a range of pupil diameters. The pupil sizes were controlled by the operating light levels and accommodation (4-dioptre step changes) was used to measure the pupil responsiveness. In this case, the stimulus is the accommodation as the subject of interest is the motor range of movement brought on by the near pupil reflex. The contraction amplitudes are particularly large for the pupil sizes between 4 and 6 mm and smaller for very small pupil sizes ≤ 3 mm or those of ≥ 7 mm, figure 4.6.[139] Similarly, pupil responsiveness is least for extreme pupil sizes, <3 and >6. The responsiveness seems to peak between 4-6 mm, figure 4.7.

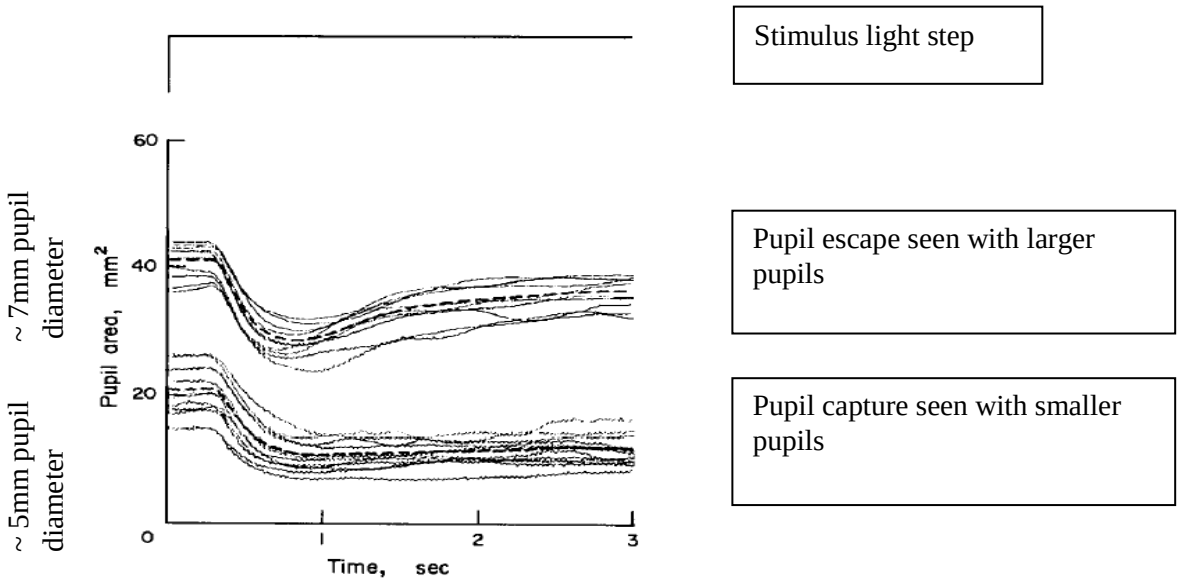


Figure 4.5. Pupil size is adjusted by accommodation. Equal dim light step stimulus or sustained stimulus of 0.035 ft-L is presented from dark adapted level (1x10-4 ft-L). Solid lines represent single responses. Dash lines represent mean values. Experiment of Sun and Stark 1982.[140]

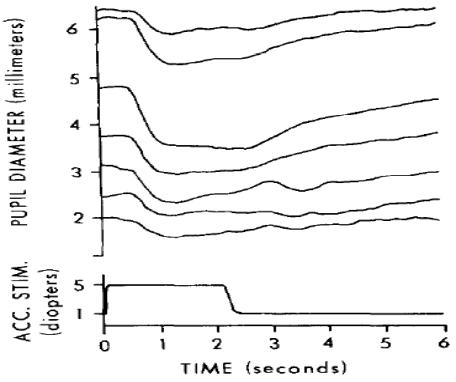


Figure 4.6. Accommodative responses of different pupil sizes to a 4 D accommodative step stimulus. Semmlow and Stark.[139]

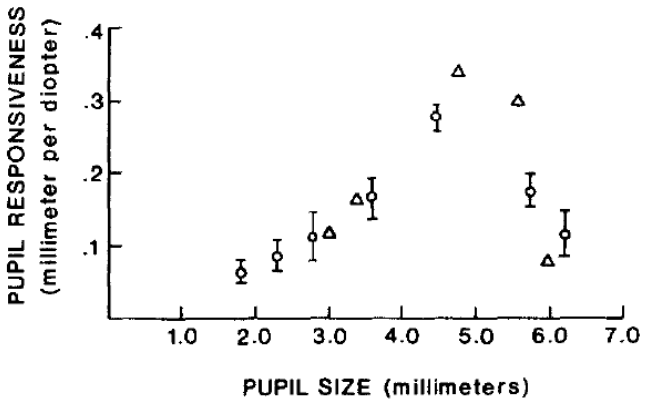


Figure 4.7. Pupillomotor responsiveness as a function of mean pupil size. Range behaviours average of 2 subjects determined by accommodative stimulation (circle- triangle). Experiment of Semmlow, Hansmann and Stark 1975.[139]

Again, looking at the individual contractions for large pupil sizes of young adults, Loewenfeld and Newsome[180] noted that the contracting pupil by light stimulation slows its rate of contraction when it reaches a certain size. There is a small inter-individual variability of this *critical pupil size*, for the same subject it is a constant value and lies somewhat between pupil size of 2.5 mm and 3.5 mm. This critical value is well above the pupil's absolute limit of contraction, not affected by age, or the intensity of the light. Also for both large and small reflexes by a bright and a dim light stimuli, the pupil changes its speed at the same critical reversal point.[180] The authors noted that this is also true for the dilator reflexes, above the critical pupil size; pupil dilating response by the darkness stimulus becomes progressively inextensive and slow.[180]

Loewenfeld's study seems to suggest that there is a range of linearity in the pupil responsiveness between the two critical sizes closer to the extremes, but Stark's Study suggests that it is alinear throughout all range of pupil sizes, figure 4.7. However, both authors agree that the changes are most pronounced at pupil sizes close to the extreme ends of the range or near to the extreme ends of movement. It is still not clear whether or not the pupillomotor responsiveness of the middle range size pupils have linear or curvilinear relationship with size. The differences of the two studies may be due to the differences in the nature of the experiments themselves – mechanical vs pharmacological, accommodation response vs light response, or due to measurement inaccuracies or small number of samplings in both groups. Nonetheless, it is unquestionable that the pupil reaction is size dependent and differences are more pronounced near the lower and higher end of the pupil sizes but not reaching the extreme ends.

What causes this non-linearity in the pupillomotor response? The above findings suggest that this pupil size effect is not related to retinal adaptation,[181] the amount of light reaching the retina, [181] or to a form of peripheral nervous system operated mechanism feeding back to the brain. [181] The range response behaviour is consistent for light response, accommodation response, and vergence response;[139;181] This is not affected by age.[180] In pharmacologically tested pupils (cocaine, cyclopentolate, physostigmine, and guanethidine)[180] the effects manifest at a specific pupil diameter for each individual, and the amplitude and velocity are affected more than the duration of

the reflex. These all point towards a mechanical limitation. One explanation to this mechanical phenomenon is a length-tension relationship of iris muscles.[139] During pupil contraction, the area of the outer ciliary ring (containing dilator muscle) enlarges in its area as the pupil becomes smaller but the area of the inner ciliary ring collarette (containing sphincter muscle) remains relatively constant despite the increasing area taken up by the iris tissue.[182] Consequently, as the pupil becomes smaller, compaction of the iris tissue in the collarette poses a mechanical limitation to movement of the iris, resulting in a non-linear plateauing of the contraction in response to the stronger light stimuli.[182] Stark and Colleagues[140] described this pupil behaviour as "memory-dependent" non-linear system. Other terms also used are the "floor-effect" of the iris.

Pupil size and increasing light intensity (short flashes)

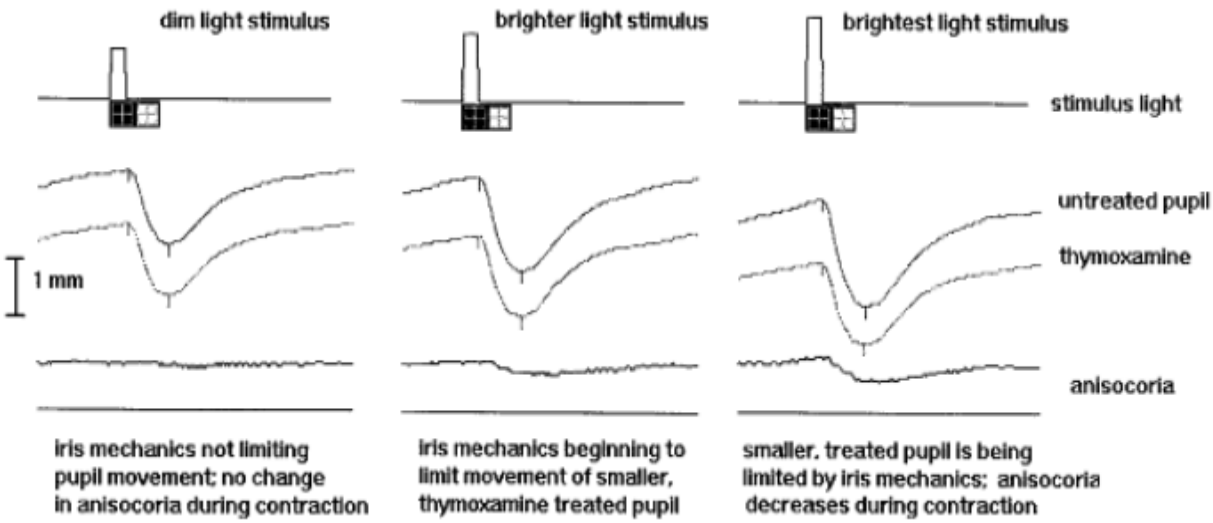


Figure 4.8. Effect of stimulus light intensity on the thymoxamine treated (small) and untreated pupil. Experiment of Kardon. Duane's Foundation Ophthalmology 1997.[182] The authors used the Maxwellian optics to deliver the light stimulus so that equal amount of light enters the treated and untreated eyes.

When increasing intensities of short flashes of light are presented to a normal eye and an eye which is treated with thymoxamine (alpha adrenergic blocking agent which works by competative antagonism of noradrenaline thus making the pupil miose), it is seen that the dim light stimulus produces equal amount of pupil constriction to both treated and untreated eyes, figure 4.8. As the stimulus light gets brighter, the treated small pupil has less room for movement before encroaching onto the mechanical non-linear zone and fails to constrict as much as the untreated eye (anisocoria reduces). The untreated pupil "catches-up" with the treated pupil at the peak contraction. [182] The

experiment of Kardon agrees with the theory of a mechanical factor limiting the pupil movement and it reinforces that the smaller pupil has less range of movement before it reaches the zone of mechanical limitations.

Pupil size and light reflex parameters relation: interdependence of parameters.

In the experiment by Semmlow and Stark (1975),[139] they plotted the plane phase trajectories of velocity of pupil movement against pupil diameters, figure 4.9. The findings suggest that the amplitude of constriction is the only major response variation that is attributed by the mean pupil size while velocity and duration are consistent with the amplitude as seen in the trajectories. A recent larger study by Bremner F[109] further confirmed that the speed and extent of pupil response are covariant and this reflects intrinsic mechanical properties of iris, and that there is no additional gain by measuring velocity as well as amplitude of constriction in clinical practice. Age and intensity determine the pupil size, and the size determines the amplitude which in turn determines the velocity. When this relationship is interrupted, for example by adjusting the effect of pupil size on amplitude, there will be no association between age and amplitude of response. The pupil dilation and recovery, however, are found to be independent of pupil size in another study by Heller.[171]

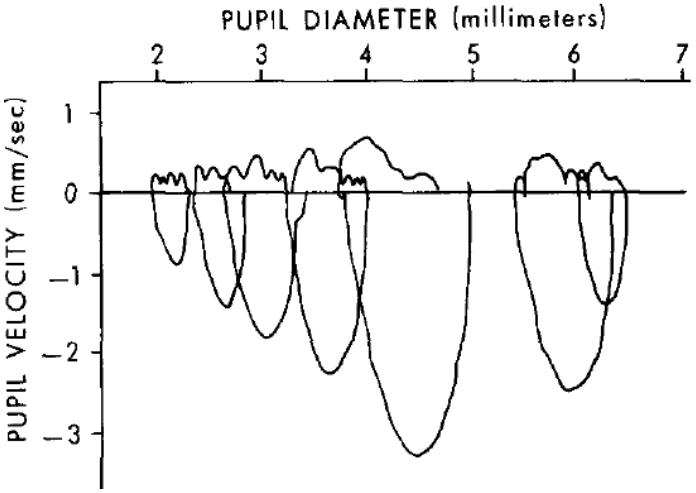


Figure 4.9. Diameter-velocity phase plane trajectories, downward contractions, upward dilations. "All constriction movements have peak velocities and duration consistent with the movement amplitude, and major response variation with mean pupil size is one of mean amplitude alone."[139] Experiment of Semmlow and Stark 1975.[139]

Pupil size and stimulus frequency relation

Pupil size also has a correlation with the variation in frequency of ON-OFF or sinusoidal stimulus light. If "gain" is described as the percentage change of pupil aperture by percentage change of light, [140] when subjected to increasing frequency of ON-OFF or sinusoidal light stimuli, the gain produced by the small pupil reduces with increasing frequency (an equivalent of pupil capture in frequency domain). This means to say that pupil changes per light intensity is higher for the low frequency stimulus than for the high frequency stimulus. The large pupil, however, has an increasing gain followed by reduction in gain (an equivalent of pupil escape response in frequency domain) with increasing intensities (figure 4.10). Low frequency responses are much reduced for the large pupil.[140] Although small pupils have main gain over large pupils for all ranges of frequencies, at extreme frequency of stimulus, both pupils have very little gain.

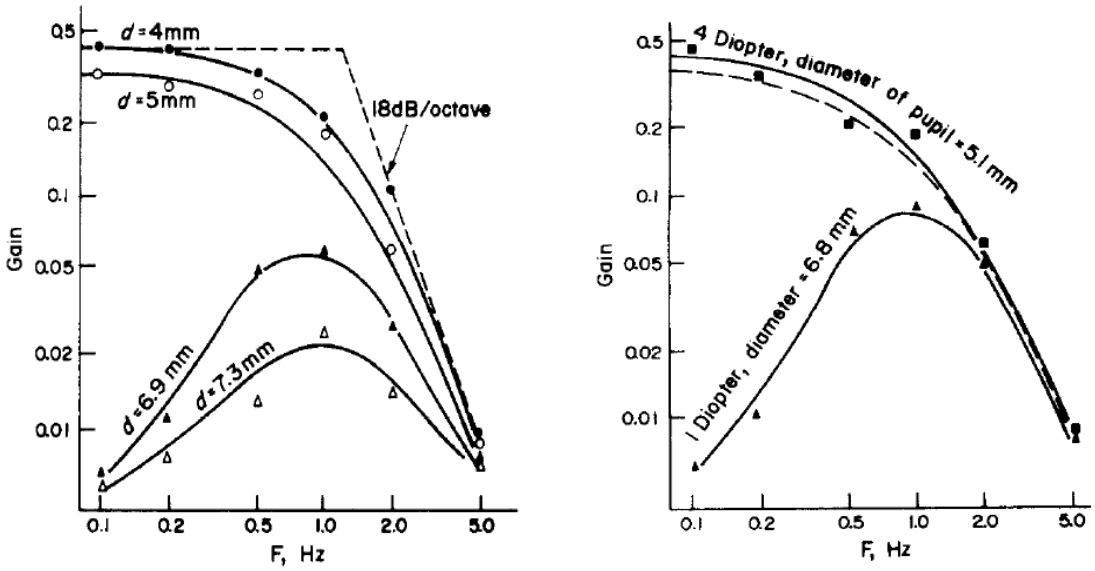


Figure 4.10. The frequency-gain relation for large and small pupils. Left diagram is when the pupil size is controlled by operating light level. Right diagram is when the pupil size is controlled by accommodation targets. Experiments of Sun and Stark 1982.[140]

In conclusion, the mechanism of the "pupil size effect" is dependent on the retinal level only so far as retinal activity sets pupil size.[140] The size effect is considered to be due to the mechanical phenomenon which produces an expendable non-linearity in the working ranges of the pupil.

4.3.2 Demographic factors that affect pupillary light response

4.3.2.1 Age

Age and pupil size

Pupils of infants and old people are smaller than the rest of the population. Pupil diameter increases from infancy, is largest between age of 12 and 20 and decreases gradually thereafter, figure 4.11. The small pupil in infants is due to their having relatively small eyes, incomplete development of peripheral adrenergic transmission, immaturity of the brain and low levels of emotional activities, sympathetic discharges and central inhibition of the parasympathetic nucleus compared to a fully mature person.[183] Change in pupil size with advancing age is largely contributed by the progressively decreasing central inhibition to the EW nucleus[183] rather than the atrophy of the iris muscle which only happens at a much later age. The rate of change of pupil diameter with age is approximately 0.043 mm per year at low light levels and 0.015 mm per year at high light levels.[178]

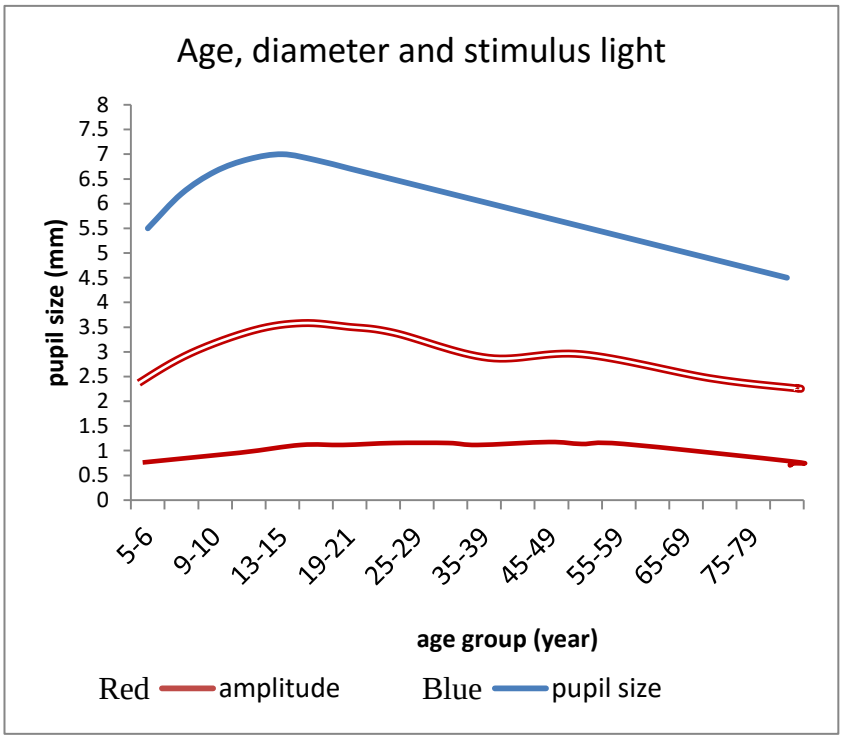


Figure 4.11. Age pupil size relation showing gradual increase in pupil size from young to about 20 years of age and gradual decline in size with age. Red tram line (=) represents pupil constriction amplitude with 3 seconds stimulus light, and red solid line (▬) with 1 second stimulus. The constriction amplitude generally corresponds to the pupil size, with longer duration of light, more mechanical input is required but with shorter stimulus less variability is noted across the age group. This graph summarises Loewenfeld's graphs on age related changes in chapter 10. Reflex integration: Pupillary Consequences. Pupil. Anatomy, Physiology, Clinical Applications 1999.[183]

Age and light reflex

Children between age of 3 and 13 have relatively large pupil but inextensive pupil light reflex. This is due to strong emotional factors centrally inhibiting the EWN for parasympathetic pupil constriction outflow. [183] This is overcome by the use of long lasting or bright illuminations.[183]

For those above 60 years of age, anatomical changes in the iris (such as degree of iris atrophy) as well as diminution of inhibition to EWN are responsible for relatively smaller pupil with inextensive reflexes. Reflexes are also more attenuated with a rapid rather than slow rate of stimulation.[183] The inextensive reflexes in these cases may be overcome by the use of weak or short flashes that do not require much mechanical work. If a strong light is used, their small initial pupil size will limit the amplitude of pupillary constriction.[183]The pupillographic features of flattened reflexes of the older (>60 groups) are similar to those of the young subjects who are fatigued but these changes in the latter are transient and reversible.

In summary, the primary change in the constriction part of the pupillogram in association with age is due to the size of the pupil. Other changes such as constriction amplitude, velocity and duration are related to pupil size effects. As with small pupils, lower intensity and low frequency stimuli can maintain its constriction and reflex shape. Latency before constriction is found less affected.[184;185;185;186] The deficits in the dilation part of the pupillogram are considered to be associated with the sympatheticdeficit.[184]

4.3.2.2 Gender

Pupil size is found to be independent of gender[178;187]. No significant gender effect is also found in pupil reflex characteristics.[188;189] One recent study has reported slight male preponderance in the contraction anisocoria in situations when contraction anisocoria is larger with right eye stimulation than the left eye.[190]

4.3.2.3 Lateralisation

There is not enough evidence to conclude that there are significant differences in pupil size and the PLR measurements between the left and the right eye. Although one recent study[191] suggested that there are differences in relation to the cortical lateralisation of

the central autonomic nervous system, the study did not measure both eyes simultaneously, and the structure of the study was weak for this conclusion. Lateralisation on the contraction anisocoria, however, was commented by another study stating that stimulation of right eyes produced a larger contraction anisocoria than stimulating the left eyes, and that this effect is more pronounced in males than in females.[190]

4.3.3 Internal factors that affect pupil response to light

4.3.3.1 Central and autonomic nervous system

Pupil size is regulated via the autonomic nervous system.[192]The pupillary reaction to light is subjected to the supra nuclear influences such as cortico-thalamo-hypothalamic influences, psycho-sensory influences and tonic inhibitory inputs from the cerebral cortex to the EWN.

Wakefulness or pain, for example, will stimulate the oculosympathetic pathways and inhibit the parasympathetic Edinger-Westphal nucleus. This keeps the pupil dilated. Conversely, drowsiness causes disinhibition of the sympathetic inhibition to the EWN with resultant miosis of the pupil. Likewise, the diminution of sympathetic inhibitory input to EWN results in the pupillary constriction during sleep. Upon awaking there is a restoration of the reduced reflex described by *psycho-sensory reconstitution phenomenon or arousal phenomenon.*

A pupillographic tracing of a normal alert person with intact sympathetic and parasympathetic nervous system will show a regular oscillations of the pupil size with an average frequency of 1 Hz in steady illumination.[193] This pupillary unrest is called ***hippus*** (also see 2.1.1). Although the term "hippus" was meant for "the pupillary unrest of abnormal degree" in nineteenth century for various neurological diseases, it has become a term for a normal physiological behaviour.[194] Any supranuclear stimulus that disturbs the sympathetic and parasympathetic balance will result in alteration in the frequency and amplitude of the spontaneous oscillations as well as the size and shape of the light reflex.[175]

Pupil size in darkness is proportional to the level of central sympathetic tone.[192] Pupillary oscillation in darkness can be used as a marker for monitoring sleepiness[193] and pupil diameter correlated with individual's subjective feeling of tiredness.[195] Hypersomniacs have much higher amounts of pupillary oscillations in darkness than normal, figure 4.12.[193] As the state of alertness decreases, pupils (a) miose (b) fluctuate (sleepiness waves) and (c) becomes less responsive to light.[196] These features are important as there is little control over the patient's state of alertness during the pupil test.

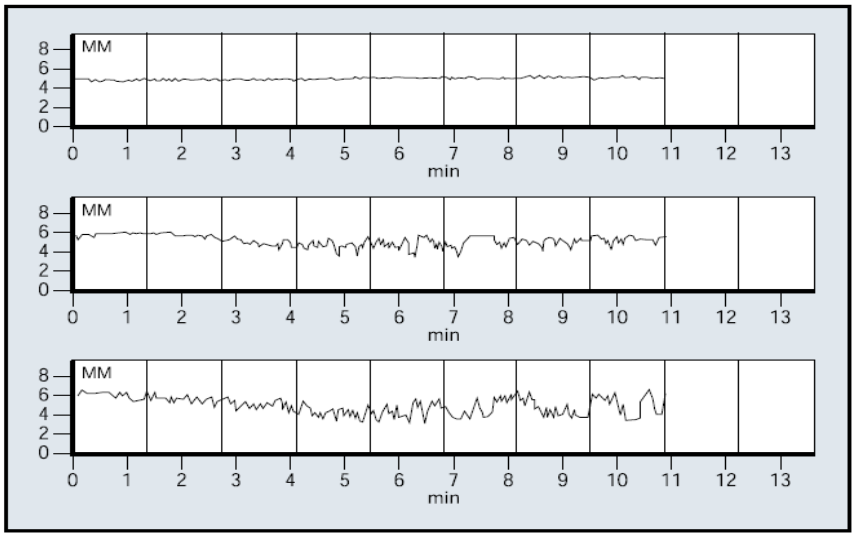


Figure 4.12. The pupillograms of a normal person and 2 hypersomniacs. The normal person has small regular spontaneous oscillations whereas hypersomniacs show slower, deeper and irregular waves. Adopted from Kawasaki et al. 1999.[197]

4.3.4 External factors that affect pupil response to light

4.3.4.1 Background light (retinal threshold and adaptation)

A rapid increase in illumination level brings about a marked pupillary constriction while the same increase of illumination brought about gradually so that the retina has time to become adapted to the new conditions will induce little or no change in pupil diameter.[198]

The visual system has a remarkable ability to shift its optimum operating level depending on retinal luminance, thus allowing it to function over a range of luminance. Visual adaptation spans at least over 10 log units to maintain stable visual sensitivity. In sustained stimulus (or background stimulus) the pupil adapts to the new light level.

Although it may not dilate back to the size in the complete darkness, the baseline pupil gets larger to an equilibrium diameter. The pupil thus sets its base to the new level. The time it takes to reach the equilibrium level varies with the condition employed but for illumination between 100 and 1,100 lux (2 to 3 log units) it takes about 15 minutes on average.

When the adaptation is changed in this manner and the intensity remains constant, the maximal pupillomotor effect changes over from yellow to light green, thus producing a counterpart of the Purkinje phenomenon called ***pupillomotor Purkinje phenomenon.***[199] This is because spectral sensitivity for the rods is at green and for cones is at yellow. By adaptation, rods begin to function along with cones.

Pupillary *threshold stimulus* is the minimum stimulus required to elicit pupillary contraction. Low threshold is associated with high pupil sensitivity to light. The threshold stimulus required to elicit pupillary contraction varies with the region of the retina stimulated as well as being a function of age.[189] A minimum pupillary reaction can be achieved with illumination corresponding to the *absolute perceptual threshold*. Pupillary movement can also be obtained by a d*ifference* in the intensity of two lights *or differential threshold* for light.[199] The difference in the intensity of 2 lights, the alternation of which brings about a perceptible pupillomotor response, is 95:100.[199] It is independent of the absolute values of the stimuli but varies with their gradient.[200]

Under dark adaptation, the fovea shows a decreased sensitivity compared with surrounding retinal areas due to the lack of rods in the fovea. Therefore, in dark adaptation or when the stimulus light has low intensity, the pupillomotor responsiveness is higher in the periphery compared to central retina but the amplitude of contraction produced is lower than it would be for the central retina.[194] In mesopic and photopic adaptation, the pupil responds in the central field where cones and ipRGCs are densely populated; the temporal field response is usually greater than the nasal field response.[182] Pupillary sensitivity of the retina is therefore very similar to visual sensitivity.[194]

4.3.4.2 Light Stimulus

4.3.4.2.1 Intensity

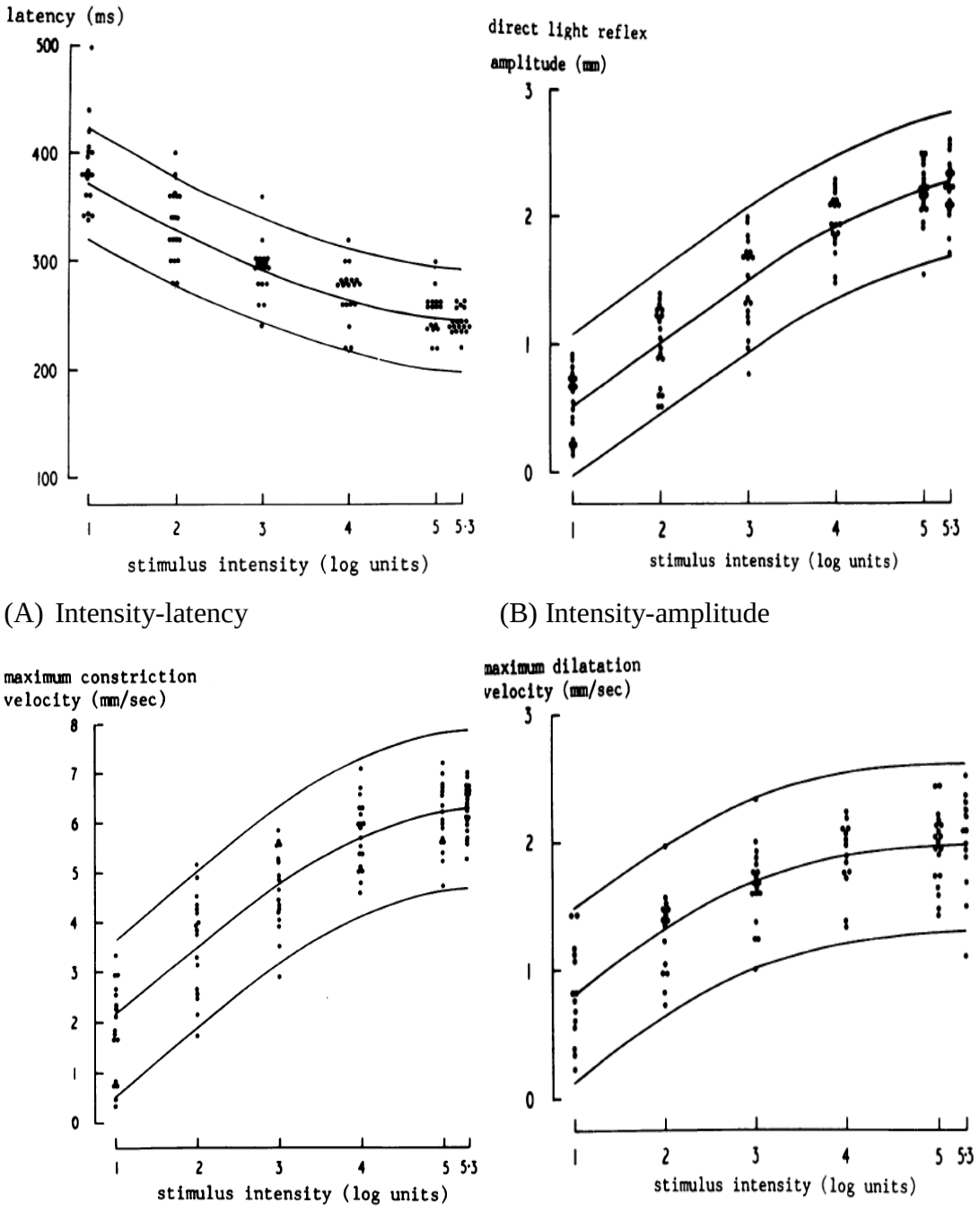
Intensity of light equates the amount of flux of photons reaching the photoreceptors and drives the pupillomotor response. However, the influence of the intensity on the pupil reflex does not depend on the physical energy of the light alone but varies with a number of factors - the colour of the light, the duration of the light, the speed at which the stimulus is delivered, the area of the retina that receives the light (stimulus size), distribution of the retina (e.g. fovea vs macula vs periphery) as well as the adaptation of the retina – the effects of which are interlocked.[83] Although, in theory, the product of intensity and the area of the retina stimulated can be regarded as a unit that brings about a constant amount of pupillomotor response, when other conditions are standardised, it is not always the case. This is because of the differences in threshold and supra-threshold values of the pupillomotor responses across the distribution of the retina. For example, a very bright small light may not produce the same amount of pupillomotor output as a very dim light lit over a larger area since the latter may not pass the pupillomotor thresholds. Also, it is important to consider the possibility of light scatter and the stray light that will inadvertently involve in the pupillomotor drive especially when the larger area is stimulated. Media opacity such as cataract can cause light scatter, and small pupils can potentially alter the intended area of retinal illumination. If the size of illumination is small less robust constrictions may be obtained compared to a larger size stimulus.

Logarithmic increases in light intensity are associated with roughly linear increases in pupillomotor effectiveness with the exception of except for in the highest and the lowest intensity levels where the increments are modest.[83] Higher intensity would mean higher firing rate of the retinal neurones,[201] and thus proportional increase in the efferent drive to the iris sphincter muscle as far as the muscles of the iris allow. High intensity stimulus is thus associated with shorter latency time before constriction, high constriction amplitude, high velocity of constriction (mean or peak), prolonged constriction time[83] and high velocity of redilation (mean or peak).[202;203] Low intensity of stimulus light, conversely, produces a reflex which has long latent period (usually >0.5 seconds), slight, slow and short contraction.[83] These reflexes are called the *low*

intensity reflexes, and are commonly found in afferent pathway diseases such as glaucoma.

When the intensity of light is increased over a range of approximately 3 log units above the scotopic visual threshold the pupillary contractions become gradually stronger and less variable.[194] When the stimulus light is further increased the reflex begins to grow markedly in amplitude, velocity of contraction and duration of contraction until the maximum value is reached which is normally at about 7-9 log units above the threshold.[83;194] Very powerful light flashes fail to add further to the amplitude and speed and they do not reduce the latency further but they greatly prolong the contraction; after such stimuli the pupil may remain in spastic miosis for several seconds.[83;194] It is considered that the after-image is related to delay in redilation of the pupil.[194]

At lower intensities (intensities hovering around the threshold levels) there is higher variability in the retina sensitivity and higher variability in the pupillomotor response which is evident both inter and intra individually.[83;194] But when a number of reflexes are averaged, the results are less variable.[83]Ellis CJK[203] (1981) tested 19 healthy subjects for the effects of intensity against latency before constriction, amplitude of constriction, maximum constriction velocity and maximum dilation velocity. The spread of inter-individual variability for 1 to 5.5 log units of intensities can be appreciated from Ellis's diagrams where 95% of the normal limits are shown for the segment of intensities that the author used, figure 4.13.



(A) Intensity-latency (B) Intensity-amplitude

(C) Intensity-maximum constriction velocity (D) Intensity-maximum dilation velocity

Figure 4.13. Effects of stimulus intensities on pupil light reflex parameters. Experiment of Ellis CJK[203]

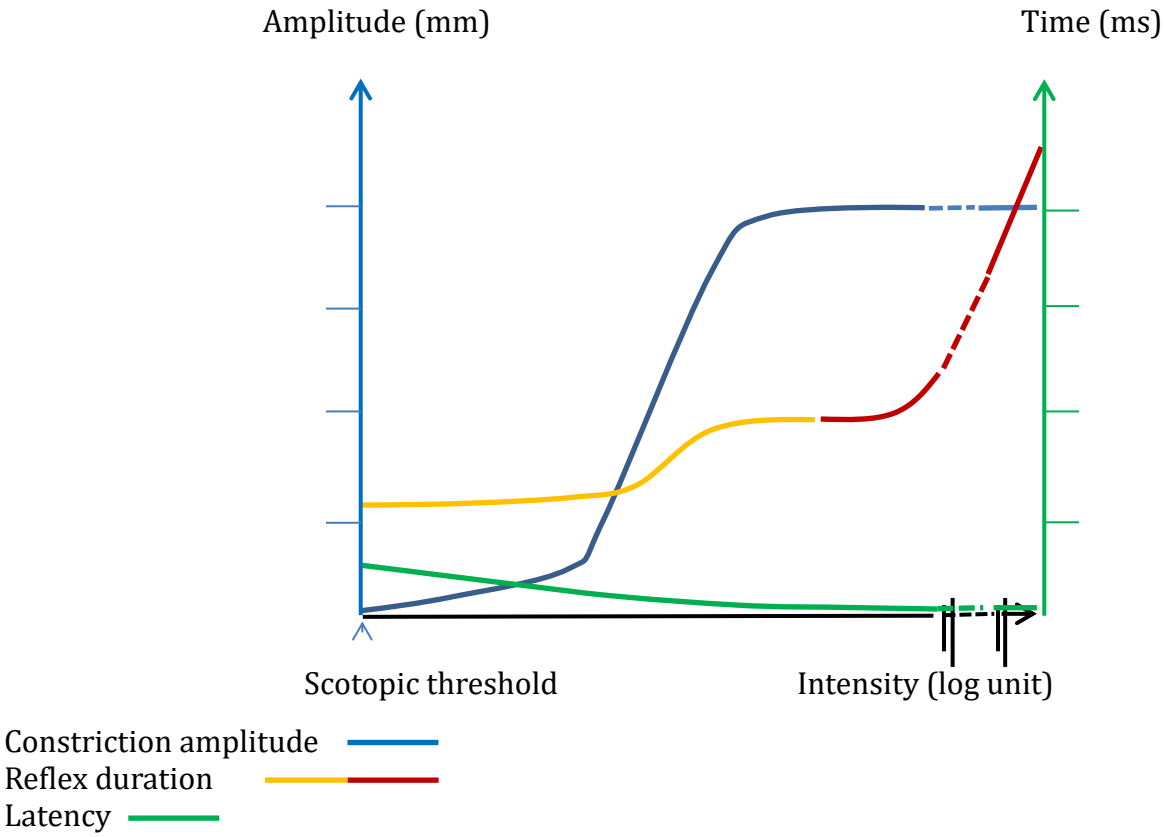


Figure 4.14 .Intensity- response curve depicting constriction amplitude (blue line), latency time (green) and reflex time mainly contributed by increase in constriction time with increase in velocity (yellow) by increase in redilation time (red). The diagram redrawn from Loewenfeld. The Pupil. Anatomy, Physiology and Clinical Applications 1999. [83]

Intensity and latency

Latency decreases with increasing intensity to a minimum at about 9 log units above the scotopic visual threshold and further increases in intensity are no longer able to shorten the latency.[83;203] The intensity-latency relation seems to be mildly curvilinear respecting the equation $Y= a + bX + cX^2$ (X= intensity, Y= amplitude).[188;203] There is a wide range of latency time (200 – 500 ms)[182;203] reported in the literature for those stimulus intensities of 1-5 log units. Latency time has higher variability between subjects at the lower intensity levels. In a fully dark adapted eye, if increasing flash lights are presented, latency time may decrease from high maximum of 500 ms to as low as 200 ms in very bright light. Latency time becomes prolonged with dimmer stimuli, in the range of 20-50 ms further delay for every 1 log unit decrement of light intensity.[182;203] Latency time of direct and consensual light responses are similar for an individual person, figures 4.13 and 4.14.[203]

Intensity and amplitude

Pupil amplitude of constriction increases with increasing stimulus intensity.[83;182;188;201;203] The entire response function of intensity-amplitude curve resembles a "S" shape when log unit intensity is plotted against the amplitude of constriction. The initial small modest increase, just above the level of pupillary threshold, follows a low plateau, and followed by a sharp rise, and then gradually lessening increments per unit of stimulus intensity until the curve flattens entirely which is normally at about 9 log units above the absolute visual threshold, figure 4.14.[83] The amplitude increases linearly over at least a 3 log-unit range of log intensity light stimulus in the middle segment of the curve.[182] The intensity-constriction amplitude relation respects the equation $Y= a + bX + cX^2$ (X= intensity, Y= amplitude).[188;203]

The inter-individual variability of constriction amplitude is high at the lower end of the intensities. Therefore, it is important to factor in the issues of variability if very low intensity stimulus light is to be used for experimental purposes. For clinical purposes, higher intensity light gives more pronounced pupil reaction with less variability than low intensity stimulus.

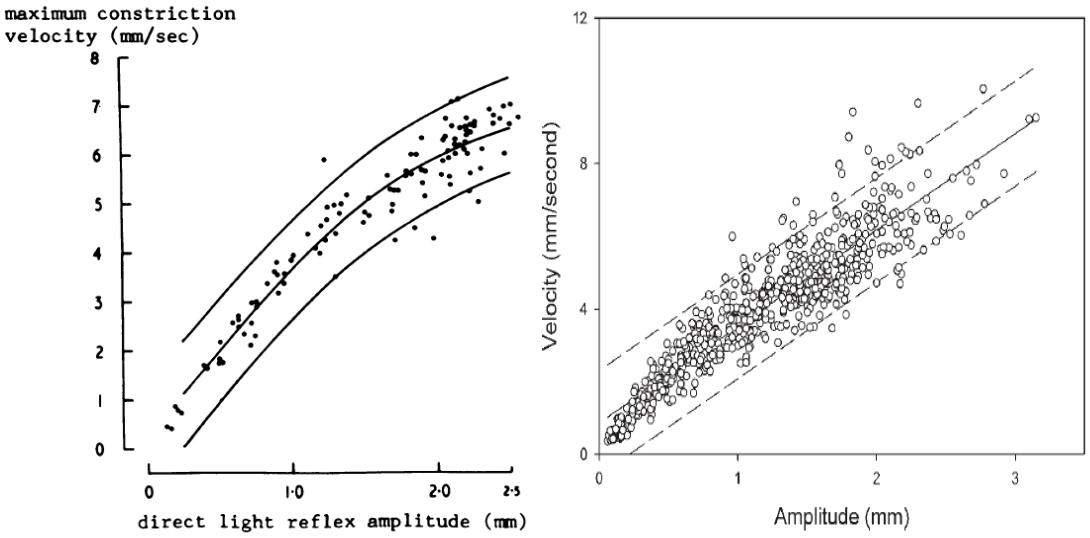
Intensity and duration of reflex

When intensity is increased in even steps from scotopic visual threshold, duration of reflex (from the beginning of pupillary constriction to the end of pupillary redilation) increases but has two phases, figure 4.14. In the scotopic range, there is only slight prolongation of reaction, but when the cone threshold is exceeded, contractions are much more prolonged until the next plateau is reached, figure 4.14. The change in the reflex time after this phase is mainly due to the prolongation of redilation since redilation becomes progressively weak but there is no further change in the constriction time (from the beginning of light to peak contraction).[83] When the intensity is about 9 log units above the absolute threshold, there is little or no redilation but only contractions that last for several seconds even after the stimulus is offset.[83;194]

Intensity and constriction velocity

Intensity also has a relation with mean or average constriction velocity indirectly through the amplitude of constriction in association with pupil sizes as described

previously in section (4.3.1.2). This means to say that the amplitude of constriction and the constriction velocities are interdependent, and the increase in velocity is proportional to increase in amplitude for each level of light intensity, figure 4.15. The effect of intensity on velocity goes as far as the effect of intensity on amplitudes. Therefore, intensity-velocity curve is expected to be similar to an intensity-amplitude curve.

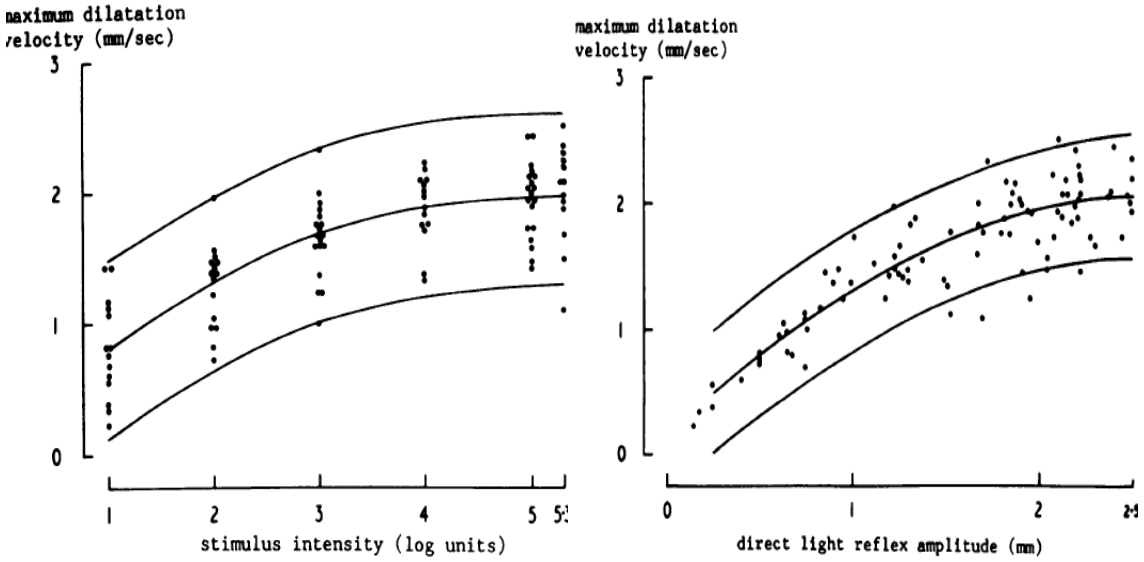


(A) (B)

Figure 4.15. Amplitude velocity relationship. (A) Experiment of Ellis[203] on 19 subjects with intensity range between 0 and 5.2 log units[203] (B) Experiment of Bremner[109] on 43 subjects with intensity range between 0 and 4 log units.[109] It can be seen that relationship between the amplitude and velocity is fairly linear up to 4 log units [Velocity = 0.86 + (2.65 x Acceleration)], above which there is a degree of plateauing effect on the velocity of constriction.

Intensity and dilation velocity

Maximum or mean dilation velocity increases with increase in the stimulus intensity. The intensity-velocity relation follows the equation $Y= a + bX + X^2$. Similar to maximum constriction velocity, maximum dilation velocity also has a proportional relation to the amplitude of pupil constriction, figure 4.16. [188;203] These relations may be attributed to the pupil size effect.



(A) (B)

Figure 4.16. (A) Intensity and dilation velocity (B) constriction amplitude and dilation velocity relations.[203]

4.3.4.2.2 **Stimulus size**

A larger size stimulus is associated with more neuronal firing for the pupillomotor response. Stimulus size, however, does not work alone. Intensity, duration, as well as retinal adaptation status all contribute to the effective pupillomotor response. It is described above that retinal sensitivity (threshold) level is different between fovea and the surrounding area of the retina in the dark adapted eye.

A small size stimulus can differentiate the sensitivity of fovea and the surrounding area more than the larger size stimulus. With a larger size stimulus, the retinal sensitivity profile is less different across the retina.[201] With larger size stimulus more robust pupillary constriction can be produced even in the peripheral retina with stimulus intensities within the range where scatter of light is negligible.[201]

4.3.4.2.3 **Duration**

It is mentioned above that the parameters of stimulus and the light reaction are interlocked and stimulus intensity, duration and area of stimulation all contribute to the retina effectiveness. The short flashes of light for a given intensity deliver less energy than the longer flash of light. How does the pupil respond to the changes in the stimulus duration? The relation between the duration and the effectiveness is complex. Its

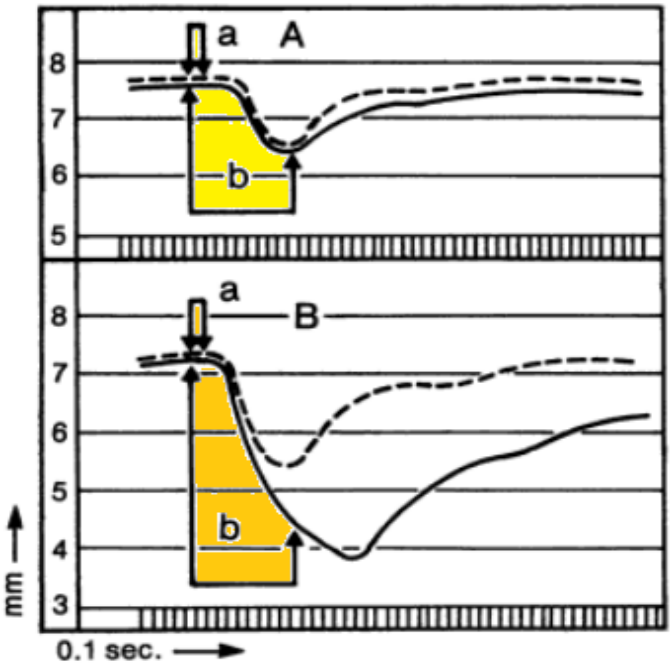
relationship is different for the short flashes of light and for longer sustained stimuli because the longer stimulation allows adaptation as another factor to manipulate the correlation. For the purposes of this thesis, only the relationship for the short flashes of light is discussed.

When the dark adapted eye is exposed to light flashes of a very short duration, the pupillary threshold is very low and distinct pupillary reactions can be obtained well within the first log unit of the stimulus luminance above the scotopic visual threshold.[194] ***Temporal summation*** is the ability of the system to sum up the energy impinging upon it within a given period of time.[83] The photoreceptors have this ability to sum up the energy in the time manner before the response is actioned. Within certain limitations and criteria, there is reciprocity (interchangeability) between the intensity of light and its duration. For example, for a fixed period of duration, intensity has a positive correlation with the responsiveness; and conversely, for a fixed intensity, the duration has a positive correlation with the responsiveness; therefore increasing either one has the same result. For the same response or effectiveness, the product of the intensity and duration has to be constant: intensity x duration = total energy delivered (area = constant, within critical period).

The period of during within which the reciprocity exists can be termed as ***critical period***. The critical period is said to be between 1 and 100 ms.[83] If the duration is reduced from 100 ms to 1 ms, which is the lowest for the duration attainable, the intensity will reciprocally increase to a hundred fold (2 log units) for its highest limit for reciprocity. Above this intensity, the effectiveness rises with the intensity only.[83] Duration no longer contributes to the pupil responsiveness for a certain range of intensity. When the test is done in the darkness, critical duration is between 75 to 100 ms but in light- adapted eyes the critical duration is shorter, between 30 to 70 ms.[83] For the near-threshold stimuli the critical period for intensity-duration reciprocity is similar for the pupil and for the visual detection which is at about 70 ms.[83]

What about for those intensities larger than 2 log units above the scotopic visual threshold? Reciprocity of the intensity and duration holds true for reactivity near the threshold. Out of the critical period, for the lower range of intensities (~3log units above the threshold) the pupil response is a shallow (smaller constriction) and slow (longer

duration) and the changes in duration has no effect on the response, figure 4.17. This is typical of a ***low intensity reflex light***. For stronger stimuli (~4-9 log units above the threshold) the duration begins to take part and produces stronger and longer contractions with longer durations; however, latency and the speed of contraction are unaffected in this case.



A. Low intensity lights produce reflexes with long latency, slow and shallow constrictions, regardless of duration.

B. High intensity lights produce stronger reflexes with shorter latency and faster velocity and larger constriction. Longer duration of light further extends the amplitude and constriction time but does not alter latency or velocity.

Figure 4.17. Light reflexes elicited by short (a, 100 ms) and long (b, 1000 ms) light flashes. (A) 3 log units above visual threshold (low intensity light) is used. It can be seen that the response for the 100 ms and 1000 ms are the same – shallow, short contractions. For 1000 ms, for this dim light stimulus, the pupil begins to dilate before the stimulus is terminated (B) 9 log units above the visual threshold (high intensity light) is used. Both durations produce the same but short latent periods and peak high contraction speed; but the long stimulus causes continued contraction for a longer time so that reflexes become much more extensive than those of short duration stimulus. Experiment of Loewenfeld 1999.[83]

On the pupillogram, with shorter duration of stimulus the constriction and primary redilation phases tend to blend together and become contiguous.[170] If the stimulus duration is long enough (≥ 0.4 second) and the intensity is adequate, all the phases of the pupil reflex shape described above may be demonstrated, figure 4.3. For light flashes shorter than about 0.4 seconds, variability of reflex shapes is no longer visible on the pupillogram, as pupillograms will simply show "V" shape contractions and dilations with variable amplitudes.[83]

The variability due to hippus (pupillary unrest, pupillary noise) is less extensive in the dark than in the light.[170;194] In *continuous* light stimulation, hippus tends to come on at about 1.5 seconds from the beginning of light stimulus.[170] If the stimulus duration is long, for example 3 seconds, it is likely that traces of hippus are caught in the pupillogram. This gives no added value to pupillographic studies because hippus is merely noise and is highly variable. Unless all phases of the pupillographic reflex shape are to be studied, short flashes of stimulus light are more favourable than step-like sustained stimuli. If the duration of stimulus further increases, for example > 4-5 seconds, the mean diameter of the pupil begins to increase due to light adaptation, but pupillary unrest and oscillations carry on unchanged.[170]

For situations where stimulus duration is longer than the latency before constriction, for example figure 4.17 B, the pupil is already constricting while the stimulus is still ON, and therefore it is not unreasonable to question whether attenuated light is delivered to the eye (due to the constricting pupil) to set up a complex stimulus situation which can be held responsible for the reflex shape obtained.[83] However, it is known from the intensity amplitude curve, figure 4.14, that (because intensity is in log unit) hundred folds of reduction of stimulus intensity are required for a significant change in the amplitude of contraction. A small amount of attenuation of light will not cause reduction in amplitude or set off a new reflex shape that is irrelevant.[83]

4.3.4.2.4 **Frequency of the stimulus and the pupillogram**

Repeated short light flashes in rapid succession produce pupillary movements (contraction followed by dilation) for each flash light. With increased number of stimuli per unit time (increased in frequency of stimulus), the diameter of the reacting pupil becomes smaller. With further increase in frequency, summation happens and the sphincter muscles are driven into tonic contraction, and the pupil becomes less and less capable of responding to individual stimuli with a separate reflex movement.[83] The human smooth iris muscle reaches this summation at a low stimulation rate. The wavelets of contractions and dilations become shallow, and at higher frequencies, they fuse.[83] The maximal range of pupillary oscillations tested in the literature is between 3–9 per second.[83] Some authors use sinusoidal stimulation where the intensities are alternated instead of intermittent flash lights. The response to sinusoidal stimulations

tends to be less extensive since the changes are more gradual and the lower intensity in the sinusoidal stimulations is not as low as darkness intervals.[83]

When the pupil is subjected to the very quick flashes of light, there is a phenomenon of "queuing". The latency of the first light is always longer than that of successive contractions. If the stimulus frequency is increased, much more attenuated contractions happen since the pupil diameter gets smaller with longer latency.[83]

4.4 INEQUALITY OF DIRECT AND CONSENSUAL RESPONSES

Contraction anisocoria is a phenomenon in which the direct light response is larger than the consensual light response upon receiving a stimulus light. It is a light induced anisocoria and production of such an anisocoria favouring the direct response, upon unilateral central stimulation of either eye, is termed "consensual deficit" or "alternating contraction anisocoria."[83;204] A small degree of contraction anisocoria is a normal finding in most healthy subjects. [111]In contrast to anisocoria (difference in the resting size of the pupils due to differences in the *efferent* limb of the reflex arc), alternating contraction anisocoria reflects the *afferent* limb of the reflex arc.[166] Contraction anisocoria may be bilateral (direct response more than consensual response in both eyes) or unilateral (direct response more than consensual response in one eye only, the other eye has equal responses).[111;194] Unilateral contraction anisocoria is more common than the bilateral type.[194]Therefore, the word "alternating" can be misleading in the latter case. Most authors nowadays address "alternating contraction anisocoria" as "contraction anisocoria."

Contraction anisocoria may be diagnosed when the difference of the pupil diameter change in the two eyes is greater than twice the spatial resolution.[111] A few recognised features of contraction anisocoria are that it has a mean value of approximately 0.075 mm[111] but this absolute value may be misleading if different approaches are used to measure the amount. In percentage value, it is approximately 6.1% of light reflex amplitude (or 0.05 mm for 1 mm contraction).[111;205;206] Some describe contraction anisocoria when pupillary contraction of one eye is 0.4% to 2.8% larger than the contralateral eye.[190] There is a high degree of repeatability seen with contraction

anisocoria even if the light response is repeated a year apart.[111] Contraction anisocoria can occur in the presence or absence of prior dark adaptation.[111] When the intensity of stimulus light is raised (either full field or half field), the contraction anisocoria increases proportionally with reflex amplitude.[31;111]

The duality of the pupillary pathways accounts for the anatomic basis of the consensual response to light. The dissimilarities of the direct and consensual pupil reaction are considered to be due to the predominance of the crossed fibres over the uncrossed fibres at both decussations: at the optic chiasm[31] and the EWN.[29;31] In cats, the predominance of the *crossed fibres* at both decussations is markedly greater thus giving rise to much larger direct light responses than the consensual responses.[207] In human the differences are small and the amounts of contraction anisocoria are small.[111] Until the advances of automated visual technology and the use of pupillometry, the small consensual deficit is easily overlooked.

Smith and Ellis (1979) investigated 150 normal subjects for this light induced anisocoria by a light stimulation placed centrally but slightly to the side of the visual axis using video pupillometry.[111] They reported the prevalence of contraction anisocoria in normal subjects to be 85%. To specifically test the hemifields, Wyatt and colleagues (1981) presented the discrete stimulation to each hemifield and found the nasal retina (temporal field) to be more sensitive than the temporal retina (nasal field) producing more pupillary constriction.[206] In the report documented by Cox (1984) the temporal field stimulation (nasal retina) produced a direct pupillary response that is larger than the consensual response and, conversely, nasal field (temporal retina) stimulation elicited a larger consensual pupillary response than a direct response.[208] Post-chiasmally, Kardon (2000) demonstrated the absence of pupillary responses when hemifield stimulations were placed in the blind homonymous hemifield of patients with optic tract lesions. This further confirmed the hemifield organisation of the pupillary fibres at post chiasm regions like visual fibres.[29;31] All these studies used video pupillometry to be able to record the subtle differences of the direct and consensual responses.

Chapter 5

Relative Afferent Pupillary Defect – Pupillographic Approach

5.1 WHY TEST RAPD WITH A MACHINE? ADVANTAGES AND DISADVANTAGES

It is described in chapter 3 how the clinical swinging flash light test is performed and the relative afferent pupillary defect quantified. However, a number of factors can induce variability in clinical practice. Although a moderate to large amount of RAPD is easily identified, it is often difficult to detect or quantify a subtle RAPD by clinical methods.[115;209]

It is apparent that the clinical method is more of an "art" in detecting a RAPD. There is inter-observer variability in the choice of stimulus, the way the stimulus is presented, the choice of outcome measures as well as the interpretation of the findings. The choice of stimulus light can vary from a pocket pen torch to the indirect head light used in the retina clinics. The room light conditions are also vastly different. Some examiners stimulate the eyes for up to 3 seconds each, some for about 1 second, while others vary their speed during the test to detect a subtle RAPD. In order to elicit the afferent differences, some examiners compare the constriction phase of the two eyes, some the direct and consensual responses of the same eye, while others change their practice on a case to case basis. The accuracy of clinical method, therefore, largely depends on the skill and experience of the examiner.[209] In experienced hands, a small RAPD in the region of 0.3 log units can easily be detected but in less experienced hands a significant RAPD can be missed.

The accuracy of the clinical swinging flash light test is challenged by various factors such as hippus, initial anisocoria, dark irises, small pupils, inadvertent unequal amount of time spent on each eye leading to unequal retinal bleach, different angle of light applied to each eye, patients who do not relax their accommodation fully and continually, and other factors.

The element of subjectivity by the observer in the test inherently weakens the test's accuracy; for example, there is a tendency of over-estimation of the test when the large pupil fails to constrict (because it is more obvious to see) than the small pupil does so.

Therefore, in experienced hands, it is the test that has all the answers at "bed side" examination but in un-experienced hands it can generates confusing results.

The variability of the test is also contributed by different settings of the test environment in the clinics and the lack of standardisation of the light source, retinal adaptive status and the technique itself. The test light does affect the amount of RAPD estimated by the swinging flash light test.[110;210] A denser NDF is required to balance the afferent defect when the bright test light is used.[210]

The subject can also induce variability in the clinical test. When the subject is instructed to fixate on a distant object, the examination room may not be large enough to relax accommodation, the room may be too dark for the subject to find a distant target to fixate on, or the subject himself may not be able to relax their accommodation during the swinging flash light test when the examiner is working very close to the eyes with the light and neutral density filters.

False positive results may be obtained due to unequal illumination,[116] and retinal bleaching[211] during clinical test. Although the same light stimulus is presented, there may be a dissimilar amplitude or velocity of pupil reaction simply due to the inherent moment to moment physiological modulations of pupillomotor output by the higher cortical areas.[116] When a few swings of light, typically 2 to 3, are presented to the eye until the smaller reaction to light is observed in the predicted eye for an RAPD using SFLT it is very easy to wrongly deduce the physiological asymmetry as pathology. With the clinical method, it is impossible for the examiner to recall pupil reactions performed more than 3 times or so.

Conversely, false negative results may be obtained when a subtle pathological RAPD is masked to the observer by the change in the pupil reaction due to higher centre influence. This easily happens with the clinical test because the clinician can only do 2 to 3 swings at a time. A small RAPD may be missed when the examiner is not expecting it because one or two swings of alternating light may give seemingly equal pupillary reactions.[116] There is also a problem with end point determination.[212] Clinical

determination of RAPD is typically weighed by the last few consecutive light reflexes which can cause bias in the estimation of the relative defect.[116]

The quantification of RAPD clinically using a neutral density filter (NDF) has its own technical difficulties. The smallest RAPD that can be recorded with confidence by the use of NDF is 0.3 log units. While very dense RAPD such as above 1.2 log units is being evaluated, the filters required to use are so dense that it is necessary for the examiner to look around the filters to see the pupil move.[23] This off axis examination imposes inaccuracy in assessing pupil size and reaction. Quantification by the NDF in many instances is biased by the test light used. The use of filters, especially when dense, can induce unequal retina light bleaching between the eyes. Furthermore, there is always a question of whether a reduction in the incident light across the entire retina is necessarily the same afferent defect as the one to be matched. In the pupillographic method, it is not required to use the NDF to match the worse eye because the pupillary response differences can easily be quantified *directly* from the recorded pupillogram. Moreover, the results obtained by the clinical NDF method are categorical data in 0.3 log units' steps, and subject to underestimation. For example, results between 0 and 0.15 log units will be classified as 0 log unit and those between 0.3 and 0.45 log units will be classified as 0.3 log units.[118]

Due to these disadvantages many researchers look into more objective, reliable and reproducible methods of detecting RAPD. Pupillographic estimation is the most accepted and recognised method documented in the literature. Automated pupil recording eliminates the issue of inter-observer variability. It has an added advantage of being able to standardise the test - the stimulus, the way it is presented, focusing and accommodation target for the subject as well as the test environment – since these variables can all be predetermined to the requirement. Whilst the clinical test is limited to 2-3 swings of light, a large number of ON-OFF stimuli can be applied to the eyes with the automated methods. The odd shape reflexes due to higher centre influences (for example when the subject is sleepy) are thus easily compared to the rest of the pupillograms and, if required, can be omitted. Averaging a large number of repeatable and reproducible images acquired by the automated method minimises the confounding effects of physiological modulations and variability. In addition, a wealth of

information regarding pupil reflex, shape, pupil dynamics, and responses can easily be obtained by automated pupillometry allowing clinicians to observe and diagnose various conditions and diseases with pupil manifestations with ease and accuracy. A simple observation of pupillary responses by the swinging flash light method, on the other hand, only provides the semi-quantitative[115] clinically useful information. The pupillographic method aims to enjoy both - the benefits of a comparative relative test and the ease, precision, repeatability and reliability offered by the automated recording.

5.2 DO WE NEED TO REPLICATE CLINICAL METHOD?

Thompson HS[213] replicated the clinical test using the pupillographic technique used by Lowenstein and Loewenfeld in 1966 on one of his patients with unilateral optic neuritis. He applied 3 seconds of light to the left and then the right eye with zero inter-stimulus-interval which is possible when the light is applied by the automated method. The following diagram, figure 5.1, depicts the results of his automated test. When the left eye was stimulated both pupils constricted but when the light was presented to the right eye there was no constriction but pupillary escape was noted. It is easily identified from this pupillogram the abnormal eye.

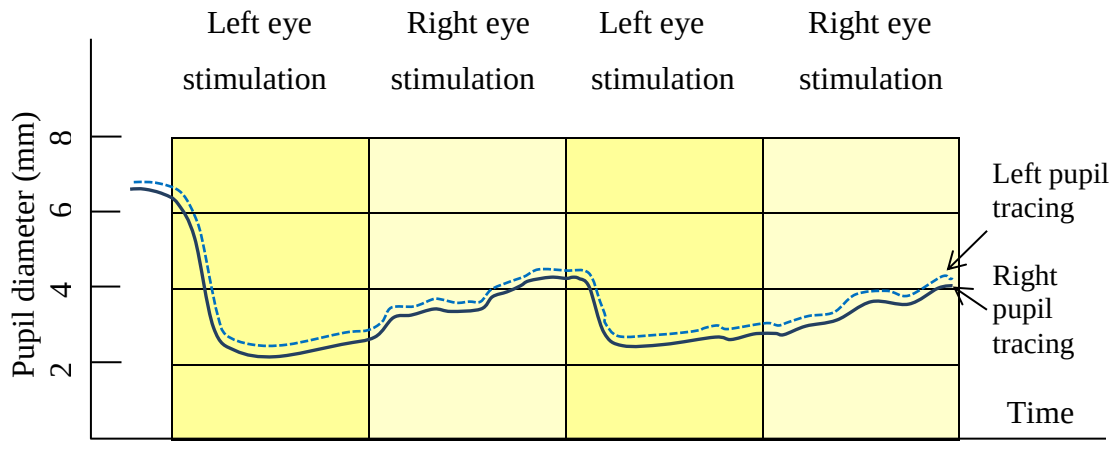


Figure 5.1. Simulating clinical swinging flash light test by the pupillographic method on a patient with right optic neuritis. Alternating 3 seconds light is applied to each eye in turn. Diagram redrawn from Thompson 1966.[213]

It can be seen that the pupil diameter at the beginning of the light stimulus depends on the amount of constriction the pupil has made from the first eye stimulation and how much it has recovered. The final pupil diameter at the end of stimulation of the

good/better eye is smaller than the final pupil diameter at the end of stimulation of the bad/worse eye because the good eye constricts more than the bad eye. The pupil diameter at the beginning of light stimulation is therefore larger for the good eye than the bad eye. The larger pupil allows more light to enter the eye and thus augments the pupillomotor drive coming from the stimulation of the good eye. The consequence of this is that the consensual pupillary response is larger than the direct light response for the worse eye and the opposite is true for the better eye.[115]Cox TA (1986)[105] simulated the RAPD by the use of NDF. He also used the swinging flash light algorithm (1-3 seconds ON, 0 to 0.3 sec OFF) to estimate the RAPD. Cox reported that the difference between the direct and the consensual responses of an affected eye increases with the density of the filter. From this finding Cox proposed that the direct and the consensual response of the affected eye could be compared to detect a RAPD in clinically instead of looking at the direct responses alone.

Do we need to replicate the swinging flash light test?

The concept of pupillometry in the measurement of RAPD is fairly new in the literature. As it evolves, it is becoming more and more apparent that the pupillometric means of quantifying the differences in the afferent pupil pathway abnormalities is *not* an automated version of the clinical method of swinging flash light test per se, but it is an independent approach to measuring an afferent pupillomotor input inequality. With the graphical method, the end-point is not the pupillary escape but the differences in the parameters of the recorded pupillograms. The observer can alter the ON-OFF duration as appropriate to the test requirement. Often short stimulus duration, usually of ≤ 1 second, is all that is required for the comparison.

In the clinical method, in order to make a mental comparison of the two pupillomotor pathways possible, it is required that the inter-stimulus interval (ISI) is very short (the light has to be swung as quickly between the eyes as possible). This allows the stimulating light to be applied to the other eye when the pupillary response is still active from the first eye stimulation making the comparison possible. The duration between stimuli is short and negligible. Figure 5.1 shows that when either eye is stimulated repeatedly, there is no time for the pupil to recover to the pre-stimulus diameter, or thereabout, before the next stimulus. For a pupillometric test which often delivers a

large number of pairs of alternating stimuli, having zero ISI is not beneficial. The number of successive stimulations can cause "summation" of the pupillary reactions whereby the pupil diameter progressively decreases, leading to having a smaller initial pupil before each set of stimuli. This has 2 important unwanted knock-on effects - (1) with smaller diameters, the pupil range of constrictions get more and more restricted and (2) the "queuing effect" causes progressively longer latent period - making the comparison test less efficient. Also, smaller amplitudes with variable recordings introduce more error during analysis. This method of continuously stimulating either eye, however, works for the non-automated clinical methods. This is, in fact, a desirable effect for a clinical swinging flash light test because the larger pupil at the beginning of stimulation to the good eye augments the pupillomotor input coming from the good eye, and the smaller pupil diameter at the beginning of the stimulation of the bad eye further attenuates the pupillomotor input and the corresponding reflex. This exaggerates the differences between the good and the bad eye making it easier for the clinician to detect the pathology. Because the clinician only uses 2-3 swings of light, the effects of summation and queuing are negligible. If longer pause duration is allowed in the clinical swinging flash light test, and the pupils are allowed to return to the initial pupil sizes, it will not be easy for the clinician to observe the differences in the afferent input. [105] In pupillometry, however, the duration of inter-stimulus interval (OFF duration) is intentionally set to a period to allow for the recovery to allow for the pupil diameters get back to the acceptable level before the next stimulus is applied, for more regular pupil sizes to be obtained with each stimulus, and more regular pupillograms to be achieved for comparison, figure 5.2. A number of repeated stimuli (ON-OFF stimuli) in pupillometry also mean more data is obtained for more accurate analysis.

The graphical presentation of the pupillometry – a pupillogram, has its own variables that can be used to quantify the afferent asymmetry. While the pupillary escape is the end point for the SFLT (indirect marker of asymmetry), pupillographic RAPD (pRAPD) *directly* quantifies the differences in all the available parameters of the pupillograms. In *binocular* pupillometers, in particular, the dynamics of both pupils are *simultaneously* recorded and thus a real-time differences are continually appraised – a feature which cannot be ventured by the traditional non-automatic methods.

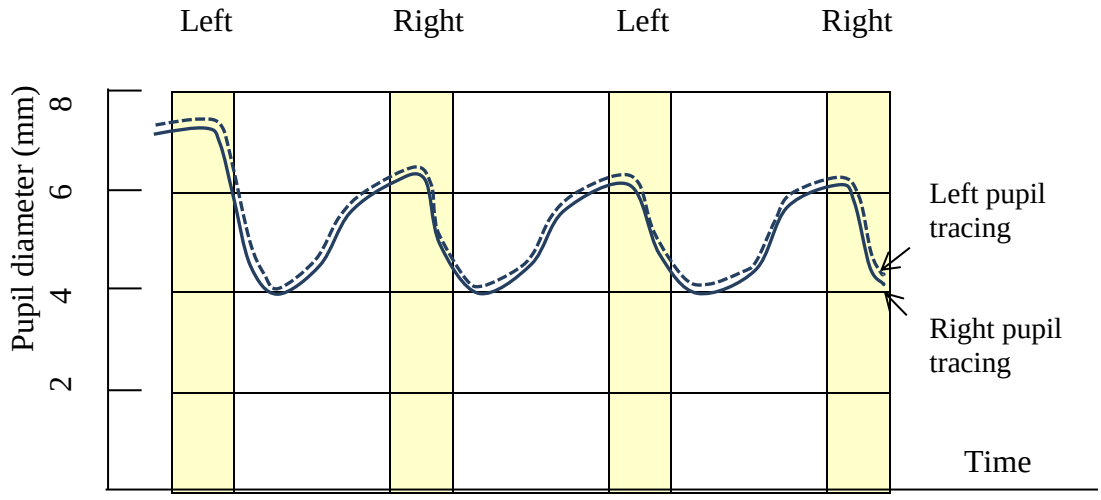


Figure 5.2. An example of a pair of pupillogram recordings of a *binocular* pupillometer. The right and the left eyes are alternately stimulated. Yellow shaded areas represent stimulus light. Inter-stimulus areas represent darkness presentation (OFF stimuli) for both eyes. After a short latent period both pupils constrict and re-dilate to a fairly constant pupil diameter before the next stimulus is applied to the other eye.

However, there is not yet a designated method or an instrument commercially or easily available for the estimation of RAPD pupillographically. Most pupillometers that are documented in the literature are research-based or custom-built and not commonly available to use for measuring RAPD. While the researchers are perfecting their instruments, there is still a variation in the use of the stimulus parameters and the outcome measures in estimating the pRAPD. A difference in opinion also exists among the investigators as to what aspect of the pupillogram is best for detecting and quantifying the relative afferent pupillary defect. A number of factors seem to affect the development and evolution of the pupillometry such as (a) requirement to consider the physiological variation in the pupil size and its dynamicity and age dependency which affect the feasibility, reliability and repeatability of the test, (b) the clinical needs – (i) whether the pRAPD test is targeted for the diagnosis or for screening or both, (ii) the type and the nature of the disease(s) or pathology(ies), (c) the level of accuracy required (sensitivity and specificity balance), and in addition (d) the availability of the test to the clinicians or health care professionals. It may be that there will be different types of pupillometers available in the future each with different purposes to serve various clinical needs.

5.3 PUPILLOGRAPHIC FEATURES OF AFFERENT PATHOLOGIES (LOW INTENSITY RESPONSE)

When the optic nerve, retina or the optic tract is damaged, the pupil responses to light in that eye are diminished and the pupil behaves as if the light is dimmer in the affected eye. The light reflexes produced in eyes with afferent lesions thus manifest a characteristic PLR reflex morphology distinguishable from that of normal light reflexes. These include: longer latent period, smaller (less-extensive) and slower pupillary constriction, shorter duration of constriction, slower and smaller recovery compared to those of a normal eye, [214] figures 5.3 and 5.4. Keeping all the other stimulus parameters the same, reducing the stimulus light intensity to the normal eye can reproduce the reflex features obtained in the eye with afferent lesions. In other words, the only abnormality of the affected eye in terms of the PLR is that it requires more intense light than the non-affected eye.[123] Because of this remarkable resemblance to low-intensity reflexes, Loewenfeld and Lowenstein termed the reflexes of eyes with afferent disease "**low-intensity reflexes**" [214] When reflexes of equal amplitude in both the affected and non-affected eyes are compared, the corresponding pupil dynamics such as latency and constriction velocity do not differ significantly.[123] The relation between the pupil dynamics is not disturbed. Figure 5.3, shows the features of low-intensity reflexes: the top part of the diagram depicts the normal pupil dynamics while the bottom illustrates a smaller, slower reflex with longer latent period. An example of *a pair* of pupillograms obtainable by the *binocular* pupillometer, which records the pupil images of the left and the right eyes simultaneously, is seen in figure 5.4.

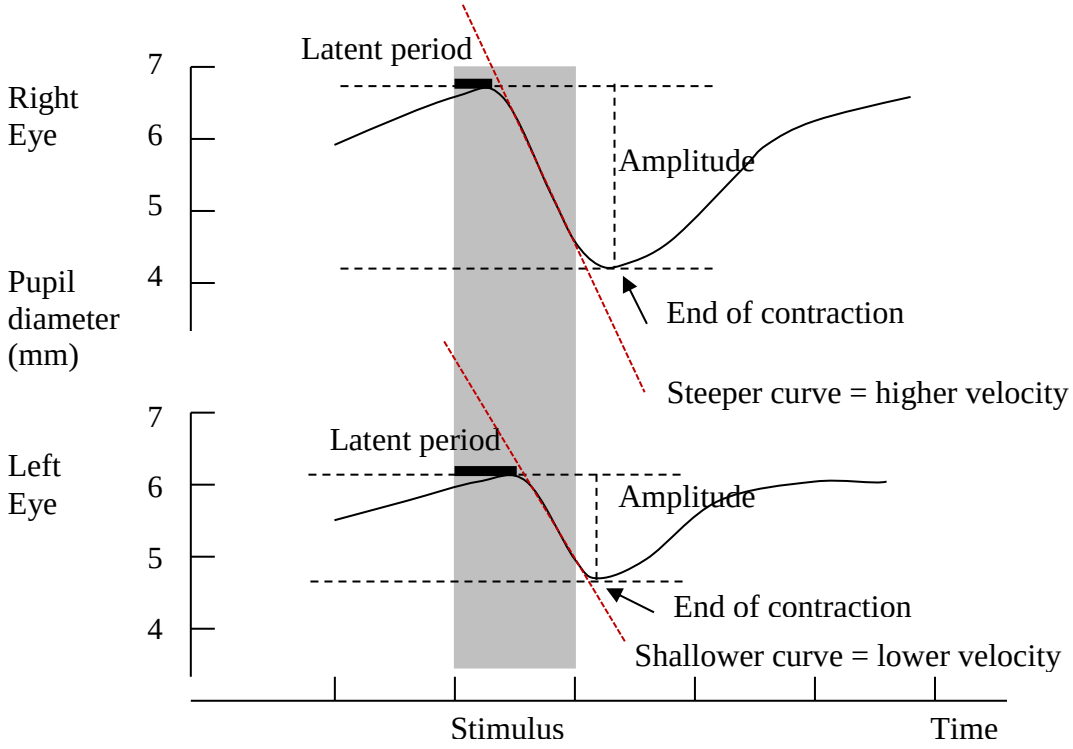


Figure 5.3. Features of low-intensity reflex (lower diagram) are compared with that of a normal reflex (upper diagram): longer latent period, smaller constriction amplitude, slower velocity/ acceleration of constriction (red dotted line) and dilation, shorter constriction time. By reducing the light intensity of the right eye, it is possible to match the reaction produced when the left eye is stimulated.

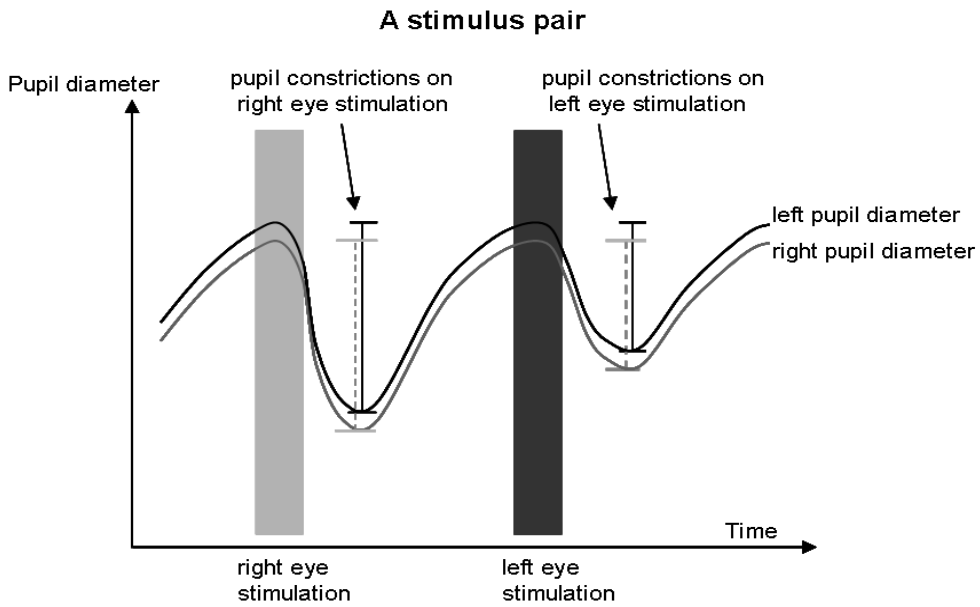


Figure 5.4. Simultaneous recordings of a binocular pupillometer, direct and consensual reflex of the right stimulus and the left stimulus are seen. Stimulation of the right eye produces a normal reflex in both eyes. But stimulation of the left eye produces less extensive low-intensity reflex.

5.4 APPROACHES TO ESTIMATING RAPD PUPILLOGRAPHICALLY

5.4.1 Adaptation before stimulus

In pupil literature, pupils are either dark or light adapted prior to stimulation. The purpose of pre-stimulus adaptation is to set both pupils into an equal adapted state (light or dark), to set off with equal retinal sensitivity and also to adjust the pupils to the required size before the stimulus is given. The choice of light or dark adaptation and the duration of adaptation depend largely on the nature of the study. A quick flash light used during the measurement of RAPD brings about the rapid pupil reaction. This reflex reaction is often tested when the eyes are dark adapted. The dark adaptation before a pupillary flash light test has a number of theoretical advantages. A larger pupil at the beginning of test will give a larger constriction of pupil for a better comparison. A good level of dark adaptation will reduce the visual threshold to the scotopic visual threshold. It is better to test the sensitive retina than the light bleached retina. Dark adaptation also sets the baseline visual threshold for both eyes. Most authors therefore dark adapt the subjects before their pupil light tests. However, there are some disadvantages with dark adaptation. It may be impractical to dark adapt the subjects and may not be feasible to dark adapt for a long length of time prior to pupillometry testing in the clinic. Also it is always difficult to know how long of dark adaptation is required for a specific test. The range of dark adaptation time varies considerably among authors, table 5.1. Some authors thus light adapt instead especially when they do not require the pre-stimulus pupil size to be large and all they need is to set pupils into one baseline level before stimulus. Also light adaptation can be performed quickly.

Authors	Year	Dark/ light adaptation duration
Thompson et al.[213]	1966	3-5 min dark adaptation
Ellis CKJ et al[111]	1979	30 minutes dark adaptation
Jonas JB et al.[162]	1990	5 min complete darkness
Bergamin & Kardon [215]	2003	3.1 apostilbs of light for 30 seconds (light adaptation)
Lankaranian et al.[212]	2005	5 min dark adaptation
Kalabukhova et al.[209]	2006	Experiment performed in the dark with 15 seconds of light adaptation before test.

Table 5.1. Some of the examples of the duration of adaptation used in the literature.

The factors to consider when deciding for pre-stimulus adaptation are (1) the nature of the study (studies testing cone functions may not require as long dark adaptation time as

testing rod functions; studies on pupil dynamics may require a larger pupil than a small pupil working in a nonlinear range) (2) the subject (adaptation may reduce pre-test inter-subject variability), (3) the concerned outcome measures (for example, effects of adaptation on the quality of data and its variability), (4) the test duration and its feasibility for specific use (longer adaptation may be possible in the laboratory based studies but not in clinical studies).

5.4.2 Stimulus Parameters

Various algorithms of parameters have been used by different investigators to optimise the accuracy of estimating the RAPD pupillographically. They were tested on available instruments and devices. Often the questions arise with regards to finding the optimum intensity and duration of stimulus, inter-stimulus interval, colour of stimulus light, stimulus configuration, number of repeats and test duration are all different.

5.4.2.1 Stimulus duration and intensity

Dimmer light produces smaller pupillary constriction and brighter light larger constriction. Likewise, a short duration stimulus produces a smaller pupillary movement compared to the longer duration stimulus. The reflex shape obtained is intended to be reflective of pupillary *reflex* action to the acute change to a higher level of brightness form the pre-stimulus level (which acts as a stimulus). In other words, it is not the absolute value of luminosity of stimulus used that matters but the difference between the luminosity of the stimulus light and background pre-stimulus light level that is important in delivering the required pupillomotor drive. Because the retina has the ability to adapt and change its sensitivity the continued illumination produces the adapted state as well as the reflex state. A short duration of stimulus light, *a flash light*, should be used for the purposes of measuring true reflex to the stimulus applied. A flash light is the one duration of which is not long enough to allow retinal adaptation to happen. For the purposes of RAPD measurement the standardisation of stimulus light is more important than choosing one particular light level as a standard one for any method of measurement.[83] This is because, even when the duration or the intensity of light is fixed, the response to a particular light level varies with the retinal sensitivity and the level of dark adaptation which are variable for different individuals. Moreover, the experimental set up, the test environment and the system for the stimulus delivery

are also different between investigators. For example, those using the Maxwellian open-loop optical system will be able to stimulate a larger and more standardised area of retina than those without. It is noted that there is no uniformity in the units that the authors used to describe the stimulus light intensity. Table 5.2 describes the examples of the stimulus intensity utilised by some of the authors.

Authors/ years	**Intensity light levels**
Thompson 1966	~ 15 foot-candles
Ellis 1979	1,2,3,4,5 and 5.3 log units
Cox 1989	1 x 10^3 ft-L
Kawasaki & Kardon 1995	Starting intensity 3700 apostilbs equating to 1.0 log unit; reduced by 0.3 or 0.1 log unit steps
Volpe 2000	23 milliwatts/cm2 and 2 milliwatts/cm2
Bergamin & Kardon 2002/3	Decibel attenuations: 0 (37,000 ASB = 11,770 cd/m^2) to 45 dB (1.17 ASB = 0.37 cd/m^2) with 5 dB increases in between.
Bergamin & Kardon 2003 Latency	5 intensities separated by 0.5 log units over 2.0 log units range (10-30 dB attenuation)
Lankaranian & Spaeth 2005	0.4 lux
Kardon, Kawasaki & Miller 2006	37000 apostilbs (0 log unit attenuation), 0.3 or 1 log units steps of 6 light levels (37 000, 3700, 370, 37 apostilbs)
Kalaboukhova 2006, 2007	1000 cd/m^2
Wilhelm 2007 Ocuserv Tubingen	2 arrays of 12 green light-emitting diodes, cornea illumination of 60-80 lux

Table(5.2) summarising stimulus intensity levels used by some of the authors.

While the minimum pupillary reaction can be achieved with illumination corresponding to the minimum absolute threshold; the alteration of two lights of different intensities can also bring about the pupillary response. The pupillary response is independent of the absolute values of the stimulus light but varies with their gradient /differences in the intensity of the two stimuli (differential threshold). Therefore, when a NDF is inserted in front of a light source to alter the intensity to the next level and the pupillary reaction is measured, the amount of NDFs used corresponds to the intensity that brings about the pupillary response.

As a general rule, the intensity and the ON-OFF duration required are those sufficient to produce a reflex shape required to differentiate the pathology from the normal by a pre-determined method. It is also dependent on which part of the pupillogram that is of interest. As discussed in the previous chapter 4 (a) there is a critical period duration which the intensity and duration have reciprocity, this is roughly below 100 ms duration for the dark adapted eyes, (b) for a near threshold intensity of light (a very dim light) the duration has very little play in the pupillary response (c) for a brighter light, (for example at 3-9 log units above threshold), however, a longer duration of stimulus increases the amplitude of pupillary reaction and reaction time within its limit, but the latency and the speed of contraction remain unchanged, and (d) hippus can be caught in the pupillogram after about 1.5 seconds duration of stimulus. Bearing these in mind, if one is interested in comparing the *amplitude* of pupillary constriction of the two eyes and also avoiding potential hippus in the pupillogram, it is best to choose a short duration of stimulus (< 1.5 seconds) with bright intensity. This gives an adequate amount of constriction for comparison. With a short duration, a more defined sinusoidal wave pattern can be obtained, making it easier to define the beginning and the end of the constriction phase. If it is a situation where the initial constriction phase is of interest, a bright stimulus is used instead of a dim one because a normal eye can escape at lower intensities.[216] A bright stimulus at least 3 log units above the visual threshold may be chosen for a stronger pupillary reaction and for less variability. The longer duration may also be used to extend the constriction phase for a better analysis but duration of < 1 seconds is usually enough for a good constriction phase without being interfered by hippus.[209] If one is only interested in the latency before constriction, short duration with intensity well above the visual threshold, with the inter-stimulus interval long enough to avoid summation will be suitable. If the intention, however, is to elicit the pupillary escape, a longer duration stimulus (eg 3-5 seconds) may be used because this will allow the effects of asymmetric afferent disease to be expressed themselves after peak contraction (dilation phase), causing "pupillary escape."[22;215;216]

Table 5.3 lists the examples of the duration of the ON-OFF intervals and the number of repeated stimulus pairs that have been used by different authors in the literature. As discussed, the pupillometers as well as experimental set ups are different for each author.

Authors and year	Repeats	Alt/ Seq	ON (sec)	OFF (sec)
Thompson 1966[213]	-	Alt	1	3
	-	Alt	3	0
Ellis 1979[111]	6-10	Seq	0.1	7.9
Cox 1989[115]	6-8	Alt	3	0
	8- 10	Alt	1	0
Kawasaki 1995[116]	195- 235	Alt	2.8	0.2
	195- 235	Alt	0.2	2.8
	195 -235	Alt	0.02	0.98
Volpe 2000[217]	4	Alt	0.2	1.0
Bergamin 2003[215]	4	Alt	0.2	2.8
Bergamin 2003[218]	8	Alt	0.05	2.5
Lankaranian 2005[212]	6	Alt	3	1
Kalaboukhova [209]	10	Alt	1	0.5
	10	Alt	0.5	1
	8	Alt	1	1
	6.68	Alt	1	1.5
	6.6	Alt	1.5	1.5
Kalaboukhova[117]	11	Alt	0.5	1
	11	Alt	1	0.5
Wilhelm 2007 [118]	6	Alt	2.5	0.5

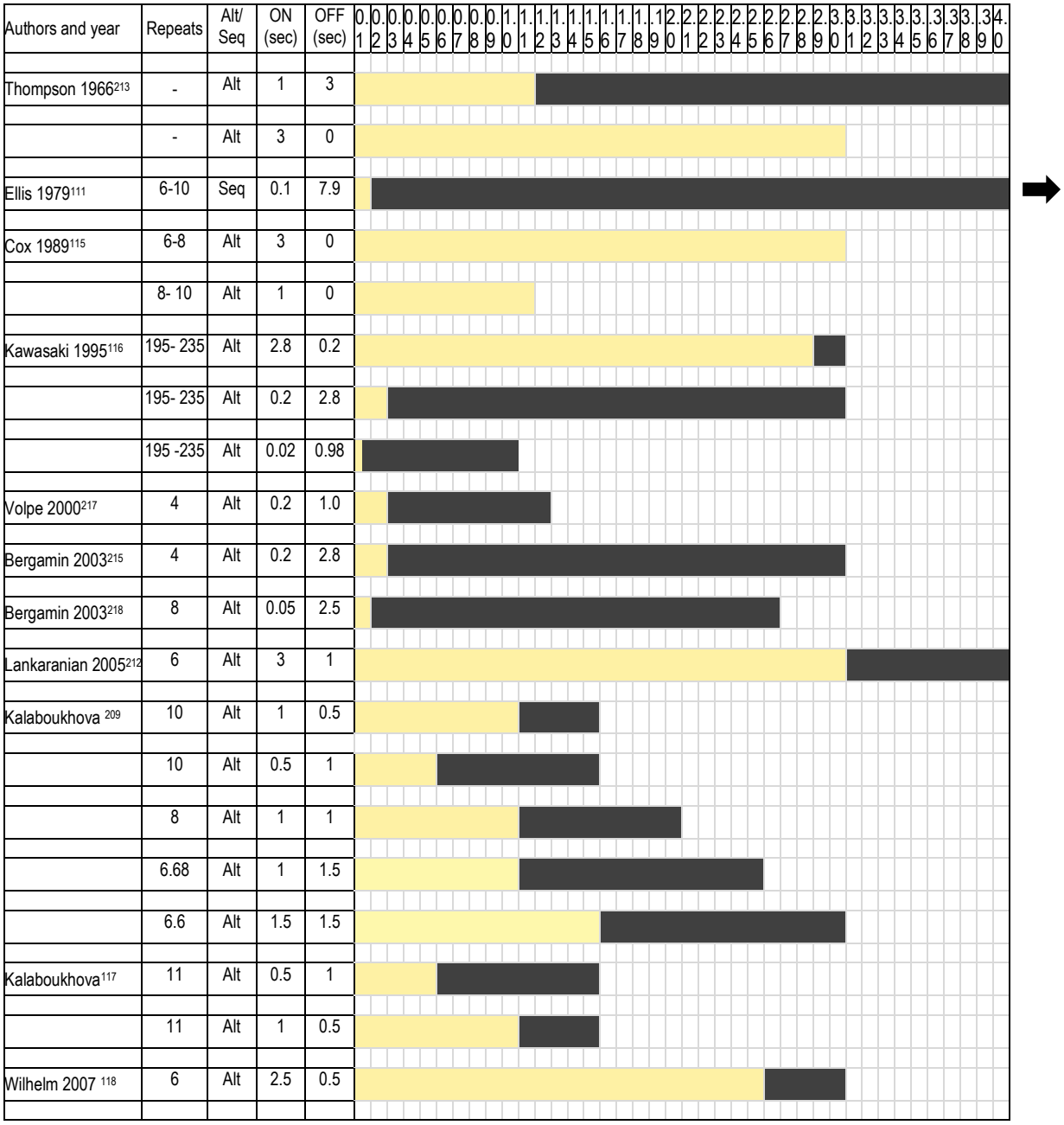


Table5.3. Summary of duration of ON-OFF stimuli used to estimate pRAPD by some of the authors in the literature. Alt = alternating stimulus, Seq = sequential stimulus.

Kawasaki and co-authors tested various pairs of ON-OFF durations (2.8s-0.2s, 0.2s–2.8s, 0.02s-0.98s) and commented that the variability is lessened when the dark interval between alternating light stimulus is short (0.2s-0.98s ON-OFF configuration in their experiment).[116] The authors hypothesized that a short dark interval between the light stimulation permits more interaction between sequential stimuli at the midbrain pupillomotor centre and allows less time for supranuclear influences.[116]

Kalaboukhova and colleagues (2006) tested a cohort of normal and glaucoma patients with shorter stimulus ON-OFF combinations (0.5s–1s, 1s–1s, 1s–1.5s). They achieved 82% sensitivity and 87% specificity in distinguishing glaucoma patients from normals with 1s-1s or 0.5s-1s stimulus-pause combinations.[209] With a stimulus duration of 1 or 1.5s, they noted various reflex contractions occurring after the initial contraction, especially in normal subjects. These waveforms sometimes interfered with their calculation of RAPD and thus recommended using a short duration stimulus of 0.5s for a better reproducibility. The authors recommended 0.5s–1s stimulus pause combination at a light intensity of 1000 cd/m^2 to be the best suited for the detection of glaucoma.[209]

5.4.2.2 Number of stimulus light levels

In theory, one light level is all that is required to elicit the light reflex. However, using more than one light level has shown to have practical advantages over using a single light level for the following reasons: -

(1) The greatest level of asymmetry of pupillomotor input is not the same for every light level. This is because of differences in the level of damage in the affected eye. For the eye with severe optic nerve damage, a higher light intensity is required to elicit the pupillary reaction. In cases of subtle afferent defect, the pupillary reflex inequality will be apparent at lower intensities. Therefore in a pool of patients with optic nerve pathology. It is often seen that, in some patients, the asymmetry is greatest at lower intensities, in some at midrange intensities, with yet others showing the greatest asymmetry only at the brightest intensities.[123;203;215] Therefore, asymmetry is better detected if a range of light levels is used.[215]

(2) For the same level of stimulus there are inter-individual variations in terms of amount of light reaching photoreceptors. The size of pupil responses varies among individuals. These differences are due to: -

   a. Age differences - Age related changes can begin as early as 20 years of age. Pupil size and lens opacity are the two that vary with different age groups. A small pupil can restrict the amount of light entering the eye while lens changes can either scatter or block the light. These can effectively change the amount of light reaching the retina as well as the area being stimulated.

b. Differences in the retinal sensitivity – this is primarily due to variation in the number, quality and the distribution of the photoreceptors and ganglion cells.

c. Differences in the qualities of neuronal transmission.

d. Differences in other factors including genetics of individuals.

Using the clinical method, the clinician modifies his technique on a case by case basis. As described in chapter 3, for the sluggish pupil, they may hold the light for a longer time and for large pupils they may hold the light for shorter time. Using the automated methods, having more than one light level gives the flexibility of being able to accommodate various pupil sizes and dynamics. When a large sample of population is intended to be studied by pupillometry, using a range of stimulus levels is better at detecting any asymmetry.

(3) The intra-individual physiological variability of the reflex outcomes can also be better assessed by using more than one light level.

(4) In mathematical terms, multiple light levels provide a series of data which can be plotted in a form of a line or an area. This makes more accurate calculation of a RAPD estimate since a comparison made of two lines or areas is more robust than merely getting a difference between two data points. For example, the intensity-dependent abnormalities can be optimally detected by using all three parameters of the linear correlation (slope, offset, correlation coefficient) of the responses of each eye.[215]

(5) There may be a better allowance for control of the test environment if more than one light intensity is used against the background light level.

Table5.4 describes examples of the number of light levels that the authors have used in the past.

Authors/ year	**Stimulus pairs per light level**	**No. of light levels**	**Total pairs**
Ellis 1979[111]	6-10	6	36-60
Kawasaki, Moor, Kardon 1995[116]	15 – 47 x (13 – 5 intervals)	3	195 - 235
Volpe 2000[217]	4	1	4
Bergamin & Kardon 2003[215]	4	10	40
Bergamin 2003[218]	8	5	40
Kardon, Kawasaki, Miller 2006[31]	12	6	72
Kalaboukhova 2007[117]	6-10 x 3	1	18 -30
Wilhelm 2007[118]	6	7	42

Table 5.4 Example of the number of light levels used by some authors to calculate pRAPD in the literature.

5.4.2.3 Number of repeat stimuli (frequency) and test duration

Recording one pupillogram is not sufficient. It is required to repeat the stimulus a number of times to provide more samples and more robust average results. In the experiment done by Kawasaki and co-workers (1995),[116] when RAPD was based on only a few light alternations (stimulus pairs), there was excessive variability in its measurement (95% confidence interval> 0.5 log units). The confidence level was 0.4 log units if 10 stimulus pairs were tested but only 0.1 log units if 100 stimulus pairs were used. They needed to use ~ 200 stimulus pairs to reduce the 95% confidence interval to < 0.1 log unit (RAPD+/- 0.05 log unit).[116] A standard deviation of 10% of the mean is normally considered a quite typical result if a subject is stimulated repetitively. This is true for both latency and constriction amplitude.

It is without question that a number of repeated stimuli are required for the more accurate estimate of RAPD. However, if the test duration is too long, there is a danger of exhausting patient without additional benefit. In the experiment done by Kawasaki and colleagues, their 95% CI levelled off after about 4 minutes of testing and approached 0.1 log unit.[116] Conversely in the initial 30 seconds of testing with only 5 to 6 alternations the 95% CI was wide at >0.3 log unit. (Kawasaki 1995). Applying 4-5 stimuli and calculating mean values is a good compromise that should be helpful in all pupillographic studies.[219]

5.4.2.4 Size and position of light stimuli

Retinal ganglion cells are densely populated in the centre of the retina. The central retina is therefore most effective for both visual and pupillomotor input.[95] In order to get 30 degree full field one has to use Maxwellian view. It is technically challenging and open-loop optics is not widely available. The maximum field of illumination for non-Maxwellian users is about 23 degrees. Table 5.5 describes some of the examples of the stimulus size and position utilised by some authors.

Authors/years	Maxwellian?	Stimulus size	Stimulus position
Ellis 1979	No	2 mm beam at the mid pupillary point	7^0 31' lateral to visual axis
Cox 1989	No	visual angle subtended by each light source = 1^0 27'; 10 mm diameter beam at the pupil	6^0 temporal to fixation, horizontal meridian
Kawasaki & Kardon 1995	Yes, 1.5 mm at the pupil plane	30 degree full field (4 of 15 degree square visual angles onto the retina)	Along visual axis
Bergamin & Kardon 2002/3	Yes, 1.5 mm at the pupil plane	30 degree full field, 4 orange squares -> 1 mm diameter at pupil plane by MV	Along visual axis
Lankaranian & Spaeth 2005	No	8 degree square	Along visual axis
Kardon, Kawasaki & Miller 2006	Yes, 1.5 mm at pupil field	30 degree full field	Along visual axis
Kalaboukhova 2006, 2007	No	5 degree round	10^0 temporal to visual axis

Table 5.5 Table summarising the stimulus sizes and positions utilised by some authors.

5.4.3 Outcome measures from the pupillogram

What segment of the pupillogram can be used to estimate a RAPD? A typical pupillogram has 2 phases: pupil contraction phase upon light stimulation and pupil dilation phase when the stimulus is withdrawn. The parameters of the pupillogram which have been used in the literature to estimate the RAPD include (figure 5.5):

i. Latency before constriction[123]
ii. Amplitude of constriction[31;111;115-117;123;209;212;217]
iii. The minimum size after constriction[115]
iv. Velocity of constriction and maximum constriction velocity (first derivative)[123;215]
v. Acceleration of constriction (second derivative)[117]
vi. Duration of constriction
vii. Latency before dilation
viii. Amount of redilation[115]
ix. Velocity of dilation and maximum dilation velocity (first derivative)
x. Acceleration of dilation (second derivative)
xi. Final size after redilation[115]

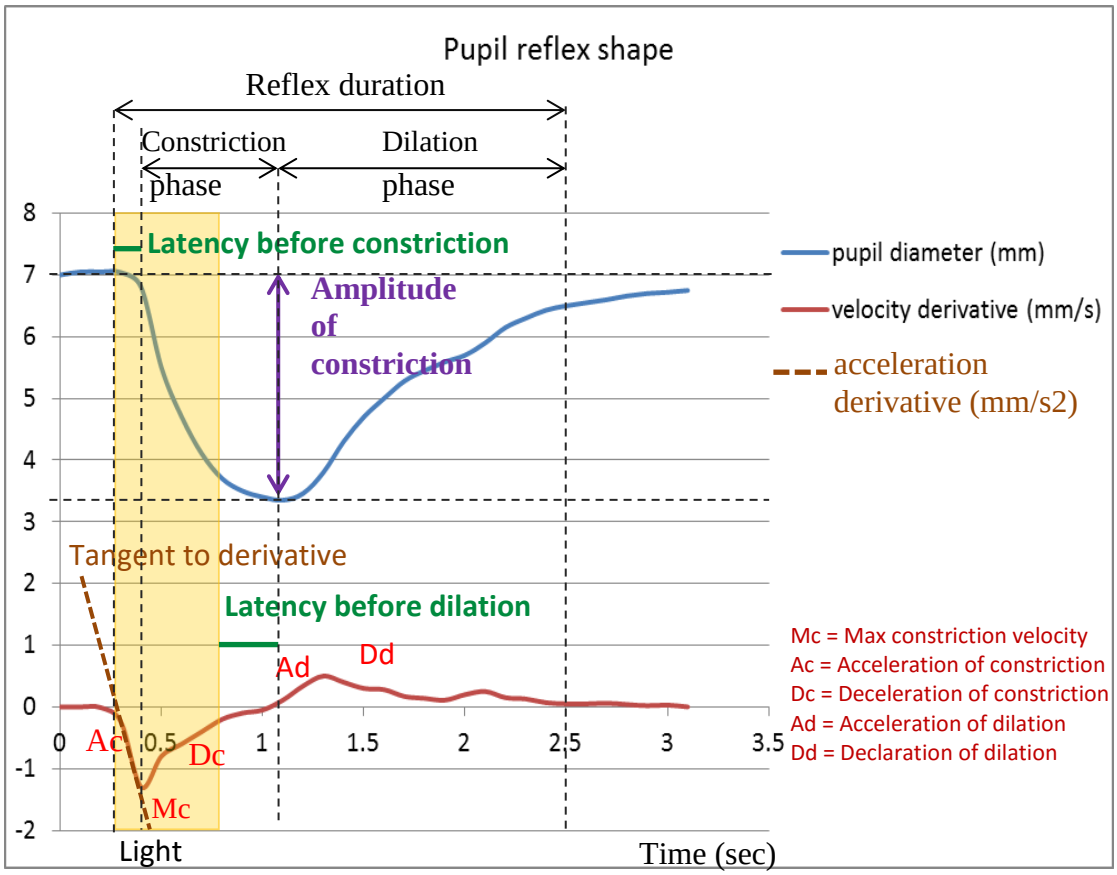


Figure 5.5. A schematic drawing of a pupillogram and its derivatives: velocity and acceleration.

As described above, the pupillogram of the eye with the anterior pathway pathology has low intensity reflex characters which include smaller amplitude of pupillary constriction, longer latency, and slower speed of pupillary constriction, slower pupillary dilation and shorter constriction time. These lead to having a smaller less extensive pupillogram. The RAPD can be calculated from any combination of any segment of the pupillogram or its derivatives. Although utilisation of all of the parameters could potentially strengthen the validity of the pupillographical estimation of RAPD, this has not been tested. The pupillometric studies published in the literature are fairly new and authors are still investigating each segment for its suitability and performance in estimating the RAPD. Pupillometers and the test algorithms are also different for different investigators. Therefore, the software that incorporates all parameters will be specific to the pupillometer and its corresponding test algorithm.

Amongst the parameters, the constriction phase is most commonly used to estimate the RAPD in the literature. This is because the constriction phase is less influenced by the physiological higher centre influences[83] or the presence of physiological anisocoria.[115]

Direct comparison of the parameters of the pupillogram of an affected eye with that of the eyes of the normal population does not provide a high sensitivity in discriminating normal from diseased eyes. (See also section 3.1.1 Absolute pupillary light test vs relative pupillary light test).The sensitivity for an *absolute* afferent pupillary defect is only about 50%.[123] This is because of a high variation in these values in the normal population. In addition, it will not be possible to replicate the test set up and the same test environment used for normative data collection in the clinical practice. However, when pupillometric parameters are compared between the left and the right eye of the same subject (inter-ocular relative test), a clear discrimination can be made between the normal and the diseased.

5.4.3.1 Spatial parameters in estimating RAPD

5.4.3.1.1 Pupil constriction amplitude

The pupil constriction amplitude is measured on the ordinate of the pupillogram, the pupil diameter from the onset of light to the point where pupil attained its maximum constriction, figure 5.5. It is usually expressed in millimetres. The main factors that affect the amplitude of pupillary contraction are the intensity, the duration and the starting size of the pupil. Other factors that affect these 3 parameters also affect the amplitude of constriction.

The pupil constriction amplitude is commonly used for pRAPD estimation. The advantages of using the pupil constriction amplitude in RAPD estimation are as follows.

(a) Unlike latency, constriction amplitudes are easily determined from the pupillogram.

(b) Being part of constriction phase, the constriction amplitude is less subject to the physiological noise.

(c) The pupillary constriction amplitude has a positive correlation with the intensity of stimulus light. The relationship is in a sigmoid shape (section 4.3.4.2.1). Therefore a differential of contraction amplitudes can be obtained with a number

of stimulus light levels which span across the mid linear segment of the sigmoid curve, providing more parameters for RAPD calculation.

(d) The constriction amplitude can be easily related to constriction seen clinically.

The following table (5.6) summarises the definition of pupil constriction amplitude by some authors.

Measurement of contraction amplitude	
Ellis 1979[111] Ozeki & Tsubota 2013[220]	The maximum change in pupil size.
Kawasaki, Moore, Kardon 1995[116] Kardon, Kawasaki & Miller 2006[31]	From the time at which the maximum acceleration of contraction occurs to the point at which the pupil velocity becomes zero.
Volpe 2000[217]	From the maximal pupil diameter to the minimal pupil diameter.

Table 5.6. Definition of constriction amplitude by different authors.

The pupil constriction amplitude is smaller in eyes with optic neuropathy or afferent pathway diseases. In bilateral disease, eyes with more advanced disease will have less constriction amplitude. The smaller constriction amplitude is also scaled with the reduction in the velocity of pupillary constriction and the prolongation of the latency before constriction. The amount of estimated pRAPD by taking the *absolute* difference between constriction amplitudes increases with increase in the intensity of the stimulus.[123] In other words, larger RAPDs may be detected at higher light intensities.

5.4.3.1.2 Other spatial parameters

Among spatial parameters of the pupillogram such as constriction amplitude, minimum pupil size, final size after redilation, and amplitude of redilation, Cox TA reported that the pupil constriction amplitude is the best indicator of small pupil defects.[115]

Bergamin & Kardon tested various segments of the pupillograph with a range of light intensities to detect which segment is most affected by the disease.[215] They hypothesize that the afferent neuronal firing rate is likely to correspond to different time points of the pupillogram. The time windows are based on landmarks corresponding to contraction onset, maximum contraction velocity, peak contraction, and maximum

dilation velocity. The authors concluded that the pupil size measured between the time at which maximum contraction velocity occurs and the time to peak constriction to reach the smallest pupil size (i.e. the late phase of constriction) provides the best response parameter for the pRAPD estimation.[215]

A more precise method of measuring the amount of pupillary contraction is to map out the area of the pupil during the light stimulation rather than measuring the horizontal or the vertical pupil diameter. Kalaboukhova and Lindblom (2006,2007)[117;209] used a custom-built pupillometer to measure the area of the pupil in terms of the number of pixels recorded from the display of a digital enhancement from the video camera image. The RAPD was estimated by taking the ratio difference between the pupil area changes of the right and the left eye stimulations. Using pRAPD estimate by this method, they achieved high sensitivity and specificity (87% and 90%, AUC 0.93) in discrimination asymmetrical glaucoma patients from that of the normal eyes.[117]

5.4.3.2 Temporal parameters in estimating RAPD

5.4.3.2.1 Latency

The latency of a pupillogram is the time period between the onset of stimulation by light and the onset of the pupil movement in reaction to stimulus,[218]and is described as temporal factor on the abscissa of the pupillogram, figure 5.5. It includes the time it takes for the afferent input to reach the neuronal centre, efferent output to reach the iris, as well as the time it takes for the iris muscles to contract. The latent period is thought to reflect the delays in visual processing proportional to the amount of afferent damage.[218]

Latent period has two components: *the minimal irreducible latent period* that is mainly contributed by the motor system of the iris and the *additional variable delay* due to the properties of the retinal discharges and their processing by the brain.[83] The minimal irreducible latent period is about 180-200ms range.[83] The maximal latent period is reported to be around 500 ms. There are theoretical advantages of using latency time for the measurement of RAPD. The latent time is prolonged in conditions that affect the afferent pathway. Unlike amplitude of pupillary constriction to light, it is less affected by the central factors or the mechanical properties of the iris.[218] In addition, age has

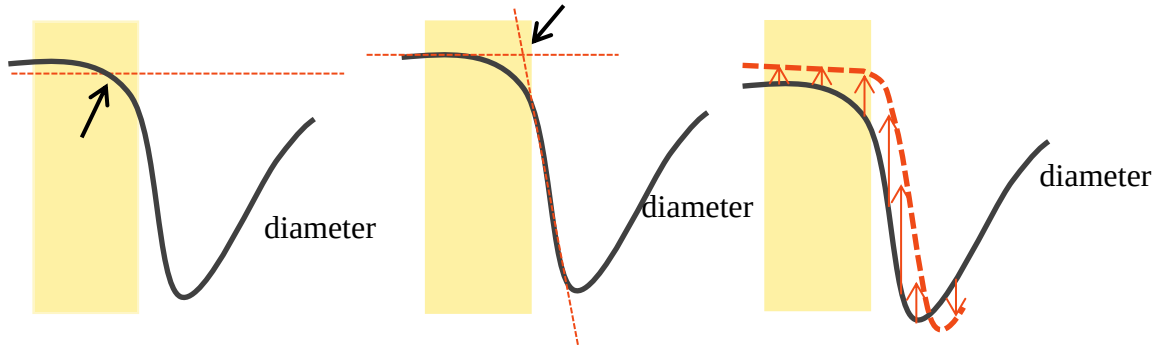
little effect on latency. As with other parameters of the pupillogram the inter-individual variation of latency is high but it is well-matched between the eyes of the same subject especially with higher intensities of stimulus[218] and thus suitable for a comparison test.

There are a number of factors that affect the duration of latency time: the quickness of the sphincter muscles to contract, the intensity of the stimulus light (the higher the intensity the shorter is the latency), the duration of stimulus light (longer duration of flash light within its critical period will shorten the latency), the area of retina stimulated (larger area associated with shorter latency time), the speed of the light stimulus delivered to the retina (quick stimuli is associated with short latency time), the colour of the stimulus light (in dark adapted eyes green light gives shorter latency than yellow light), the retina adaptive status (dark adapted eyes have shorter latency), and the retinal location (foveal stimulus in the light adapted eyes and the periphery stimulus in the dark adapted eyes for a shorter latency).[83] For a given intensity and duration, latent period is inversely proportional to the duration of the dark interval (inter-stimulus interval, ISI) that proceeds its stimulus. This is thought to be due to "queuing effect".[83]

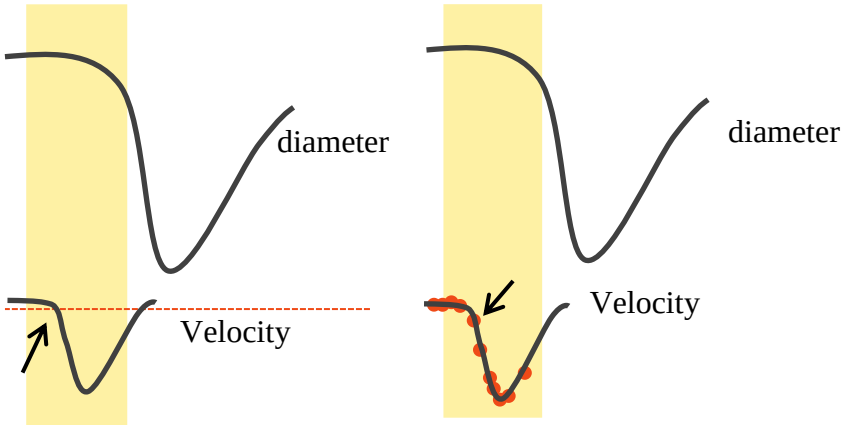
Latency time is highly variable for each person, between 200-500ms over the day.[83] The exact aetiology of variability is unknown but many factors listed above as well as the background hippus, chance absorption of photons by the photoreceptors are all likely to contribute to this intra-individual variation. Also, differences in the test environment and the experimental set up as well as the parameters used may contribute to large range of latency reported in the literature.

The contraction of smooth iris sphincter muscle does not cause an abrupt change in the pupil tracing and it often makes it difficult to determine endpoint of the latency period on the pupillogram. The background hippus can also interfere with the determination of beginning of pupillary constriction when low intensities are used. The reflexes of the low intensity stimulus are inextensive and easily interfered by the pupillary unrest movements and this often imposes dilemma to the observer especially when the cut-off point is set at a sensitive point. Different authors have used different approaches in determining the start of pupil motion in response to light, including:

- observation of deflection on the graph to identify significant velocity[220]
- amplitude threshold crossing i.e. the point where amplitude begins(Brogmann 1972),[221] figure 5.6(a);
- velocity threshold crossing i.e. the point at which the velocity changes(Feinberg and Podolak 1963),[222] figure 5.6(b);
- intersection of 2 straight line fits (Friedman1967, Alpern 1963), one through the latent period and one through the part of the constriction where velocity seems constant, [223] figure 5.6(c);
- velocity deflections from zero method (Pfeifer 1982) – differences of pupil sizes between 2 consecutive points are calculated. The point at the beginning of 3 consecutive negative differences is considered the initial time of pupil response.[224], figure 5.6(d);
- mathematical curve fitting procedure (Lee 1969),[225] figure 5.6(e).



(a) amplitude crossing (c) intersection of 2 straight line fits (e) mathematical curve fitting



(b) velocity crossing (d) velocity deflection from zero

Figure 5.6. Schematic drawings of methods of determination of the end of latency period. A black arrow shows the point where the latency ends for each method.

Each method has its own drawbacks. Observation methods are subjective. Both Amplitude and velocity crossing methods are inaccurate and do not indicate the real start of constriction but some moment after that.[221] The amplitude crossing method depends on the movement range of the pupil and it is limited by the iris mechanics, ie a small pupil will have delayed onset on contraction by this criterion.[218] The use of velocity to determine the onset of pupil movement (velocity threshold crossing) depends on the amount of amplitude because amplitude and velocity are closely related and both are dependent on the size of the pupil and the range of its movement.[218] The two lines intersection method (one through the baseline pupil diameter and the other during the contraction phase of the reflex) may not represent the real onset of movement. The determination of the slope of lines is variable and it depends on the pupil movement preceding the onset of stimulus.[218]

Bos J (1991)[221] has suggested the following methods which are independent of amplitude of constriction: (a) if the data sample rate is high enough (~200 Hz) pupil velocity deviation from zero can be used as this would give an accuracy of about 5 ms at best; (b) for data samples with lower sampling rates (<50 Hz) a curve fitting method which includes complex second order mathematical modelling may be used to yield an accuracy of about 5 ms. Averaging of the sample data was not recommended by the author because averaging potentiates the inaccuracy in summating the movement from the real mean values.[221]

In studies by Kawasaki(1995)[116] and Bergamin and Kardon (2003)[218] latency was defined as the time period from the onset of stimulus to the time when the greatest absolute acceleration in the tracing occurs. Bergamin and Kardon recorded the reflection of image with an infrared video recorder with a sampling rate of 1000Hz and omitted the time point if the change in pupil size exceeded 0.1 mm/s. This is because human pupil cannot move faster. Figure 5.7, depicts the graphical presentation of the determination of the latency end point. Bergamin (2003) suggested filtering the data instead of using the raw recorded data. They suggested using Savitzky-Golay filter for the video sampled pupil data because it does not apply an abrupt frequency cut off, selectively filtering high-frequency noise but preserving the low-frequency components

of the biological slow pupil movement. To date, there is no universal agreement on the method that gives an accurate estimate of the latency before constriction.

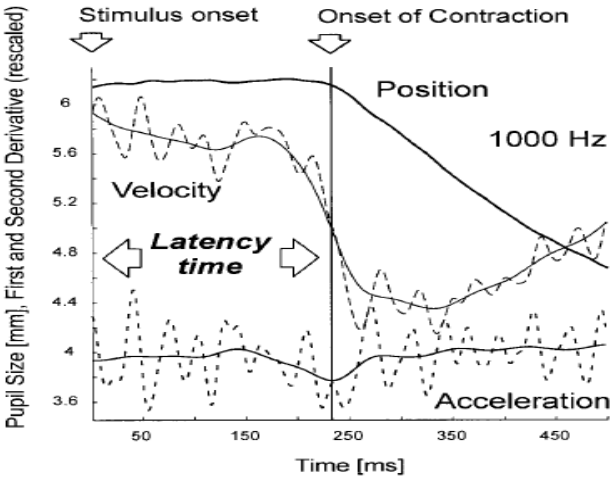


Figure 5.7. The onset of the pupillary constriction is determined by the velocity (2nd order) and acceleration (3rd order) derivatives. The derivative curves are superimposed on to the pupil diameter curve, top = pupil diameter, middle = velocity, bottom = acceleration. The dash lines represent unfiltered curve, solid lines represent filtered curve. The trough in the acceleration curve represents the highest acceleration of the pupil reaction which corresponds to the steepest point in the velocity curve. The corresponding point on the pupil diameter curve represents the time at which the latency ends. The diagram adopted from Bergamin & Kardon 2003.IVOS;44(4):1548.[218]

For an accurate measurement of the latency, it being a temporal parameter, a recording device with a high temporal resolution and sampling rate is required to precisely capture the moments of pupillary constriction. The minimum desirable sampling frequency is 60 Hz.[218;221] When second or third order derivatives of the amplitude data are used for the calculation of latency, a high spatial resolution for measurement of pupil diameter is also desirable.[218]

The latency is prolonged in patients with optic neuropathy. This goes along with smaller constriction amplitude and the slower rate of pupillary constriction (low intensity reflex). For bilateral conditions, the latency is prolonged in the affected eye compared to the less affected eye. The prolongation of latency and the reduction in pupillary constriction are proportional.[109;123]

5.4.3.2.2 Pupil constriction velocity

The constriction velocity is derived from the velocity derivative curve, figure 5.5, and expressed in mms^{-1}. The maximum constriction velocity can be obtained at the highest point of the velocity derivative curve. The velocity (or the maximum velocity) of

constriction increases with increasing stimulus intensity. In eyes with afferent disease the velocity of constriction is reduced along with the reduction in the amplitude of constriction and prolongation of latency time. As mentioned above, the amount of reduction in constriction velocity is scaled with that of amplitude of constriction. Kalaboukhova and Lindblom (2007)[117] compared the diagnostic ability of velocity based pRAPD with pupil- constriction-area based pRAPD in the diagnosis of glaucoma and found the latter to be better (AUC of 0.6 vs 0.9). In the experiment conducted by Volpe and co-authors (2000),[217] it was found that the sensitivity and the specificity of differentiating normal from those with true RAPD of any density using constriction velocity was 69% and 84%. As for other temporal measures, it demands a high sampling rate and good spatial measurement for an accurate calculation of velocity.

5.4.3.2.3 Pupil re-dilation velocity

Another temporal parameter that can be used to estimate the pRAPD is the pupil dilation velocity. In general, the dilation phase is more subject to the physiological higher centre influences and hippus. Most authors use the parameters of the constriction phase for the RAPD estimate since they provide less variable results. Kalaboukhova and Lindblom (2007)[117] also estimated the pRAPD using the ratio of the pupil dilation velocity calculated by change in the pupil area instead of diameter. The stimulus configuration was 0.5 s-1s ON-OFF combination. The sensitivity and the specificity of the pupil-dilation velocity ratio in discriminating subjects with glaucoma from that of non-glaucoma subjects are less than that by the pupil constriction area ratio (AUC of 0.755 vs 0.923) further supporting the fact that the parameters of the constriction phase are better at assessing the afferent pathologies.

In summary, based on the published literature, it appears that spatial parameters are better (or more accurate) than temporal parameters in estimating the RAPD. It may be because the measurement of spatial parameters is relatively straight forward compared to that of temporal parameters. This allows for more accurate measurements with spatial parameters. For temporal parameters, mathematical equations such as differentiation curves need to be used to quantify the assessment. In situations where there is a variation in determination of the end point (e.g. latency) the measurement algorithms are often different among the investigators. Furthermore, temporal parameters rely on

temporal resolution and sampling rate as well as spatial resolution of the equipment. For example, if the recording speed is less than 60 Hz for a pupillary reflex action, the accuracy of the temporal measurements may be questioned.[218]

5.4.4 RAPD estimation

A binocular instrument has the advantage of measuring both eyes simultaneously; data from the outcome parameters of both eyes for the entire repeated on-off stimuli can be used in analyses. Various researchers have tested on using different outcome parameters and specific test paradigms, which they believe are the best at discerning the relative afferent difference. Each paradigm for calculating RAPD is thus specific to the test strategy employed and the instrument used.

There are many ways RAPD can be calculated. These methods can be broadly categorised as follows:

(1) Simple linear difference method
(2) Ratiometric difference methods
(3) Graphical methods

5.4.4.1 Simple linear difference method

In this method, one value is simply subtracted from the other to get the absolute value difference between the two data points. Only one set of data (one of left and one of right eye) is required for this method.

For example,

RAPD = pupil response of the right eye stimulation – pupil response of the left eye stimulation.

The table (5.7) lists some of the examples of subtraction method used by authors for an RAPD estimate.

Authors / year	**RAPD (amplitude difference by subtraction)**
Jonas JB at al 1990[162]	>0.5 mm difference in mean constriction amplitude
Ellis 1979[111]	> 0.21 mm difference in mean constriction amplitude, which is

	2 standard deviations (SD) above the mean value of the normal controls in his sample.
Lankaranian and Spaeth 2005[212]	>0.25 mm difference in mean constriction amplitude, which is the upper limit of the 95% CI of the normal controls in their sample.

Table 5.7. Examples of the RAPD estimation by taking the difference between constriction amplitudes obtained by the left and the right eye stimulations.

5.4.4.2 Ratiometric methods

Here the differences between the eyes are described as a proportion. Only one set of data (one of left and one of right eye) is required for this method.

(A) Simple ratio small/large

This shows how much smaller the response to light is in one eye than the other. It can be described as a percentage proportion.

For example,

$$pRAPD = 1 - \left(\frac{\text{smaller constriction amplitude}}{\text{larger constriction amplitude}} \right) \quad X\ 100\%$$

This method shows how much one eye is weaker in response to light than the other eye.

(B) Simple ratio (large/small)

This calculates how much larger the pupillary reaction is in one eye than the other and is described in percentage proportion.

For example,

$$pRAPD = \left(\frac{\text{larger constriction amplitude}}{\text{smaller constriction amplitude}} \right) - 1 \quad X\ 100\%$$

This method shows how much one eye is better in response to light than the other eye.

(C) Simple ratio (OD/ OS) or (OS/OD)[117;209]

Instead of taking the smaller or larger responses as numerator or denominator, the left or the right eye responses are used.

For example,

$$pRAPD = \left(\frac{OD\ response}{OS\ response} \right) \quad or \quad pRAPD = 10 * \log_{10} \left(\frac{OD\ response}{OS\ response} \right)$$

Here, if the result is <1, the RAPD belongs to the eye in the denominator. This can be further translated in signed format, such as, for example, the negative value for the right RAPD and the positive value for the left RAPD by inverting the ratio which is below 1 and assigning it a negative value for the above equation.

5.4.4.3 Graphical methods

For this method of calculating RAPD, more than one data point is required. These are often obtained by the use of more than one light level. Because the different intensity levels can be obtained by the use of NDFs, the light levels can be addressed by log unit values from the NDFs. The RAPD has been calculated from the measurements of light levels by various means.

(A) Graphical method A

One method, described by Kawasaki, Moor and Kardon (1995)[116] and Wilhelm & Wilhelm (2007),[118] is to plot a graph of response against the light level. For instance when the mean response differences are plotted against the mean illumination differences expressed in log units, a linear graph can be fitted joining these coordinates. The amount of RAPD is the illumination difference when the line transects the x axis. It is the illumination difference when there is no difference in the pupillary responses. In other words, it is the additional illumination required in the weaker eye to keep both pupil responses equal.

In the example below, the eyes are alternately illuminated by a range of light levels (LR1 to LR7 for the right eye and LL1 to LL7 for the left eye in this example), alteration of which can be achieved by inserting NDFs of known attenuations in front of the light source (L), table 5.8. The inter-ocular differences in the illumination can be plotted against pupillary response differences as in the diagram (figure 5.9) elicited below. The linear plot transects the x axis at 0.2 log units. This represents the point at

which there is no inter-ocular difference in the pupillary reaction and hence signifies the amount of RAPD, figure 5.9.

NDF (log units)	RE stimulus	LE stimulus (LL)	Luminance difference (RE- LE)	Response of RE illumination	Response of LE illumination	Response difference (RE-LE))
0	LR1 = L	LL1 = L – 0	0	R1	L1	M1
0.3	LR2 = L	LL2 = L – 0.3	0.3	R2	L2	M2
0.3	LR3 = L – 0.3	LL3 = L	- 0.3	R3	L3	M3
0.6	LR4 = L	LL4= L – 0.6	0.6	R4	L4	M4
0.6	LR5 = L – 0.6	LL5 = L	-0.6	R5	L5	M5
0.9	LR6= L	LL6 =L – 0.9	0.9	R6	L6	M6
0.9	LR7= L – 0.9	LL7 = L	-0.9	R7	L7	M7

Table 5.8. Description of stimulus light levels and the outcome responses in a table.

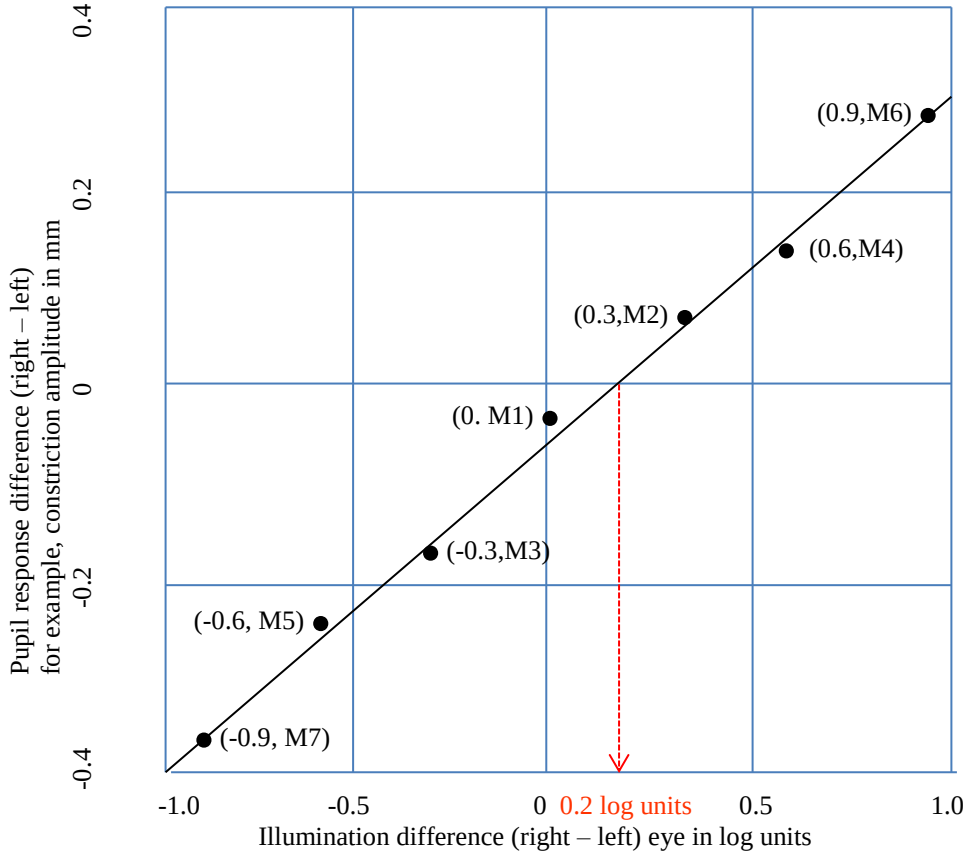


Figure 5.9. pRAPD calculation by a graphical method (intensity difference vs pupil response difference)[118]

(B) Graphical method B

Instead of plotting the differences, the response of the left and the right eyes against corresponding intensities can also be plotted to estimate differences in the output.

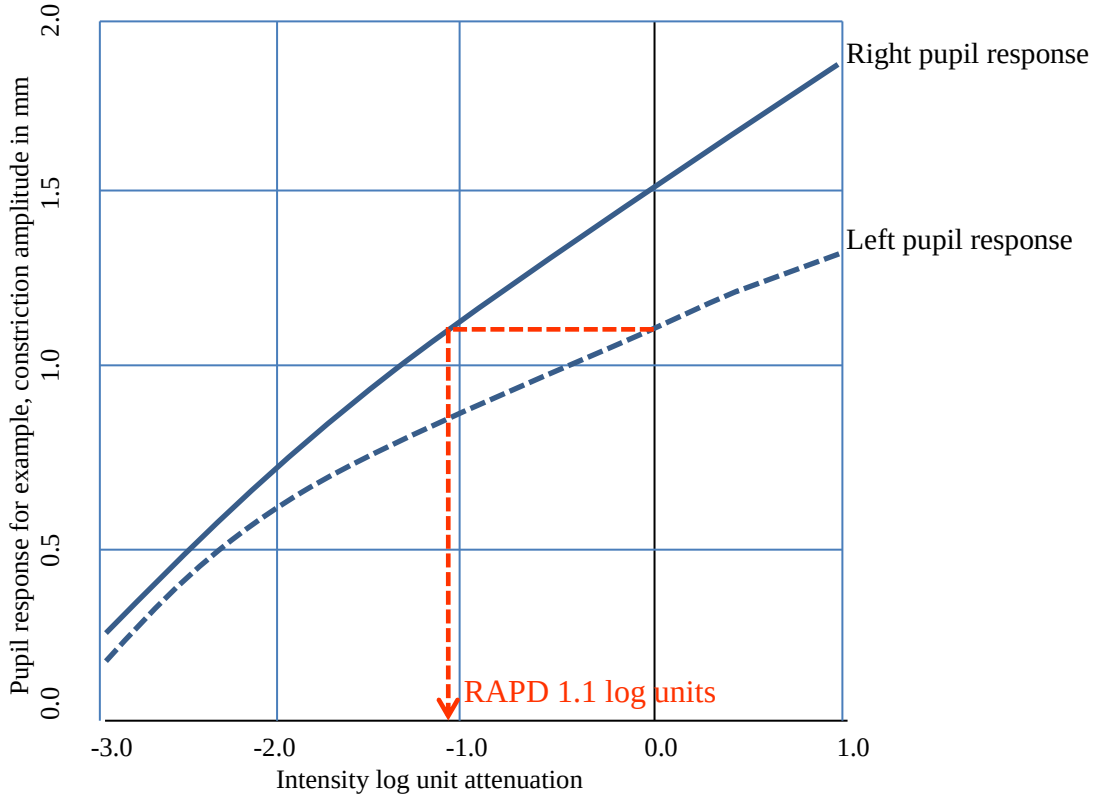


Figure 5.10. pRAPD calculation by a graphical method (intensity vs pupil response)[31]

For example in the method devised by Kardon, Kawasaki and Miller (2006),[31] the highest intensities (attenuated each time in step by a 1 log unit filer) and the corresponding pupillary light responses are plotted as below, figure 5.10.

(C) Graphical method C

The RAPD has also been derived from the comparison of the derivatives of linear correlation between the responses of the right and left eye stimulations. For example, in the experiment of Bergamin and Kardon (2003),[215] the authors test eyes using 10 different intensities. The responses of left and right eye stimulation are plotted on the abscissa and ordinates of the graph. For a normal eye, it is expected that the coordinates of the left and the right eye responses form a linear straight line, with the slope of 1, intercept of 0 and R^2 of 1.

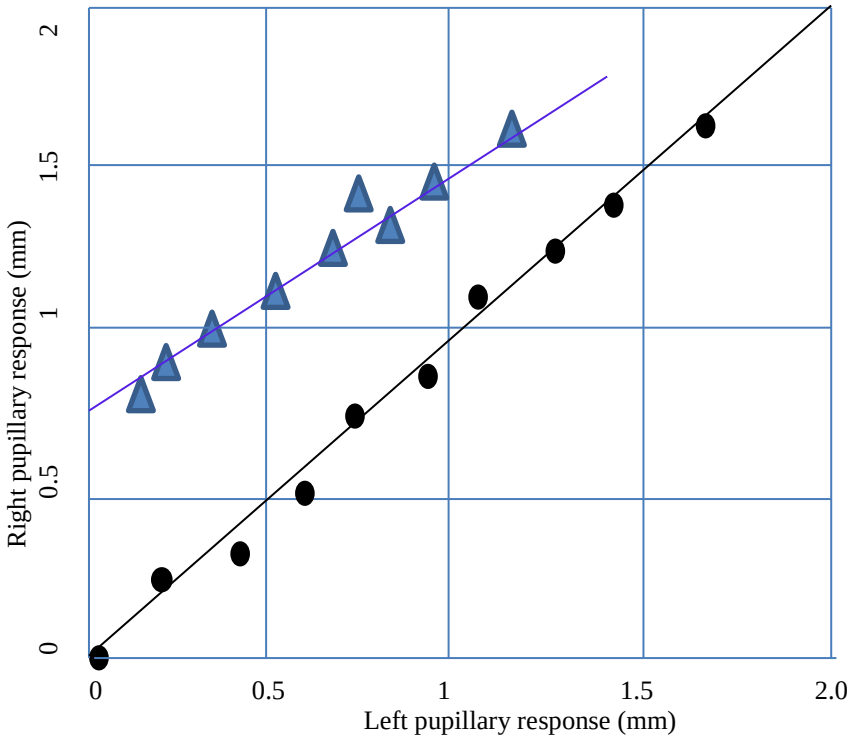


Figure 5.11. The pupillary repose of the left and the right eyes are plotted for a normal eye (black circles) and the eye with afferent pathology (blue triangles). The parameters of the linear regression (slope, the intercept and R^2) can be used as the indicators of asymmetry between the normal and the disease eye.[215]

5.4.4.4 Normalisation of the responses outcome parameters with the initial pupil diameter

As described in the previous chapter 4 pupil dynamics vary with the size of the pupil. This is because pupils have different working ranges for different sizes owing to the local arrangement of the iris muscles. Smaller pupils constrict less than larger pupils. Because the inter-individual variability of the pupil size is high for any population, using just the differences in the parameters without taking into consideration of the initial pupil diameter makes inaccurate estimates of the RAPD. For example, 2 mm of pupil constriction deficit of a 7 mm pupil has different weight to 2 mm pupil constriction deficit of a 4 mmm pupil. The difference in the initial pupil size affects the amount/amplitude of pupillary constriction which has bearings on its counterpart the speed/velocity of constriction and consequently to the acceleration of constriction.

For pupillometry, that incorporates Maxwellian open-loop optics for delivering the stimulus to the eye, the pupil size effect on the amount of photon reaching the

photoreceptors can be eliminated because a light source smaller than the size of the pupil is projected to the centre of the pupil plane. The collimated light coming from the pupil plane can stimulate up to 30 degree full field or more of the centre retina regardless of the size of the pupil. This achieves 2 things: firstly an equal area of retina is illuminated regardless of the pupil size and this eliminates the problem of unequal retinal bleaching; secondly a larger area of retina is illuminated compared to the non-Maxwellian optics system allowing more photoreceptors and ganglion cells to participate in the light response. Thus better appreciation and assessment of the afferent system can be achieved. Although the Maxwellian system eliminates the issues of unequal retinal bleaching due to differences in pupil size and maximises the area of illumination, it does not alter the mechanical size effect contributed by the iris muscles. The larger pupils have greater opportunity for movement than smaller pupils. In addition, the peculiar arrangement of the dilator and constrictor muscles causes mechanical limitation of movement in near maximal dilation and constriction.

The effect of pupil size on interpretation of PLR measurement can be lessened by incorporation of the initial pupil diameter into the equation. This can simply be done by taking the ratios with respect to the initial pupil diameters. Instead of describing 2 mm deficit in the above example, it can be described as a deficit of 2/7 for a 7 mm pupil and 2/4 for a 4 mm pupil.

For all of the methods described above, the response parameters can be normalised with the corresponding initial pupil diameter. For example, for the ratiometric methods, the RAPD can be calculated using the ratio of the normalised constriction amplitude rather than the absolute constriction amplitude as below.

(A) Normalised ratio (small/large)

This method is the same as above but it takes into account the size of the initial pupil diameter immediately before stimulus.

For example,

$$RAPD = 1 - \left(\frac{\textit{smaller amplitude / initial pupil diameter}}{\textit{larger constriction amplitude/ initial diameter}} \right) \textit{ X 100\%}$$

(B) Normalised ratio (large/small)

This is the same fashion as the last method but puts the larger amplitude over the smaller.

For example,

$$RAPD = \left(\frac{larger\ \ amplitude\ /\ initial\ pupil\ diameter}{smaller\ constriction\ amplitude/\ initial\ diameter} \right) - 1 \quad X\ 100\%$$

(C) Normalised ratio (OD/OS or OS/OD)[220]

Normalised values of right and left eye pupil diameter can also be compared.

For example,

$$pRAPD = \quad 10 * \log_{10} \left(\frac{constriction\ amplitude/\ initial\ pupil\ diameter\ of\ OD}{constriction\ amplitude\ /\ initial\ pupil\ diameter\ of\ OS} \right)$$

$$pRAPD = \left(\frac{response\ of\ OD/\ initial\ pupil\ diameter\ of\ OD}{\mathbf{r}esponse\ of\ OS\ /\ initial\ pupil\ diameter\ of\ OS} \right)$$

5.4.4.5 Direct or consensual response, or both, for RAPD estimation

A common question in calculating RAPD is whether to use the parameters of the direct response or the consensual response or both. The debate is still ongoing and no consensus has been reached because there are advantages and disadvantages inherent in each method.

Ellis (1979) used the direct responses only to compare the parameters of the constriction phase of the abnormal eye and the normal eyes of patients with optic neuropathies. The advantage of using the direct response only is that it circumvents the problem of contraction anisocoria which is present in about 85% of the normal population (also see section 4.4).[111] When contraction anisocoria is present, the direct response is stronger than the consensual response; this may be unilateral or bilateral. Although the anisocoria is typically small (approximately 6% of the constriction amplitude),[111] it is worth avoiding the confounding effects of contraction anisocoria when looking for a subtle RAPD. Physiological anisocoria can confound the result. This is because when

the direct response is smaller than the consensual response, it may be that there is a true RAPD (afferent defect) or because the pupil tested is smaller. Therefore, if only the direct response is to be used, the presence or the absence of physiological anisocoria should be tested before interpreting the results. Most authors do not use the consensual response alone to estimate the RAPD because the consensual response is more variable than the direct repose, especially in dimmer light.

There are authors who use the direct and the consensual response of one eye. For example, the RAPD may be quantified from the differences between the direct response and the consensual response because the consensual response is larger than the direct response in affected eye.[115;226] Physiological anisocoria cannot confound the results in this case because the difference of the direct and the consensual responses are in the same eye. However, contraction anisocoria can confound the results. This is because with contraction anisocoria, the direct and the consensual responses of the same eye are different.

Wilhelm and Wilhelm [118] commented that an optimal *observationa*l method of determining a RAPD includes determination of both direct and consensual responses.[118] This is because in cases of physiological anisocoria (or in pathological conditions such as 3rd nerve palsy), the direct and consensual responses of the same eye are needed to be compared during the clinical swinging flash light test. This is also true for the pupillometric studies because of the reasons described above.

Most authors in recent years used the total output or the total response of stimulation of one eye to compare with that of the other eye, table 5.9. For example, for left eye stimulation, the average of the left (direct) and right (consensual) responses are obtained; and for right eye stimulation, the average of the right (direct) and the left (consensual) responses are obtained. These average responses from the left and the right eye are then compared for the RAPD estimate. With this algorithm, both the results of the direct and consensual response, i.e. the total pupillomotor output, are compared. But, this does not eliminate the confounding effect of contraction anisocoria.

Authors/ year	Outcome parameter(s) used	Direct Response	Consensual response	Average of dir & cons responses per stimulus
Ellis 1979[111]	CA, LC, CV	√	-	-
Cox 1989[115]	CA, MinD, DA, FinalD	Direct minus consensual response of each eye		-
Kawasaki & Kardon 1995[116]	CA (horizontal pupil diameter)	-	-	√
Volpe 2000[217]	CA, LC, CV	Direct and consensual of a single right eye.		-
Bergamin & Kardon 2002/3[215]	CA per segments of pupillogram based on temporal markers.	-	-	√
Bergamin & Kardon 2003[218]	CA per segments of pupillogram based on temporal markers.	-	-	√
Lankaranian & Spaeth 2005[212]	CA	-	-	√
Kalaboukhova 2006[209]	PCA	-	-	√
Kalaboukhova 2007[117]	PCA, PCV, PDV	-	-	√
Wilhelm 2007[118]	PCA	-	-	√

CA = constriction amplitude , CV =constriction velocity, LC = latency before constriction, MinD = minimum diameter, FinalD = final diameter, DA = dilation amplitude; PCA = pupil constriction area, PCV = pupil constriction velocity, PDV = pupil dilation velocity, dir = direct, cons = consensual

Table 5.9: Summarises the type of pupillographic outcome parameters and the direct/consensual/or combined responses used by some authors.

Summary

- Afferent pathway pathology produces a limited and altered response to light which mimics the pupillary reaction obtainable from a dim stimulus –low intensity response.
- Pupillography does not replicate the clinical swinging flash light test because endpoint makers for the clinical and the pupillographic methods of defining RAPD are different.
- Derivative curves give useful information which is not provided by the pupil response curve in a pupillogram. The parameters from first order and second order derivative curves provide additional parameters for the estimation of RAPD.
- There is not yet a standardised pupillographic means of measuring RAPD. Different investigators adopt different test algorithms with variation in the stimulus configurations as well as outcome measures; the stimulus algorithm and the outcome measures being specific to the instrument that they use.
- Most authors use the parameters of the constriction phase of the reflex for the measurement of RAPD because the constriction phase is less affected by the physiological confounders.
- The pupil size effect on outcome measures can be lessened by 'normalising' measurements with the initial pupil diameter.
- There is a fundamental relationship between amplitude of pupil constriction, latency before constriction and pupil constriction velocity. This relationship is not disturbed in patients with anterior pathway deficits.
- The direct response, the consensual response or the combined responses can be used to estimate the relative afferent defect. Each has its own advantages and disadvantages. When the direct response alone is used, physiological anisocoria should be measured. When the combination of direct and the consensual responses are used, it is important to investigate whether there is a contraction anisocoria which could confound the RAPD calculation.

CHAPTER 6

GLAUCOMA

6.1 GLAUCOMA

6.1.1 Definition

Glaucoma is an evolving disease entity. Its recognition began in 1600 by a London Ophthalmologist, Bannister R, who acknowledged the hardness of the eye with associated alteration of the colour of the crystalline lens for this condition.[227] Over 4 centuries, in parallel to the advances in Ophthalmology as well as the ophthalmic technologies, imaging techniques, and visual science, various authors have attempted to define disease and classify its counterparts. Numerous anatomical, pathological and epidemiological features of glaucoma have been unravelled but yet much more to be understood.

Glaucoma can be considered a generic name for a group of diseases causing optic neuropathy with characteristic changes in optic nerve head and nerve fibre layer loss with corresponding characteristic visual field loss usually, but not always, in the presence of raised intraocular pressure (IOP). This is in contrast to the traditional thinking and description of glaucoma as a disease caused by increased intraocular pressure. To date (a) there is evidence to demonstrate that ocular hypertension alone is neither a sufficient nor a necessity for the development or progression of glaucoma,[228-230] (b) there is documented progression of disease despite sufficient lowering of IOP,[231-236] (c) and conversely, there are many ocular hypertensive patients who do not progress to having any structural or functional glaucomatous damage in the clinicians' practice. In addition to raised intra ocular pressure (IOP), other factors - such as optic nerve head perfusion, are considered concomitantly responsible for optic neuropathy in adult glaucoma.[237] Some ophthalmologists now describe glaucoma as a primary optic neuropathy, and raised IOP and optic nerve head perfusion as associated risk factors, the former being modefiable.[238-240]

6.1.2 Classification

Primary open angle glaucoma (POAG) is the most common form of glaucoma. It is different from secondary glaucoma and angle closure glaucoma in that the cause is unknown.[227] Optic neuropathy in POAG is chronic, age –related, insidious, bilateral and

often asymmetrical and progressive.[241] The biological basis of the disease is not fully understood.[241]

Normal tension glaucoma, NTG, is a subset of primary open-angle glaucoma and may represent 20 -30% of POAG patients. The IOP in this group is measured below 22 mmHg consistently. NTG patients tend to have history of vascular conditions such as cerebro-vascular disease, diabetes, hypertension/hypotension, steroid use, and vasospastic disease (Raynaud's, migraine). Optic disc haemorrhages are also commoner in the NTG patients. To date, normal tension glaucoma is considered one of the sub groups of primary open angle glaucoma.

6.1.3 Natural history of POAG

Optic neuropathy in primary open glaucoma is progressive; however, until recently the rate of progression of the disease has not been looked at. The prospective natural history study conducted by Heijl A in 2009 followed POAG patients with high pressure and with normal pressure, and secondary pseudo-exfoliation glaucoma patients from Early Manifest Glaucoma Trial (EMGT) over 6 years without treatment.[242] They concluded that there is progression of disease in the untreated group. The progression is variable with mean results higher than the median results indicating its variability. The median rate of progression corresponds to the advancing from normal visual function to blindness in approximately 70 years; on the basis of mean rate, visual deterioration to blindness is estimated to happen over 25 years.[242] Because this overall figure includes secondary PEX glaucoma patients, the progression to blindness for the POAG can be estimated to be somewhere between 25 to 70 years. The progression is more prevalent in high tension glaucoma subjects, 74% vs 56%, than in the normal tension glaucoma subjects who progress over 6 years of study period. The progression is also considerably faster in the older than in younger subjects.[242] The rate of progression for normal tension POAG patients agrees with those of the Collaborative Normal Tension Glaucoma Study (CNTG)[243;244] where 50% of the NTG patients showed progression in 5 years. According to the authors, the rate of progression is slow, and that the variability may be due to factors (such as women > men, presence or absence of migraine and disc haemorrhage) that negatively affect the course of the disease.[243;244]

The Early Manifest Glaucoma Treatment trial also addressed the natural history of IOP in early or newly diagnosed POAG subjects over 6 years follow-up without treatment. IOP was found to be stable in these patients.[245]

Summary of risk factors for primary open angle glaucoma is summarised in table 6.1.

Strong Association	Age Intraocular pressure Ethnicity Family History
Moderate association	Myopia Diabetes
Weak Association	Systemic Hypertension Migraine Vasospasm hypo- and hyperthyroidism, hypo- and hyperadrenalism, sleep apnoea syndrome, corticosteroids therapies

Table 6.1. Summary of risk factors associated with POAG

6.2 DIAGNOSIS OF GLAUCOMA

The diagnosis of glaucoma can be challenging especially in early cases. A systemic approach and thorough documentation is required so that early cases are not missed and the progression of glaucoma is not over looked.

Glaucoma leads to morphological changes in the optic disc including the intrapapillary (within the optic disc) and parapapillary (immediately around the optic disc) areas as well as the retinal nerve fibre layer.[246] Detectable functional changes include development of visual field defects, a relative afferent pupillary defect, and impairment of colour vision, colour visual fields, contrast sensitivity, flicker sensitivity, resolution

and motion detection.[247] Impaired vision, and blindness are the result in severe cases. Structural assessment and functional evaluation are used together to diagnose, to monitor the change over time, and to restage the patient.

A typical diagnostic approach includes examination of optic nerve (ON) and retinal nerve fibre layers (RNFL) to detect morphological changes, and perimetry to detect functional visual field changes. In addition, intra-ocular pressure, IOP, is objectively and closely measured because it is the primary modifiable factor in the rescue of retinal ganglion cells that are demised in glaucoma. Staging of the disease and consideration of the risk factors allows the clinician to establish a target IOP.

6.2.1 Medical history and risk factors

The diagnosis of open angle glaucoma begins with a detailed ophthalmic and medical history with emphasis on the risk factors for glaucoma, table 6.1. The detailed medical history should include: age of onset, gender, ethnicity, myopia, family history, and medical history and use of steroids. Driving status and refraction status should also be documented.

6.2.2 Slit lamp examination, optic disc and nerve fibre layer assessment

The dynamic slitlamp bio-microscopic examination is performed to assess anterior segment for features of secondary glaucoma and anterior chamber depth. The drainage angle is assessed using gonioscopic lenses. Central corneal thickness is also measured. A thorough examination of the optic nerve is a key element for the diagnosis of glaucoma. It has been shown that as many as half of retinal ganglions can be lost before the visual field test shows evidence of glaucoma. [248;249] Optic disc and nerve fibre layer assessment can be done by the slit lamp biomicroscopy or by direct ophthalmoscopy, the former is favourable because it gives desirable magnification, light level and a stereoscopic view. A normal optic disc contains central area deprived of nerve fibres called the optic cup. The shape of the optic disc is not correlated with age, sex, laterality of the eye, body weight or height.[250]

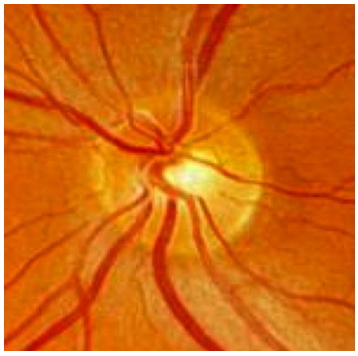


Figure 6.1. Normal optic disc.

Determination of whether the optic disc looks normal, has features of glaucomatous optic neuropathy or non-glaucomatous optic neuropathy can be addressed by the following 5 rules,[251] described in in FORG (Focusing Ophthalmology on Reframing Glaucoma Evaluation) by Weinreb and colleagues.

1. Observe the scleral ring to identify the limits of the optic disc and evaluate disc size.
2. Identify the size of the neuroretinal rim
3. Examine the retinal nerve fibre layer
4. Examine the regions of parapapillary atrophy
5. Look for retina and optic disc haemorrhages.

Other variables or descriptions that are associated with diagnosis of glaucoma are cup to disc ratio, notching of neuro-retinal rim, rim loss, vessel features (nasal displacement of retinal vessels, bending sharply at the cup margin (bayoneting) over-pass of the vessel due to advanced rim loss and reduction of vessel calibre), and baring of the lamina cribrosa.

More recently developed computer-based optical imaging techniques allow objective evaluation of the optic disc and retinal nerve fibre layer. These techniques use different optical properties and different properties of the retina to provide micron-scale measurements of many aspects of the optic disc and the structure of the retinal nerve fibre layer. A few examples of these instruments are Confocal Scanning Laser Ophthalmoscopy (CSLO), Scanning Laser Polarimetry (SLP), and Optical Coherent Tomography (OCT).[252] Larger sample diagnostic accuracy studies and modifications are still underway for their use in glaucoma management.

The most commonly used CSLO is the Heidelberg Retina Tomography (HRT), figure 6.2. This instrument uses the diode laser to scan the retina surface in the x, y and z directions and produce 3 dimensional images. The instrument allows the assessment of the optic cup and optic disc area, optic rim and optic disc area as well as volume measurement. There are a number of global parameters that are measured with HRT. In newer versions of the instrument, Moorfield regression classification software is incorporated to classify normal or glaucomatous optic discs. Longitudinal analysis can also be done using this instrument. The diagnostic precision, by means of largest area under the ROC curve, using a single or multiple global parameters published were AUC of ≥ 0.8.[253-255] In addition to global parameters, there are other HRT novel parameters (peripapillary slope, optic disc topography by retinal height, rim area adjusted for disc area etc.) which can be used to diagnose glaucoma. Their sensitivity and the specificity range from 65%-85% and 69-100%.[252;256-258] Various mathematical methods have been used to combine HRT parameters to further increase the diagnostic precision. The reported values were sensitivity of 42%-93% and specificity of 84%-96%.[254;256;259;260]

The GDx nerve fibre layer analyser is a confocal scanning laser polarimeter (SLP) that analyses the change in state of polarised laser beam (retardation) making use of the birefringent properties of the microtubules within the retinal nerve fibres, figure 6.2. It also has a number of global parameters that assess the NFL including the symmetry between 4 quadrants. Using single or multiple parameters, various studies using various cut-off points, the reported range of sensitivity and specificity of GDx instruments lies between 62% and 96%.[252;253;261-263]

Optical coherence tomographic imaging (OCT) adopts optical technology that is analogue to ultrasound B mode imaging, but it uses a light wave instead of a sound wave. Broadly, there are 2 types of OCT: time domain or TD-OCT (measurement depth are obtained after a longitudinal translation in time of a reference arm) and more recent spectral domain or SD-OCT (interferometric signal detected as a function of optical frequencies allowing for 50 times faster imaging than the TD-OCT, providing more repeatable images with better resolution). The OCT instruments measure tissue thickness and the optic nerve head thickness is analysed in various segments. The reported sensitivity and specificity range from 65% to 87 % [252;253;264] for diagnosing

glaucoma. Stratus OCT in NFL assessment has reported sensitivity of 15% (average NFL) to 85% (clock hour thickness) and specificity of 95% (average NFL thickness) to 60% (clock hour)[265] in detecting glaucoma progression as evidenced by progressive NFL loss observed in red-free RNFL photographs. [266;267] More recent studies have used SD-OCTs to diagnose glaucoma. Their reported AUCs of ROC are also ≥0.8.[268-270]

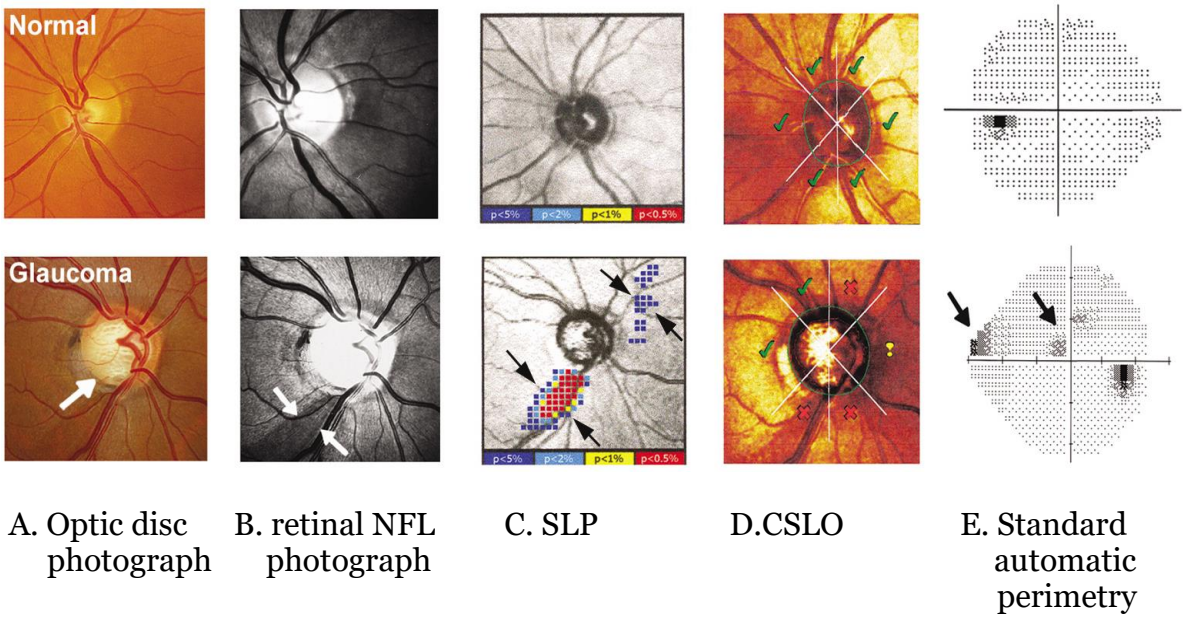


Figure 6.2 SLP (scanning laser polarimetry), CSLO(Confocal Scanning Laser Ophthalmoscopy). Adopted from Weinreb RN, Kaw PT. Lancet 2004.[241]

6.2.3 Intraocular Pressure measurement

Intraocular pressure is the only modifiable risk factor in the management of glaucoma and therefore accurate measurement of IOP is crucial in the management of glaucoma. IOP is often said to be 'normal' if it is between 10-21 mmHg by applanation tonometry, a range determined from 95% of what the healthy eyes have in the population. IOP evaluation must be carried out in clinical context. While the IOP is measured using Goldmann applanation tonometry, the followings are considered:

(a) the central corneal thickness – thick corneas overestimate and thin corneas underestimate IOP,

(b) the cornea curvature – steep corneas overestimate the IOP while flat corneas cause underestimation, but only in the region of 1 mmHg,

(c) the tear film integrity – thick tear film can increase surface tension and underestimate the measurement in the region of about 1 mmHg,

(d) the mires – too thick or thin mires and incorrectly placed mires give erroneous IOP readings, figure 6.3,

(e) calibration of the tonometer.

Figure 6.3. Mires are correctly positioned – 2 inner circles are touching each other.

6.2.4 Perimetry

Visual field examination, or perimetry, is an important part of glaucoma assessment. Glaucomatous visual field defects are functional correlates of glaucomatous ganglion axonal loss. A small unilateral visual field defect is often un-noticeable for the patient but examination of visual field allows clinician to locate and, quantify visual function, correlating this to structural changes. In addition, any progressive changes seen with repeated follow-up can be quantified. The perimetric testing, however, has its own disadvantages: it is subjective, time-consuming and may not detect glaucoma in an early stage.

During visual field testing the threshold visual level of functioning ganglion cells at set points in the field are identified and the areas where the subject can see when the stimulus light is presented against a background are marked out. Testing conditions that can be varied include the number, size, duration and light intensity of the target stimulus. In threshold testing, stimuli of varying intensities are presented multiple times at one retinal location. Threshold is designated as the dimmest stimulus seen 50% of the time. Supra-threshold stimuli are brighter than threshold stimuli, and they are seen more than 50% of the time. Infra-threshold stimuli are dimmer than threshold stimuli are seen less than 50% of the time.

There are two basic types of perimetry used in clinical practice: static and kinetic.[271] In kinetic perimetry a stimulus of set size and intensity is moved from non-seeing to seeing areas of the visual field. The island of vision is approached horizontally, and isopters, depicting areas of equal retinal sensitivity, are plotted. Static perimetry is where the

intensity of a stationary target of constant size is varied to determine the sensitivity of specific locations in the field of vision and retinal sensitivity.[271] Static perimetry is commonly used in glaucoma for perimetric tests.

Standard automated perimetry (SAP) is a static perimetry that uses a white stimulus on a white background. Although, this method is insensitive to loss of retinal ganglion cells[248;249] especially early in the course of the disease,[241] it has been used for more than two decades in routine clinical practice to quantify the patient's visual field, to diagnose as well as monitor disease progression. This is because, SAP is available in most practices or hospital settings, extensive research on large samples has been done on the performance of SAP and some improvements have been made, inter-individual and inter-centre variability have been considered in the algorithm, and there is a large normative database for SAP. Most importantly standardisation has established it use of SAP in glaucoma management.

Among non-standardised perimetry, selective perimetry which isolates specific retinal ganglion cell populations gives promising results in identifying glaucoma earlier than standard visual field testing by SAP. Short wavelength automated perimetry (SWAP) employs a (Goldmann equivalent size V) blue stimulus light against a high-luminosity yellow background, and selectively tests retinal ganglion cells that target the koniocellular sublayers of the lateral geniculate nucleus.[241] In longitudinal studies, it can detect glaucoma as many as 5 years earlier than standard perimetry.[272;273] as well as detect glaucoma progression before it can be detected by white-on-white perimetry[274] However, SWAP is better in detecting glaucoma in young patients than older patients, and using blue lights seem to cause disturbance for older patients with lens opacities. It also has wider inter-subject variability than white-on-white SAP.[275]

Frequency doubling perimetry (FDP) uses frequency- doubling illusion that is attributed to a small subset of magnocellular ganglion cells. The magnocellular ganglion cells are thought to be damaged earlier in the disease. Some studies have shown the use of FDP in detecting early glaucoma and its possible use in glaucoma screening.[275;276]

Until the problems with newer or non-standardised techniques are rectified, and these new machines are made available, SAP will still be the first choice in the clinical practice.

6.2.5 Staging of glaucoma

Staging the severity of glaucoma is of great interest to both ophthalmologists and researchers because it facilitates diagnosis, documentation of progression, treatment response, and characterises glaucoma, making further research possible.

Severity of glaucoma can be staged by means of structural changes such as changes in the optic nerve head and the nerve fibre layer, or functional changes such as perimetric deficits or a combination of both. However, the method or instrument needs to be robust, reliable, sensitive, with good inter-observer variability, user friendly, less time consuming and supply useful information. Perimetric testing is the benchmark for testing visual function in glaucoma. A number of staging systems have been proposed using perimetric results such as H-P-A (Hodapp, Parrish and Anderson) method, Mill's modified H-P-A method, staging methods used by AGIS, CIGTS, and USP GVFSS (University of Sao Polo Glaucoma Visual Field Staging System). The main problem with staging by perimetry is that it requires a reliable perimetric test. It is also time consuming in the clinic, laborious for the clinicians, and fails to account for subtle defects or pre-perimetric glaucoma and other non-glaucomatous pathologies can produce visual field defects. Some use a parameter that is not widely available. Each method/device seems to have limitation in clinical practice and so far there is no one method that correctly and efficiently categorises every stage of glaucoma. Newer instruments such as CSLO (HRT) or SLP may be a better staging tools compared to subjective observation of the optic nerve morphology and staging (inter-observer variability), but the non-availability of these instruments limits their applications.

Spaeth G and colleagues devised an algorithm that stage the disease by observing changes in retinal axons at the optic nerve head, incorporating disc size and the focal rim width. It groups the discs into small, average size, and large size, before assessing the rim width in relation to the disc size, eliminating errors due to large or small discs. Unlike the cup/disc ratio, which focuses on the excavation, the DDLS is based directly

on the thickness of the neuroretinal rim and takes into account the optic disc size. Therefore, the DDLS estimates the glaucomatous damage of the optic disc more precisely than the currently used method. This method has better inter and intra-observer agreement compared to grading with Armaly's CDR assessment.[277;278] It is highly reproducible[279] and correlates strongly with the degree of field loss.[280] Unlike perimetric staging, it can be done relatively quickly in the clinic. The disc damage likelihood scale (DDLS) is denoted as follow (table 6.2).

	Narrowest width of rim (rim/disk ratio)				Examples		
DDLS stage	For small disk < 1.50 mm	For average size disk 1.50-2.00 mm	For large disk > 2.00 mm	DDLS stage	1.25 mm optic nerve	1.75 mm optic nerve	2.25 mm optic nerve
1	0.5 or more	0.4 or more	0.3 or more	0a			
2	0.4 to 0.49	0.3 to 0.39	0.2 to 0.39	0b			
3	0.3 to 0.39	0.2 to 0.29	0.1 to 0.19	1			
4	0.2 to 0.29	0.1 to 0.19	Less than 0.1	2			
5	0.1 to 0.19	Less than 0.1	0 for less than 45°	3			
6	Less than 0.1	0 for less than 45°	0 for 46° to 90°	4			
7	0 for less than 45°	0 for 46° to 90°	0 for 91° to 180°	5			
8	0 for 46° to 90°	0 fo 91° to 180°	0 for 181° to 270°	6			
9	0 fo 91° to 180°	0 for 181° to 270°	0 for more than 270°	7a			
10	0 for more than 180°	0 for more than 270°		7b			

Table 6.2. DDLS newer version. Additional stage added for those above 270º of rim loss.[281;282]

The first step in using the DDLS is to identify the area where the thinnest rim lies. This forces the examiner to evaluate the rim throughout its entire circumference in order to identify the area of greatest thinning. The inner rim edge is defined as the position where the surface of the disk first starts to bend posteriorly towards the lamina. It is

important not to consider sloping of the disc commonly seen in the temporal area as rim loss.[282]

The DDLS[282] is based on the radial width of the neuro-retinal rim at its thinnest point, regardless of which quadrant. The radial width of the rim and the radial width of the disc are measured along the same meridian which represents the thinnest point on the rim. The disc size can be measured using slit lamp and appropriate corrective factors used to adjust according to the lens magnification. A slit beam is directed onto the disc and the graticules at the top of the slit lamp is used to reduce the height of the beam until it corresponds in size to the disc. A 66D gives the exact measure from the graticules.

The next stage is to measure the width of the thinnest part of the rim in the same meridian where the disc diameter is measured. The ratio (rim/disc) is then used for a unit quantity and the value is staged according to the DDLS table. If there is no rim remaining, the rim/disc is "0". The circumferential extent of rim absence is measured in degree to further categorise the severity within this subset.

The DDLS is designed to overcome several obstacles that have hampered previous staging systems. It balances ease of use with sufficient power to detect change, allows diagnosis, grouping into categories of severity, monitoring change, and determining the rate of change. But it has not solved all the issues. It classifies the stage by a category and does not separate those with rim/disc, for example, of 0.3 and 0.39. It will not detect the progression of rim loss from 95 to 170 degree since both fall in the same category. There is still a degree of inter-observer variability in assessing the rim morphology. It also does not attempt to stage anomalous or atypical optic discs. The inter-observer variability, however, is much less with DDLS compared to CDR method.

6.2.6 Diagnostic strategies in glaucoma

Glaucoma is a progressive disease and variable in its manifestation. Although a large majority of cases of glaucoma have both optic disc and visual field defects at diagnosis, glaucoma may be diagnosed based purely on having indisputable glaucomatous optic disc damage or evidence of retinal nerve fibre loss without visual field changes in early

cases. These glaucomas are sometime described as *pre-perimetric glaucoma.* On the other hand, the visual field changes may be the first tell-tale sign of glaucoma when the optic disc features are less-characteristic. However, the inter-individual variation of optic nerve head morphology is huge. There is a great deal of overlap of morphological features between normal and glaucomatous disc. Perimetric tests have repeatability issues, and their sensitivity in picking up early changes is very low. Early changes in threshold tests are shallow and come and go. When the perimetric test is normal or inconclusive and the disc looks suspicious again not conclusive, it is difficult for a clinician to diagnose with confidence and certainty whether the patient suffers from glaucoma. This situation often requires subsequent visits to detect changes and ancillary tests until the evidence for glaucoma becomes concrete. Risk factor assessment is also an integral part of glaucoma assessment.

Making use of asymmetry in glaucoma changes

Glaucoma is a bilateral disease.[283-285] Although the disease usually affects both eyes, it is often one eye followed by another, and it is highly unusual that the disease is symmetrical or identical in both. The numbers of retinal ganglion cells are not exactly the same between the left and the right eye for an individual, the locations of particular ganglions sub-serving a particular function are not exactly matched between the two eyes and the degree of vulnerability of these ganglion cells to the changes in intraocular pressure effect may not be identical. Parameters for measuring the asymmetry of glaucoma can be structural or functional or both. There are studies that endeavoured to diagnose glaucoma or associate severity of the disease by means of measuring the amount of asymmetry of optic disc appearances between the eyes.[286-290] An example of structure asymmetry assessment is the use of rim area disc area asymmetry ratio (RADAAR) measured by HRT to differentiate normal subjects from glaucoma subjects. The authors, Dua and colleagues, compared cases with normative data from elderly subjects (>65 years old) and then determine the sensitivity and specificity of this method for detecting glaucoma. Where there is functional asymmetry, relative afferent pupillary defect is traditionally used to detect glaucoma. Some researchers have studied the asymmetry between upper and lower half of retinal response to visual stimuli, and some use pupil field (stimulus to various parts of the retina location measuring the response via pupil movement).

Early changes in the optic nerve head and the retinal nerve fibre layer measured by means of one or more clinical parameters are often asymmetrical.[287;288] Some parameters are more sensitive in addressing the asymmetry than others. For example, measuring the structural optic disc asymmetry by means of RADAAR detects the asymmetry more than measuring the vertical disc height alone or categorical cup-to-disc ratio. Measuring the relative difference in the afferent deficit using an automated infrared pupillometer will detect the asymmetry more accurately than a clinical swinging flash light testing with a neutral density filter. As the former produces a continuous data accurate to many decimals while the latter gives a categorical grading by the neutral density filters as well as being subject to inter and intra observer variability. There is no doubt that detecting the asymmetry by more than one parameters -structure, function, and pupil reflex - will appreciate asymmetry more than quantifying the asymmetry by one parameter alone.

The advantage of using an asymmetry test is that it addresses the issues of inter-subject variability: age, gender, refraction, disc area, image acquisitions that can be variable between subjects if camera or topographic measures (e.g. HRT) are used, and the variability of contour placement on the optic discs with imaging techniques.

A limitation of using an asymmetry test for glaucoma assessment is the inherit inadequacy in the accuracy of the clinical assessment or the tests. For example, categorical grading of the optic disc will fail to address the asymmetry when comparing optic discs with subtle asymmetry. There may be issues of accuracy of automatic measurements, such as OCT and HRT, due to machine sensitivity, operator variability and patient cooperation. In the studies mentioned above, glaucoma is defined by visual field defects specific to glaucoma. This always raises an issue of miscategorisation for cases of pre-perimetric glaucoma. For the pathogenesis of glaucoma we do not yet know if the atrophy of the RGC happens before the functional impairment, or vice versa. We are not clear whether the excavation of the nerve head precedes the cell death with loss of function, or vice versa. Difficulty in defining the gold standards may also confound the sensitivity and specificity of the asymmetric test.

Interpreting the test results in glaucoma diagnosis [291-293]

One other important thing when using test results in glaucoma is not to forget that these tests are ordered to rule out the disease and to make a diagnosis rather than to corroborate a clinical hypothesis. The print out of the test results is the statistical description of where the subject lies in regards to the normative data. The diagnostic tests use a cut-off point which categorise a result as a normal or an abnormal result. This cut-off value is predetermined based on the findings of the normative data, the epidemiological findings, and how sensitive and specific the examiner wants the test to be. Increasing and decreasing the cut-off values will change the sensitivity and specificity of the given test. Therefore the test result is not a statement of whether the person has glaucoma or not but rather giving another clue to the physician in making a diagnosis. The first step in making a clinical diagnosis is to decide a likelihood of the patient having glaucoma based purely on the risk factors and clinical findings before ordering the test – a pre-test probability. The clinician then judges whether the ordered test result increases or decreases the probability of having a disease. A strongly positive test will increase the post-test probability and a strongly negative test will decrease the post-test probability. For obvious cases of advanced glaucoma, the diagnostic test may not be necessary for making a diagnosis; albeit these tests may be ordered to monitor disease progression. In clinically uncertain cases, however, the results of the diagnostic test can alter the post-test probability drastically and change the clinical impression. As a general rule, the physician should use more than one cut-off in appraising the possibility of the disease happening.

Care should also be taken when more than one diagnostic test is used and the tests are inter-dependent. If the tests are independent then the results of one test can be used as pre-test probability before the second test. If interdependent tests are treated as independent, the results can accentuate/ over-estimate the final post-test probability of the disease.[293]

6.3 GLAUCOMA SCREENING

Glaucoma management has an enormous impact in our society in terms of loss of productivity, number of ophthalmic consultations and health costs.[294-296] It is indisputable that the glaucoma is a potentially blinding condition and early detection and treatment can save sight and reduce health cost. The disease has an insidious onset and prolonged asymptomatic (latent) phase which makes it appropriate to consider for screening. However, to be able to conduct a mass screening, all the criteria of National Screening Criteria need to be, more or less, fulfilled.

A glaucoma screening service is not currently available in the UK or elsewhere. According to the population-based epidemiological data from Western Europe, the USA, the West Indies, and Australia 50% of POAG cases are undetected.[297] A large majority of referrals to the hospital service (99%) for glaucoma in England and Wales come from the optometrists.[298] This opportunistic case finding by testing intraocular pressure and other assessments during routine sight test for spectacles is haphazard.[297;299] The standard of primary testing for glaucoma is very uneven - not all patients over 40 years of age attend the sight test regularly, and sight tests, optic disc examination, tonometry. Referral criteria exclude patients who are in low risk categories; [297] and normal tension glaucoma cases are generally under-detected in the current situation.

Current status and the future

The model proposed in the UK by the Royal College of Ophthalmologists is that of *'shared care'* service between ophthalmologists and optometrists, where the optometrist refers the patient to an ophthalmologist for confirmation of the diagnosis and initiation of treatment. This aims to reduce cost of glaucoma management and improve the quality of care.[295;296]

In the United Kingdom, optometric case finding is the principal modality of glaucoma detection and the trend in Europe and the United States also is that optometrists are becoming far more involved in the care of their patients.[237;300;301] Because screening programme for glaucoma did not meet the UK NSC (National Screening Committee)

criteria, but case finding in people over age 40 years is, however, economically justifiable.[296] Optometrists are encouraged to incorporate disc assessment, tonometry and perimetry testing into the standard optometric examination for those over 40 years of age.

Until the national screening programme can be established, much can be done:

- Working towards maximum case finding in the light of new epidemiological evidence.
- Collaborating with optometrists and general practitioners to improve quality and quantity of referrals.
- Improving glaucoma detection by increasing attendance for eye examination, and improving the performance of current testing. The latter may be achieved by refining current practice or adding in a technology-based first assessment as proposed by Burr as this is a more cost-effective option. [302]
- Undertaking research that aims to include feasibility studies of interventions to help structure future RCTs.[302]
- Refinement of parameters for economic models including data on costs of blindness, risk of progression and health outcomes.[302]

II. GENERAL METHODS

CHAPTER 7

General Aspects of Methodology

Certain aspects of experimental methodology will be repeatedly employed throughout this thesis and hence will be described in detail here for future reference.

7.1 DIAGNOSIS OF A PERSON VS DIAGNOSIS OF AN EYE

Glaucoma is a bilateral disease.[241;284] For glaucoma case detection, it does not matter whether the disease is found in the right or the left eye or both. The aim is only to establish whether the disease is present or absent in each observed *person*. In this thesis pupil measurements are taken for both eyes for the investigation of relative differences to diagnose glaucoma.

7.2 DATA COLLECTION CRITERIA

7.2.1 Ethics

All experiments described in this thesis were conducted in accordance with the tenets of the Declaration of Helsinki.* Local ethical approval was given from the Wiltshire Ethics Committee, United Kingdom, and informed consent was obtained in all cases.

7.2.2 Subjects

Various recruitment criteria were employed during this research. In all cases, subjects were aged 18 years or over and were deemed capable of giving informed consent and comprehending the requirements of the study protocol. All subjects were recruited at the Great Western Hospital, Wiltshire, United Kingdom.

Subjects with healthy eyes were invited from the staff of the local eye department, friends and relatives of the patients who visited the eye clinic or other departments in the hospital, friends and relatives of stuff members. The volunteers were recruited only if they meet the inclusion and do not meet the exclusion criteria and have no family history of glaucoma.

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*As adopted by the World Medical Association General Assembly, Helsinki, Finland 1964, amended by subsequent General Assemblies up to and including October 2013(www.wma.net/e/policy/b3.htm).

Glaucoma patients who meet the inclusion criteria were recruited consecutively from the local glaucoma clinic at the Great Western Hospital. The patient population was divided into six categories, table 7.1. Among these, only patients who were proven to have POAG and those of pre-perimetric glaucoma patients were recruited prospectively in a consecutive manner if they meet the inclusion criteria but do not meet the exclusion criteria.

	Disc	**VF**	**IOP**	**Risk assessment**
(1) Patients with proven POAG	Abnormal DDLS ≥ 3 in either eye	Glaucomatous VF defect corresponding to the disc changes and/or GHT outside normal limit with PSD (or cPSD) below the 95% CI	N/A	N/A
(2) Patients on treatment for pre-perimetric or early glaucoma	Asymmetric disc features DDLS ≥ 2 in the worse eye	No field defect	Opening pressure >21 mmHg	N/A
(3) Equivocal patients	Suspicious disc damage (DDLS ≤3) and/or	Suspicious field, unreliable field, probable field defect but fewer than 3 field tests were done,	Opening pressure <21 mmHg	N/A
(4) OHT patients on treatment	Symmetric discs DDLS < 2 in either eye	No field damage	>21 mmHg in 3 or more consecutive measurements corrected for central cornea thickness	Has ≥ 1 risk factors (table 6.1)
(5) OHT not on treatment	DDLS < 2 in either eye	No field damage	>21 mmHg in 3 or more consecutive measurements corrected for central cornea thickness	No risk for glaucoma noted
(6) Patients who were discharged from the clinic	DDLS < 2 in either eye	No field damage	IOP ≤ 21 mmHg	N/A

Table 7.1 Definition of groups of glaucoma

A detailed medical history was taken and then measurements made of their best corrected visual acuity, intraocular pressure (by Goldmann applanation tonometry), and

optic rim to disc ratio. Automated threshold perimetry (SITA 24-2 programme, Humphrey Field Analyser II, SITA 24-2, Carl Zeiss Meditec Inc., Jena, Germany) was performed on glaucoma patients. Reproducibility of the visual field test was estimated based on the number of fixation errors and false negative and positive errors. The quality of the examination was judged as poor if one of these indices exceeded 20%. A visual field test was classified as abnormal if the Glaucoma Hemifield Test was "outside normal limits" and the pattern standard deviation out of the normal range ($P < 5\%$).

7.2.3 Healthy eyes

Healthy volunteers were defined as those

- who had spectacle corrected visual acuity of 6/9 Snellen's or better,
- who had normal looking optic discs and macula, and normal iris with no local pathologies,
- who are not relatives of glaucoma patients, or a family history (first degree relative) of glaucoma and
- who do not meet the exclusion criteria.

7.2.4 Inclusion criteria for glaucoma patients

- Both unilateral and bilateral primary open angel glaucoma patients including normal tension glaucoma were included if they meet the definition of glaucoma as per table 7.1 (1&2), and have open angles with no other features to suggest they have secondary glaucoma, and
- do not meet any of the exclusion criteria.

7.2.5 Exclusion criteria

- Best corrected Snellen's visual acuity of worse than 6/9.
- Any history of retinal, optic nerve disease (except for primary open angle glaucoma), ocular inflammatory diseases, trauma or intraocular surgeries (except for a cataract surgery without complication).
- Angle closure glaucoma and all forms of secondary glaucomas.
- Significant media opacities.
- Amblyopia (chapter 3) with Snellen 6/9 or worse.
- large eso or exo tropia >40D prism.

- Subjects taking topical or systemic medications that affect the pupil or motility such as miotic or mydriatic eye drops: parasympathomimetic drugs (e.g. pilocarpine, physostigmine), parasympatholytic drugs (e.g. atropine), sympathomimetic drugs (e.g. adrenaline, benzadrine, paredrine, cocaine), sympatholytic drugs (e.g. brimonidine, apraclonidine, ergotamine), myotropic spasmolytic (e.g. barium, calcium, histamine, benadryl, certain alkaloid related opium). [#]Patients on timolol eye drops, however, were included in the study.
- Conditions affecting the *efferent* pathway and pupil motility such as posterior synechia, iris atrophy and Adie's tonic pupils, 3rd cranial nerve palsy and peripheral iridotomy.
- Subjects with systemic (e.g. diabetes) or neurological disease that might affect the pupil.
- Subjects who have difficulty in resting their face on the pupillometer due to mechanical restraints: severe arthritis, contractures, paralysis.
- Subjects who have difficulty in keeping the face or eye still on the pupillometer: Parkinsonism, nystagmus or oculoclonus at primary position.
- Subjects who do not adequately understand verbal explanations or written information given in English and those who have special communication needs.
- Younger than 16 years of age.

7.2.6 Examiners

All the optic nerve examinations and diagnosis of glaucoma were made by an experienced ophthalmologist (GTS) and by the author (AST). In cases where diagnosis is unclear such as in early cases, medical notes were reviewed by a glaucoma specialist (IEM). Pupillometric acquisition was performed by the author (AST). Optic disc assessment was done by GTS who used projected graticule on the disc to measure the thinnest rim width and the corresponding disc diameter to estimate the DDLS.

[#]Johnson utilised the pupillography to study the effect of conjunctival instillation of 0.5% timolol on pupil and observed no effect on the pupil size or action except for the amplitude of redilation to the direct light which was found to be reduced in the timolol treated eye compared to those which did not receive timolol eye drop.[303] He found no effect on the redilation amplitude for the consensual reflexes. Because this thesis only employed the constriction amplitude of the PLR response for a RAPD estimate and the comparison was made within the same subject, glaucoma patients who were using timolol were not excluded from the study.

7.2.7 Experimental settings

All data collection was carried out in a designated quiet room with a door shut at the Great Western Hospital in Swindon, UK. The same testing environment was utilised for the entire thesis, but test light settings and background luminance were different as per test protocol. These are listed separately in the chapters concerned. For each test, the required intensity level and the background light were kept constant for the duration of each experiment. The room has a fire door to seal most of the nose coming from the clinic to provide a quiet environment and one window. When the dark adaptation was required all room lights were shut and a dark black blind was put across the entire window, only allowing the screen light which was in the region of < 0.0001 lux measured from the patient's seat. When ambient room light was required 4 fluorescent strip lights were turned on for the entire room. The total luminance of which was 4 lux.

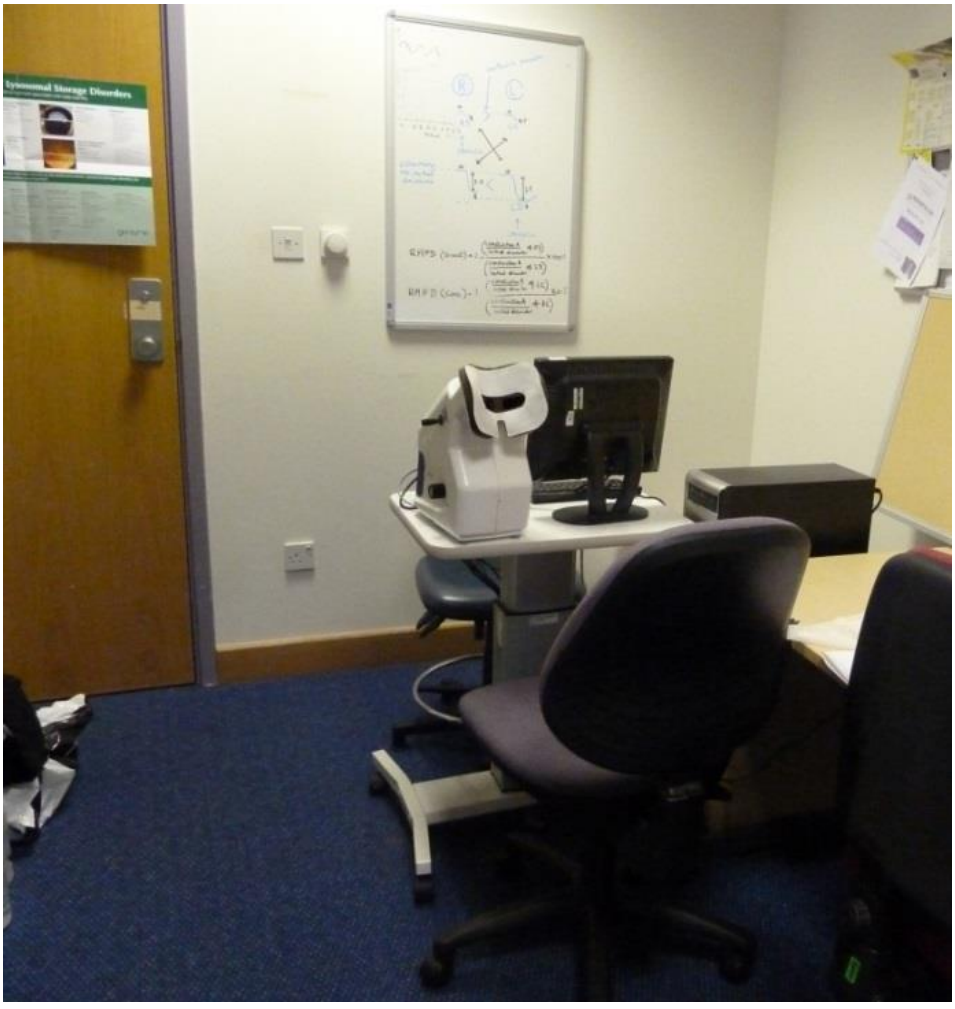

Figure 7.1. A picture of the test environment for all the experiments carried out for the entire thesis.

7.3 PROCYON P3000TM PUPILLOMETER

Figure 7.2 Binocular infrared digital infrared pupillometer Procyon P3000™

The Procyon P3000 is dynamic binocular automated infrared pupillometer. Procyon P3000 was chosen precisely because it is *not* a research instrument, is commercially available and is a simple, portable, easy-to-use, device suited for screening purposes unlike the more sophisticated pupillometers used in vision research laboratories. The instrument also provides the dynamic pupillometry; the real-time images can be viewed by the operator and the adjustment of the subject's head can be made during the test. The face rest is cushioned with soft foam that seals the ambient light entering into the instrument. Clean disposable papers are used to protect the face rest.

7.3.1 The optical diagram

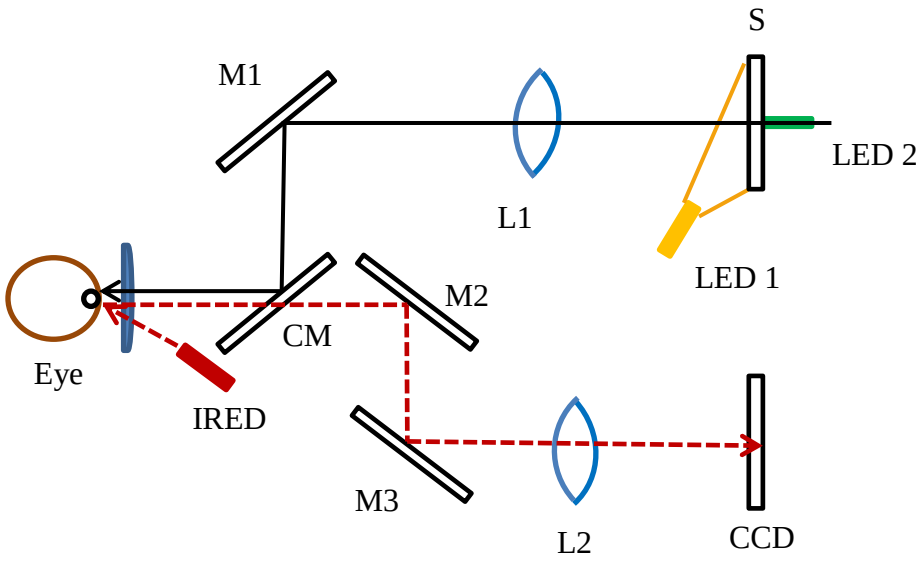

Figure 7.3. The optical diagram of the pupillometer.

The screen S is illuminated by the light emitting diode (LED1) positioned outside subjects field of view. The stimulating light from screen S are transmitted through the object lens L1 and reflected by the mirror (M1) and the cold mirror (CM) to the eye. The fixation green light from light emitting diode (LED2) in positioned behind a small aperture in the screen S positioned at the focal point of the lens L1 for its position at optical infinity.

The infrared light IRED from the infrared emitting diode is scattered on from the iris passes through the cold mirror CM1 and is reflected by the mirrors M2 and M3 and focused by the lens L2 onto a CCD imager.

7.3.2 The device specification

A video camera records the pupil movements to light stimulus of both eyes simultaneously. It has a spatial resolution of ± 0.05 mm or 0.045 mm per pixel and temporal resolution of ± 40 ms or 25 frames per second. The high frame rate allows the pupil motion to be displayed graphically with accuracy.

The stimulus for each eye is an illuminated white square, subtending an angle of 15 degrees across its width. The fixation target is a small (< 0.05º), dim (< 1mlux), green LED, projected at optical infinity, figure 7.3above.

Figure 7.4 depicts the stimulus light as seen by the patient before resting his face on the face rest which is cushioned with soft foam and covered with a disposable white sheet. The nose foam is provided right up to the bridge of the patient's nose to prevent light seeping out across the bridge of the nose. The subject then presses his face on the foam rest firmly so that it is light sealed. A single wide field view - 20 degree (h), 16 degree (w) - of the standardised controlled stimulus is achieved when the patient rests on the chin rest to view the stimulus. The accommodative target appears at the centre of the white stimulus square as a small dim green target. During darkness interval with no stimulus light on, the subject can only see the fixation target which is too low (< 0.001 lux) to cause any pupillary reaction.

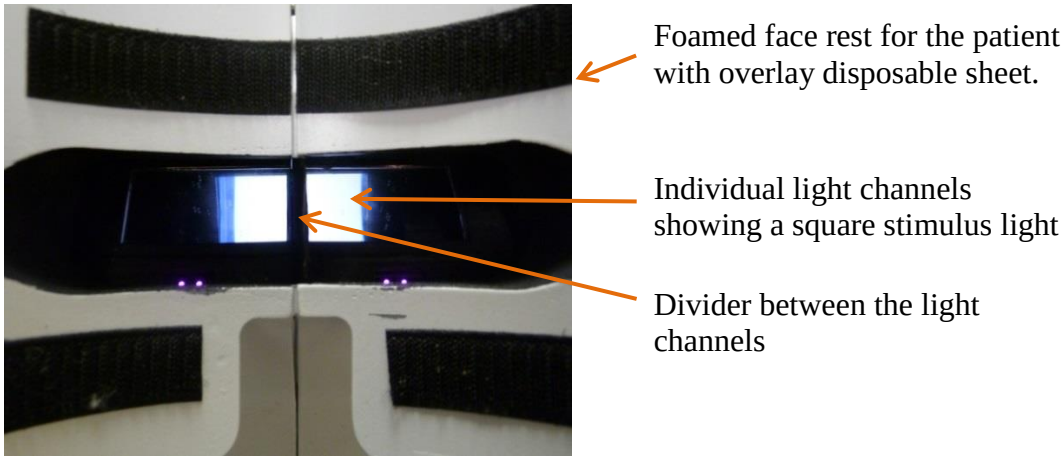

Figure 7.4. The picture of the face rest of the Procyon P3000 depicting the light channels as viewed by the subject. The stimulus is presented through the square shape channels. The central divider divides the light channels completely.

There are slots in each of the stimulus channels which allow the insertion of the filters or occluders if required. The device does not use an eye piece with separate channels but has a common light channels that is separated by the divider which falls between the eyes. When there was no NDF required during the experiment, the filter slot can be covered with a tape to provide a complete light sealed environment for the patient's eyes.

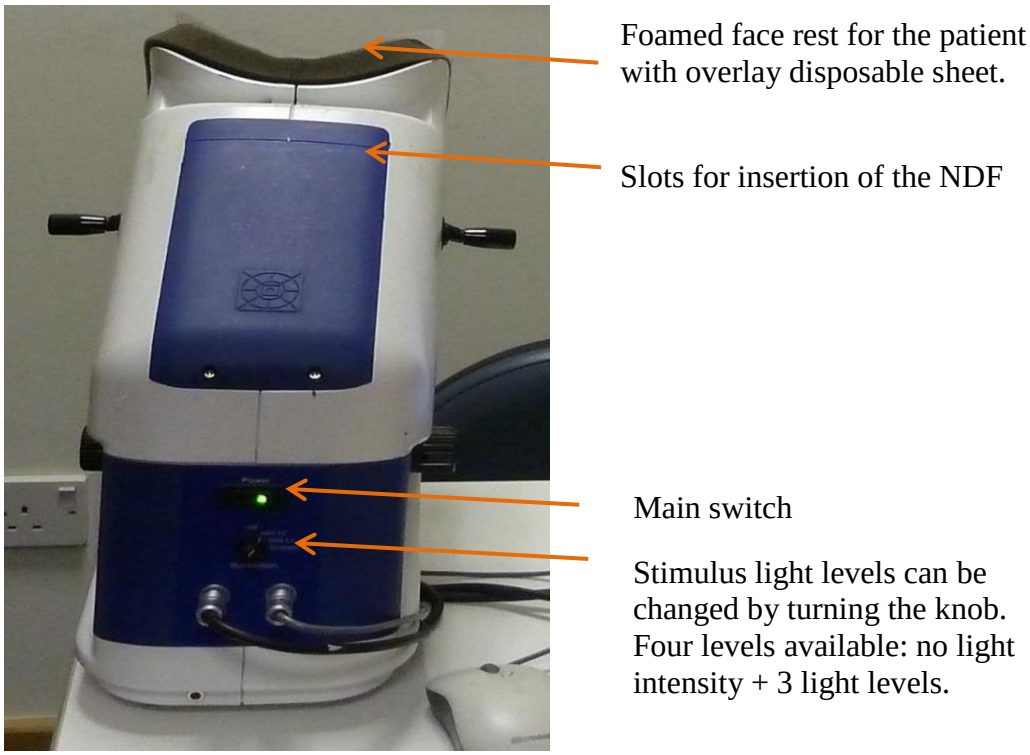

Figure 7.5. Procyon P3000 pupillometer depicting the knob for the setting required light levels, and the slots for placing neutral density filters.

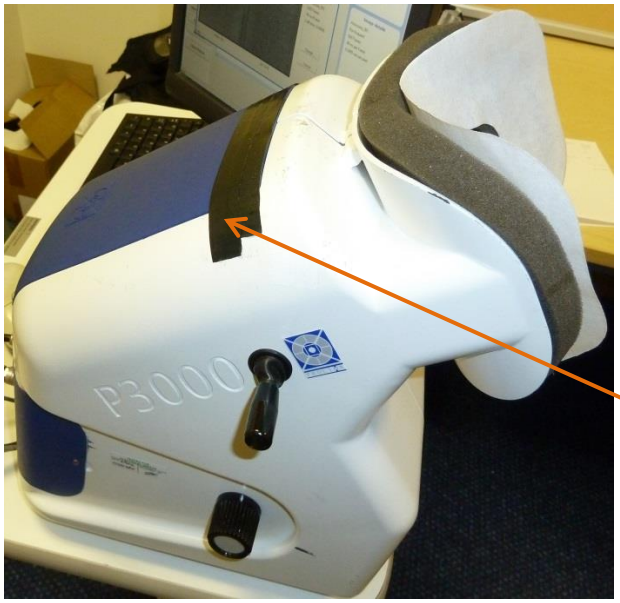

Figure 7.6. The external light coming into the instrument was prevented by the subject resting his face against the foamed face rest and by sealing the filter slots by a tape.

The stimulus parameters that could be adjusted by the software are the intensity, duration, and inter-stimulus-interval. The stimulus area, wavelength, and waveform could not be adjusted. Three stimulus light levels can be set to the required level to utilise in the pupil test. Procyon describes them as 'scotopic', 'low mesopic' and 'high mesopic' levels. The light levels were measured in lux. The stimulus can also be programmed to present either continuously, sequentially or alternately between the two channels.

During this thesis, stimulus light levels were tested to find the suitable range of light levels for the measure of relative afferent pupillary defect. The stimulus light was applied alternately and separately to each eye.

^ There are a few features in addition to the visual illumination that the device provides such as an optical moveable fixation point or accommodation target for testing pupillary accommodation reflexes, and graticule overlay facilities. These features were not utilised for the study.

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^ Further details of operation of P3000 and the user manual can be obtained from Procyon Instruments, UK.

7.3.3 Acquisition of pupillograms by the Procyon P3000 pupillometer

Prior to any pupillometric data being acquired, the subject was asked to place their face on the face-rest, which excludes ambient illumination extremely efficiently, and fixate on the dim target LED projected to an optical infinity in the centre of the screen. The subject then rested their face securely in the face rest and reminded that no light should be allowed to their eyes except for the dimly lit fixation light (size <0.05^{o}, illuminance <1 mlux) during dark adaptation. Following this, an acquisition period of 28 seconds was initiated. Eight hundred frames were captured at a rate of 25 frames per second (40 ms per frame). A sequence of stimulating visible light pulses of identical duration, intensity as per test protocol were applied through the separated left and right eyes alternatively and the measurements were taken for the pupil diameters throughout. This was achieved by the *PupilFit*^{TM} software which acquired images, controlled stimuli and fitted circles to the images. Unwanted artefacts were removed from the trace. The horizontal pupil diameter was recorded by the software and translated onto the computer by a numerical number in millimetre.

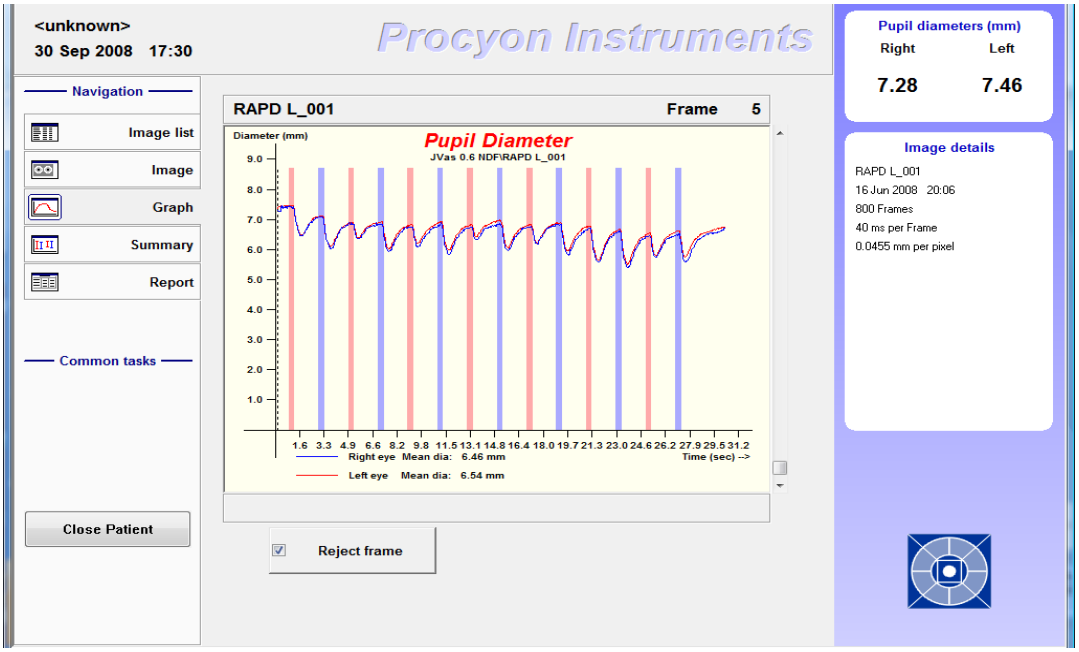


Figure 7.7. The display of the graphical presentation of the pupil movement on the monitor by the proprietary Procyon PupilFit^{TM} software. The tabs on the left provides the display options: such as in the image list, single image, graphical format, summary or a report.

The average data was displayed on the right corner of the screen, figure 7.7. The data could be displayed on the monitor in a graphical format, pictorial format (figure 7.8) or in a summary table. The operator can therefore monitor the tracing or subject's positioning in real time. The stimulus editor program was used to create new stimulus patterns for the test if required.

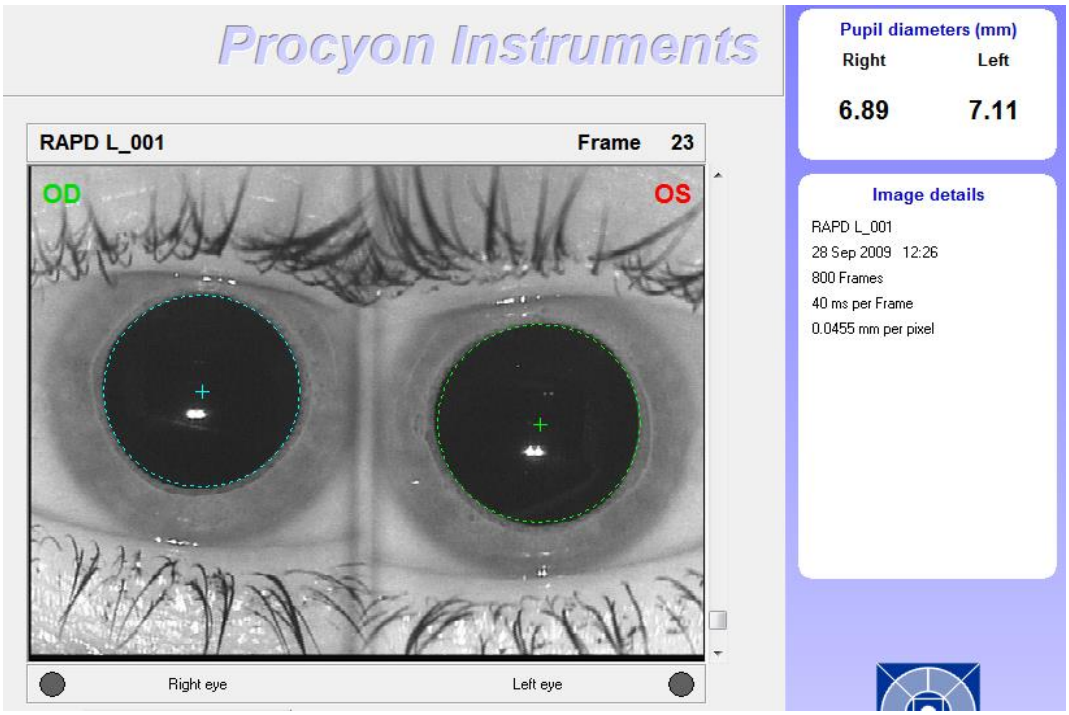


Figure 7.8. Example of an image captured by the binocular pupillometer P3000 pupillometer.

7.3.4 Outcome parameters produced from Procyon P3000 pupillometer

The proprietary software also calculates the parameters of the pupil light response directly from the graph. These include: resting pupil diameter, the pupil diameter at the end of constriction, the amplitude of pupillary constriction, latency before constriction, velocity of constriction, peak constriction velocity, redilation velocity, constriction time and redilation time. Only the amplitude of pupillary constriction was used for the purposes of this thesis, figure 7.9. This decision was made based on the findings of previous investigators who considered the constriction phase of the pupillogram appropriate for the RAPD measurement as they were more repeatable and less subject to the higher centre influences, section 5.4.3.[31;111;115;116;123;212;217] The latency of constriction was not used because of the ongoing debate on its endpoint determination as described in section, 5.4.3.2.1. The measurement of constriction velocity (a temporal

factor) demands a higher mathematical formula and is more sensitive to the device resolution as well as the noise level in the recording as compared to the constriction amplitude. The advantages and disadvantages of each parameter are discussed in the previous chapter 5.

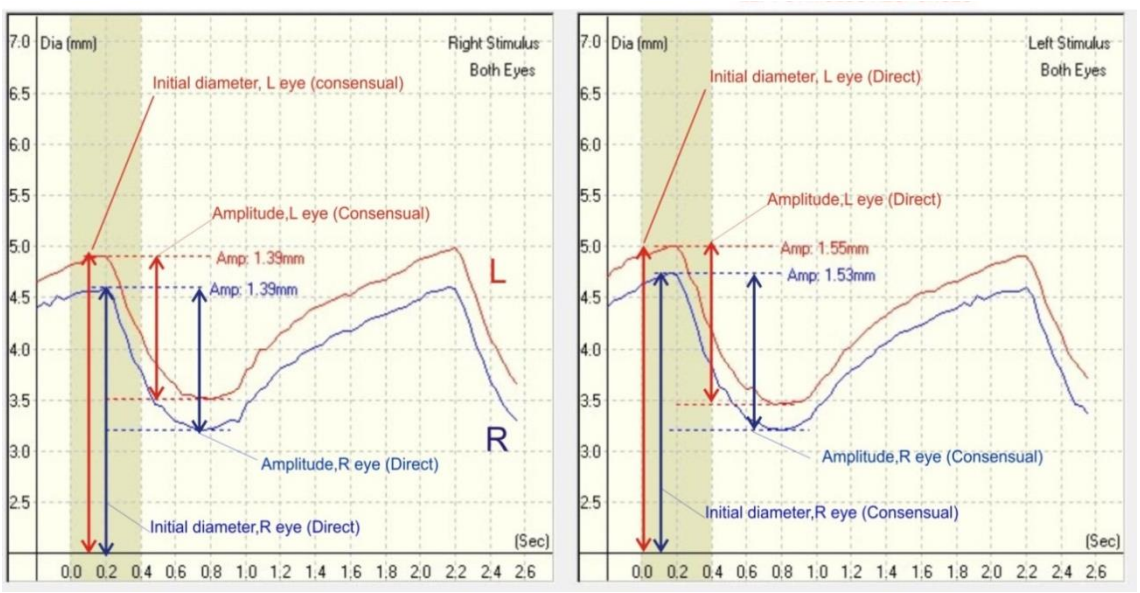


Figure 7.9. A pair of pupillograms. Direct response to the right eye (blue) and consensual response to the left eye (red) are seen in the left diagram. Direct response to the left eye (red) and consensual response to the right eye (blue) are seen in the diagram on the right. Only the amplitudes: amplitudes of pupillary constriction and initial pupil diameter prior to constriction are displayed.

The amplitude of pupillary constriction was measured between the maximum pupil diameter before constriction and minimum pupil diameter before redilation, figure 7.9. Underlying hippus can mimic the deflection that represents the onset of the pupillary constriction. In order to differentiate hippus from true constriction, the pupil tracing was monitored during the latent period. The average and 95% interval of the data were calculated. If more than 3 consecutive measurements were below the 95% confidence interval were found, the first breach of the confidence interval was marked as the beginning of the pupillary constriction.

We observed that the first pupillogram had the largest pre-stimulus pupil diameter, figure 7.7. This is because the states of adaptation of the retina to illumination change substantially following the initial pulse to each eye. The retina was originally adapted to 30 seconds of darkness and was operating in the relevant sensitivity threshold. When the first stimulus was applied with subsequent alternating sequences, the photoreceptors

set themselves a new operating threshold causing the initial pupil diameters to be smaller. The duration of inter-stimulus interval may also play a role especially if it was set to a very short period not permitting the pupil to return to its original size. The pupil constriction amplitude of the first pair, therefore, was discarded before the RAPD calculation for consistency of the data.

7.3.5 Anisocoria correction

Physiological anisocoria is present in normal individuals and in theory this can confound measurement of the relative difference in the afferent pupillomotor input. This is because a smaller pupil allows less light to reach the retina. A correction for anisocoria is made by estimating the 'equivalent neutral density filter' that would be required in the eye with the larger pupil to equalize retinal illumination. When more than one light level is used for the RAPD calculation, the amount of light reduction in the smaller eye will be proportional to the size of the pupils which in turn is proportional to the level of light used. Anisocoria correction can be dealt with in a single quantity by taking the average of each anisocoria correction for each of the three light levels. This correction is formulated and provided by Procyon. The correction for anisocoria is made before the pRAPD is calculated (using the Procyon algorithm). The formulary details of this correction have not been provided by Procyon for publication in this thesis. The laterality of the RAPD is determined to be the eye that gives lower amplitude responses on direct light stimulation. A pupillometric RAPD on the right is assigned a positive value and a pRAPD on the left is assigned a negative value.

7.4 TREATMENT OF ARTEFACTS, GLITCHES AND NOISY RECORDINGS

7.4.1 Processing acquired images

The software allows removal of unwanted artefacts from the trace. Instead of using electronic data cleansing, the operator/ author (AST) carefully looked through the pupillogram individually, and manually deleted the segment of the pupillograms which represented blinks or poor pupil circle fitting, figures 7.10 (a,b,c).

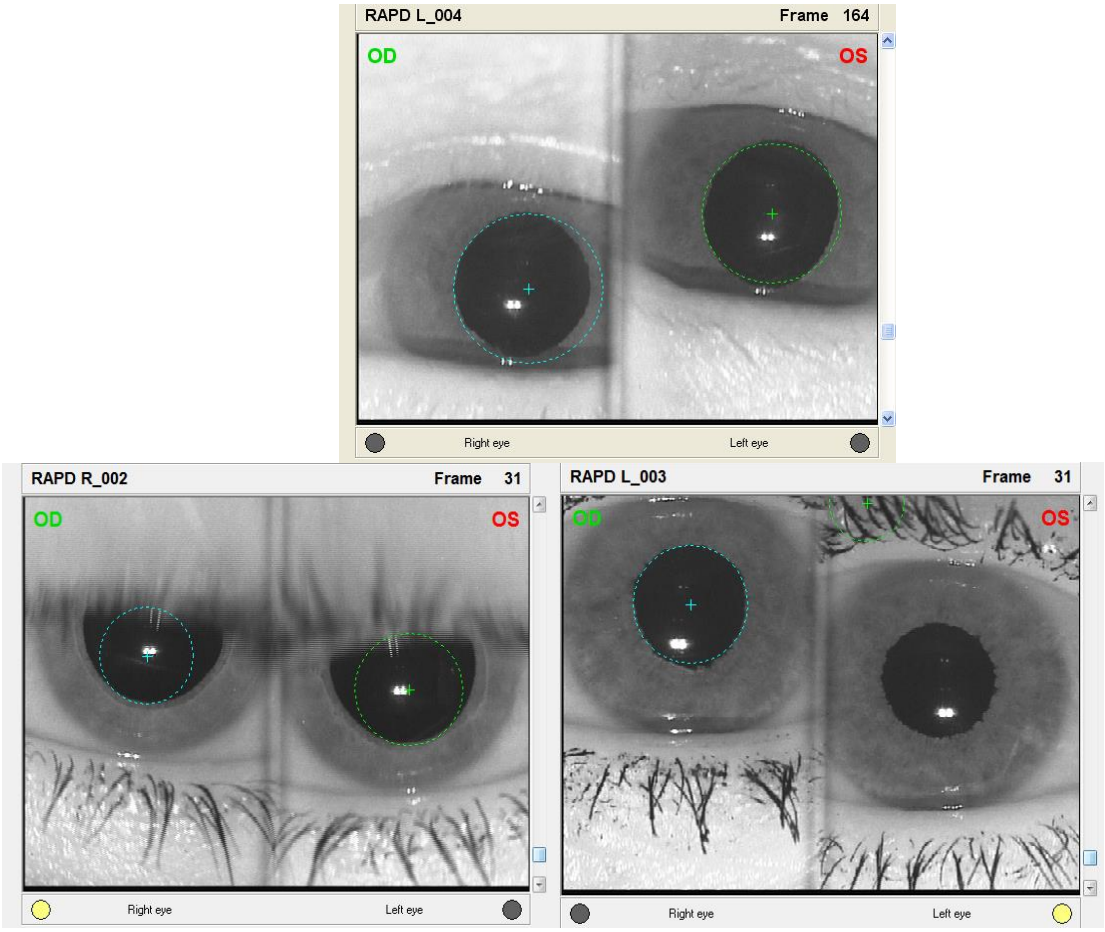
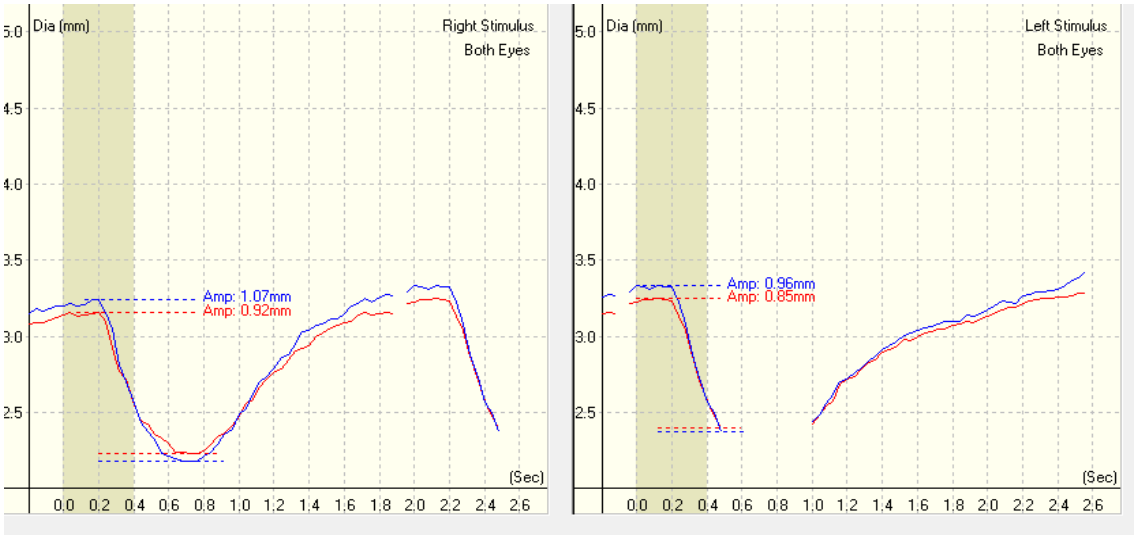


Figure 7.10(a) poor pupil circle fit of right pupil (this patient had difficulty positioning and manifest exophoria), (b) blink during pupil measurement, and (c) poor pupil circle fit of the left pupil.

7.4.2 Assessing measurement accuracy

As described above, removal of the recorded frames were required for blinks, glitches, poor fits or misfits of the pupil measuring circle. If a blink was less than three frames in duration (i.e. <3×0.04 seconds at an imaging frame rate of 25 Hz), one may interpolate "good" data from either side of the blink. But if the blinks fell at the beginning of the pupil constriction or at the end of constriction, it could cause measurement error. Before any calculation was attempted, the pupillograms were carefully checked by the author for incomplete measurement lines by the software. Figure 7.11 (b) gives an example of the horizontal dotted lines misplaced on the graph. The pupillograms with incomplete data at the beginning and end of constriction were not included for the pRAPD measurements as they do not represent the true constriction amplitudes.



(a) (b)

Figures 7.11 (a,b) Data for left pupil diameter at maximum constriction have been removed (in this case due to a blink) and therefore measurement lines are not accurately placed.

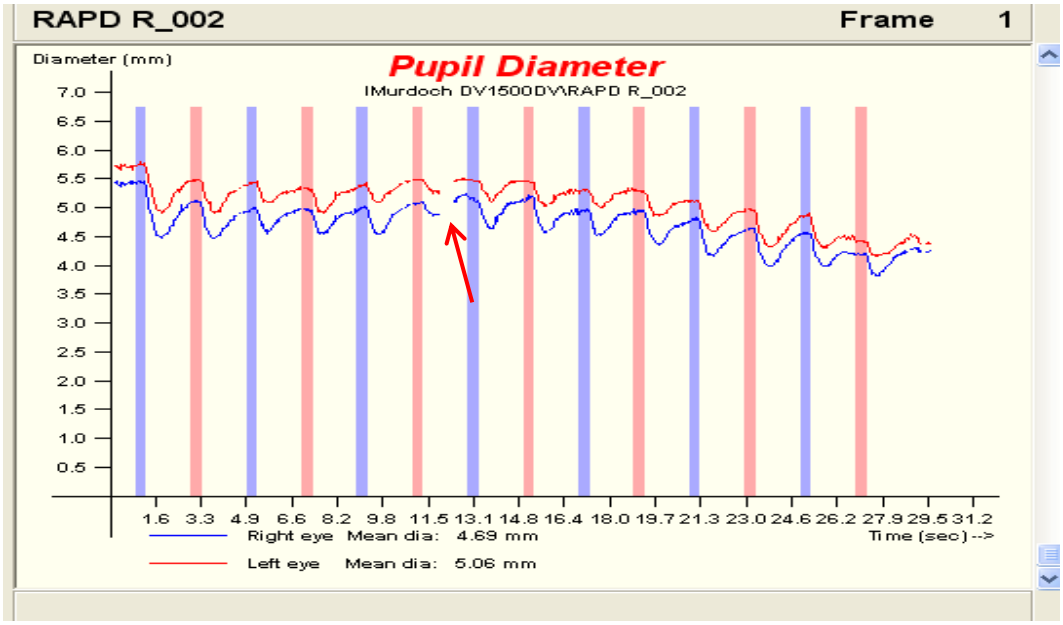


Figure 7.12. The pupillograms depicting the areas where the frames were removed (arrow).

Any isolated reflex shapes which were different from the rest of the pupillograms within the same acquisition were examined. Care was taken to differentiate between true artefact (i.e. nonsense data due to technical problems with stimulus presentation or response recording such as poor fitting of PupilFits or blinks) and *outlying points* (i.e. unexpected result but not apparently due to any technical problems with experimental set-up). The criteria were set whereby:

(1) All reflexes due to blinks, artefacts due to eye lids, poor fitting of PupilFits, off axis images, small pupil responses with no recognisable wave form for the software to calculate the amplitude of pupillary constriction were removed.

(2) The outlying reflex shapes witnessed to occur with external stimulus other than the stimulus light (for example, when someone barged through the door during the acquisition period- a startle reflex) were removed, figure 7.13. Mostly, these reflex shapes occurred as a result of higher centre influences.

(3) If there was no evidence of external stimulus other than the test stimulus and if the subject was not witnessed to be in stress or sleepy, the reflex shapes were kept for processing.

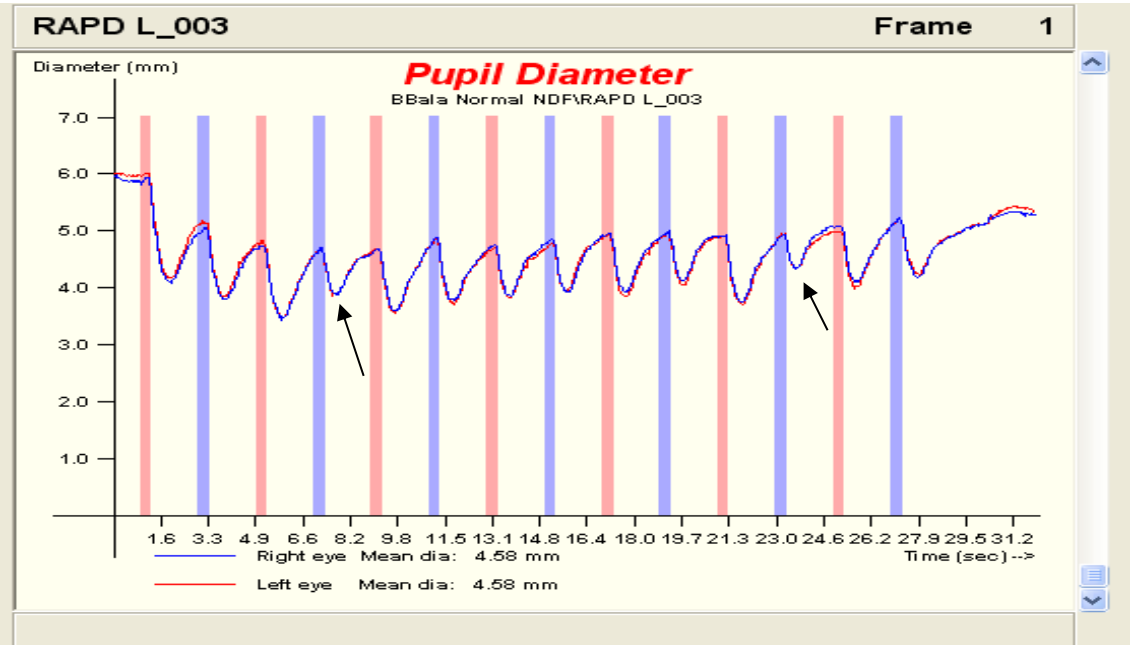
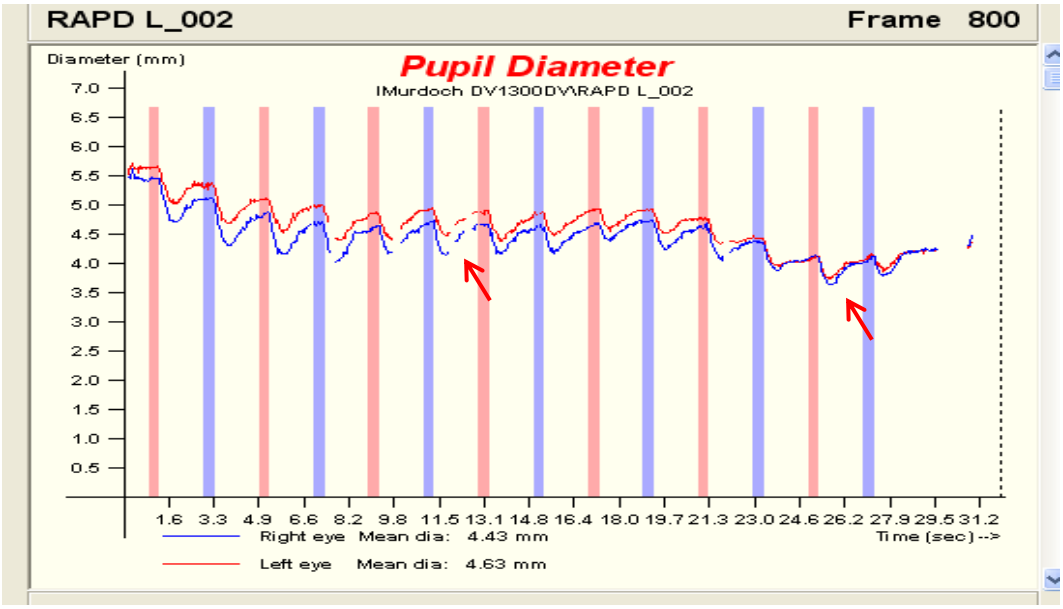
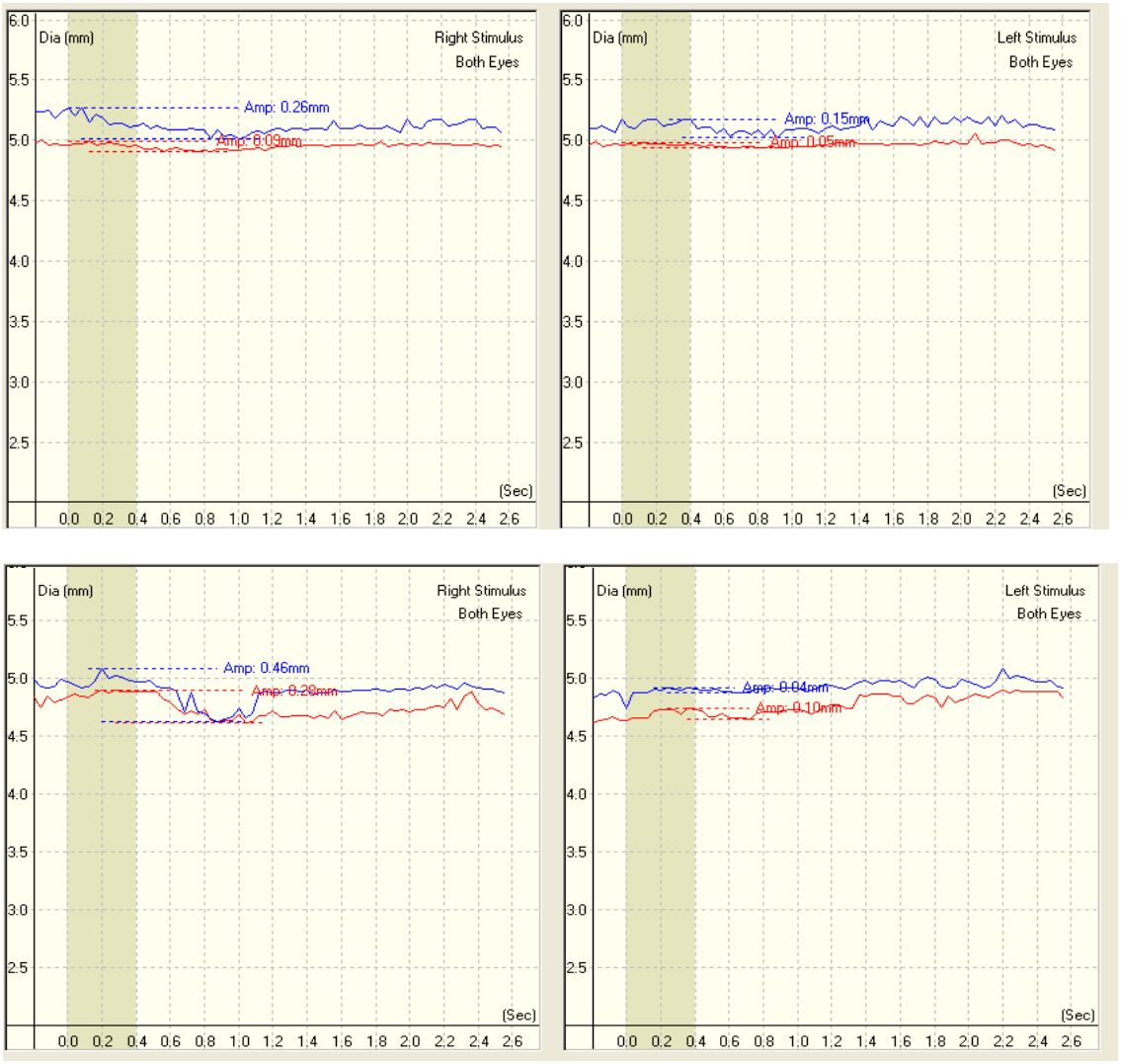


Figure 7.13 The reflex curves (arrow) which appears to be different from the rest of the pupillograms. This patient was startled by a clinic staff entering the room. The first arrow coincided with the time the staff opened the door and the second arrow when she shut the door to leave the room. These two pupillograms were removed.

An example of pupillograms from a sleepy subject is depicted in figure 7.14. The pupillograms of poorly reacting pupils gave no recognisable reflex shapes, figure 7.15. These pupillograms were also removed.



Figures 7.14 Variability in the reflex shape of a sleepy subject. The subject was sleepy and the eye lids came down causing ill-fitting of the PupilFit circles on partially exposed pupils, sharp dips were seen in the pupillogram which were removed (the first red arrow). The subject became very sleepy at the end of the stimulus (second red arrow).



Figures 7.15 (a & b). The graphs represent the poorly reacting pupils. There was no recognisable pupil reflex shape. The measurement lines were poorly fitting to the graph because of the low signal to noise ratio.

7.5 STATISTICS

The Microsoft Excel (10.0) and the Stata statistical software (version 10) were used for statistical applications.

7.5.1 Random table

The random table was used to avoid sampling bias. The following is an example of a random table generated from the Microsoft Excel sheet. There are a number of ways that the random table can be read. The method used in this thesis was to read the numbers across the page and the repeated number is skipped. If, for example, test 1 = 1,

test 2 = 2 , test 3 = 3, in the table below, the order of test will be: test 2, test 1, test 2,test 1 and test 3 and so on.

Random table:

2	4	1	2	2	1	1	3	2	3
3	4	2	3	1	3	1	3	4	4
3	2	1	1	3	3	2	4	3	2
1	1	4	1	4	1	1	2	2	4
3	3	2	2	4	4	1	2	1	3
3	1	3	2	4	3	2	4	4	1
4	3	1	1	4	1	1	4	3	3
3	4	4	2	3	4	3	2	2	2
3	3	4	2	3	2	2	3	4	1
1	3	4	2	2	2	1	1	2	2
4	3	1	4	2	2	4	1	2	4

Table 7.2. An example of a random table

7.5.2 Receiver Operating Characteristic Curve (ROC curve)

The diagnostic ability of the test may be described in terms of its sensitivity in detecting the disease (the proportion of diseased individuals who are correctly diagnosed as having a disease) and its specificity (the proportion of normal individuals who are correctly identified by the test). These two measures are closely related to the concepts of type I and type II errors. High specificity would mean low type I error and high sensitivity would mean low type II error. The sensitivity and specificity can be defined by the following formula.

Sensitivity = number of true positives / (numbers of true positives + false negatives)
Specificity = number of true negatives/ (numbers of true negatives + false positives)

From the continuous test data, it is then required to decide upon a cut-point which will distinguish “disease” from “normal”. When the distribution of disease and normal have no overlap (an ideal situation) it is possible to determine the cut-point which will produce 100%sensitivity and specificity. However, in practice, it is merely the case. The distribution of normal and disease will almost always have an overlap. Therefore, varying the cut-point will influence the sensitivity and specificity such that one is trade-off against another. An overall impression of sensitivity and specificity can be obtained by plotting the sensitivity against (1-specificity). This is termed the Receiver Operating

Characteristic Curve (ROC). The diagonal line represents the diagnostic performance which would be expected by chance alone (no predictive value). As the curve approaches the left upper corner of the plot the area under the curve (AUC) will be larger and the better the diagnostic test will be. The AUC was used to compare the performance of different test designs in this thesis.

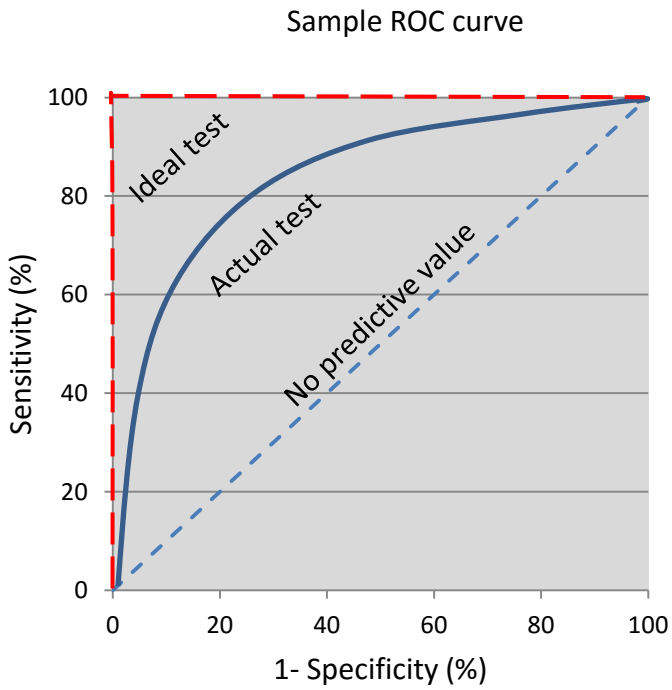


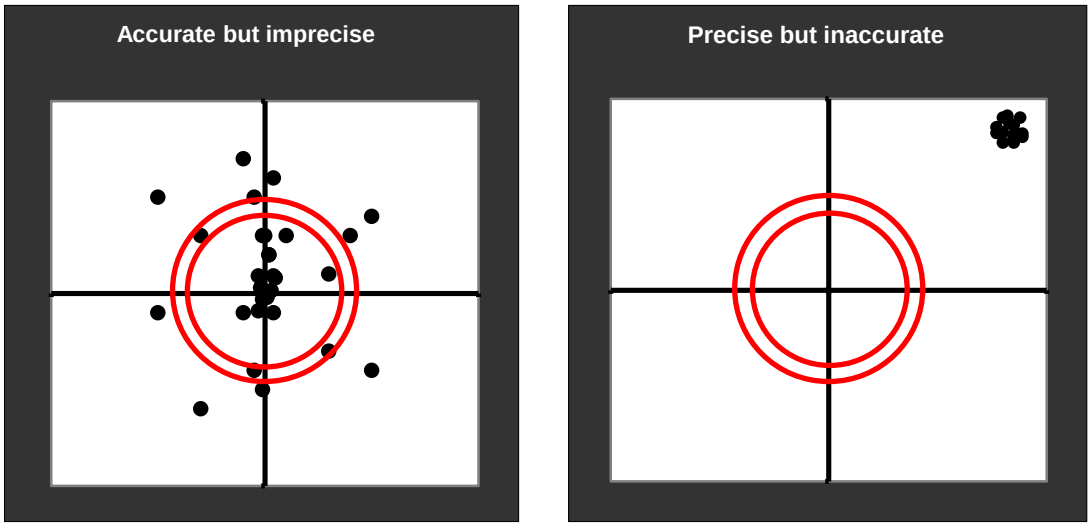
Figure 7.16. A sample ROC curve. The area under the "Actual test" curve (blue line) represents the AUC of this actual test. The red dotted line represents an "Ideal test" which would have a 100% sensitivity and 100% specificity.

7.5.3 Student t-test

This test is often used to compare groups of observation which are paired or unpaired. Paired data arise when a group of subjects are studied more than one time either by the same test or by different test. The t-test is used to test the null hypothesis that the mean test result for the group has not changed from the first test/occasion to next test/occasion. Rather than using the normal distribution which is valid only for large groups of observations, the t-test uses the similar t-distribution which is valid for any sample size. The t-test was used in this thesis to compare groups of data (continuous variable).

III. ACCURACY AND PRECISION OPTIMISATION

For an instrument that measures a biological response, the quality of the measurement is assessed by its accuracy and precision. In a broader term, the measurement is considered accurate, if the measurement values are close to the "actual value". The measurement is considered precise, if the test-retest-variability (TRV) is small, figures below (a, b).



Figures (a) An example of an accurate but imprecise test, (b) an example of an inaccurate but a precise test.

There are a number of factors that can alter the accuracy and the precision of the test. In pupil measurement this may include factors related to the instrument, to the subject, to the observer and the test itself. For example, the measurement of pupil dynamics may be inaccurate due to the measurement errors. On repeated pupil testing, the results may be different due to (a) the underlying variability in the base line RAPD of an individual, and (b) the variability of the test environment or (c) the test itself. The possible error incurred by the observer was minimised in this thesis because the measurement concerned was done using an automated pupillometer and the role of the observer was merely to acquire the pupillograms through fully automated instrument. However, the observer could make errors in interpreting and processing of results. For example, there may be a situation when the observer has to decide whether the circle of PupilFit fits accurately to the pupil margin.

The following chapters address the accuracy and precision of the test. The chapters will focus on evaluation of the instrument and calibration, optimising stimulus parameters-

including duration of dark adaptation and stimulus configuration, outcome parameters and studies of repeatability of the test.

CALIBRATION OF THE INSTRUMENT

Chapter 8

Calibration of the instrument

8.1 INTRODUCTION

The P3000D pupillometer is a step-up from the P2000 model, and both models have been widely used for the measurement of dark adapted and light adapted pupil sizes in the field of refractive surgery.[304-306] They are known to be able to measure pupil diameters either unilaterally or bilaterally with precision. The original Procyon P2000 series have been reported in previous papers to successfully distinguish Neutral Density Filters (NDF) to 0.3 log units.[217] Lankaranian & Spaeth[212] used P2000 pupillometer to test glaucoma patients in 2005. The new P3000 pupillometer has not been used for a comparative pupil response reflex test such as a test of relative afferent pupillary defect. The design of the stimulus channels in the Procyon P3000 is different to the P2000. For the purposes of this study, it is crucial that the P3000D instrument is first tested for its suitability in accurately measuring the relative afferent pupillary defect by pupillometric means. This is addressed as ***calibration*** of the instrument.

Neutral density filters of known attenuation are well calibrated by their manufacturers and the registered amount of brightness attenuation is considered accurate for each filter. They serve an excellent tool for calibrating an instrument emitting uniform light source. Neutral density filter reduces light entering onto the retina producing low intensity light reflex measurable by the pupillometer. The low intensity wave forms elicited by a pupillogram as the result of placing a neutral density filter in front of one eye is similar to the waveform produced in the eye with optic nerve pathology[214] - prolonged latent period, reduced extent and speed and relatively short duration. This property of NDF has been used in both experimental and clinical settings to simulate afferent pupillary pathway deficits.[111;118]

The value of the neutral density filter is determined by the optical density of the filter which can be described as the amount of attenuation of light by the filter. Wratten 96 (Kodak) Neutral Density Filters have been used. The attenuation has standard definition: Attenuation, A *(log unit)* $= \log_{10}$ *[Filter Transmission]*, where "Filter Transmission" is the ratio of output illumination from filter to the incident illumination onto filter. So, for example, if a filter when placed in the path of a beam of light, of

illumination 10 lux, reduces the beam to 5 lux, the "Filter Transmission" is 0.5. The Attenuation, A, is $\log_{10}(0.5) = -0.3$ log units.

1.0 log unit = 90% light attenuation, 0.9 log unit = 87% light attenuation 0.8 log unit = 84% light attenuation 0.7 log unit = 80% light attenuation 0.6 log unit = 75% light attenuation

In theory, if the light attenuation of the NDF is known the amount of RAPD induced should be predictable. Clinically, the amount of relative afferent pupillary defect (RAPD) is determined by placing NDF in front of the better eye in increment of (usually 0.3 log units) while the test light is swung from one eye to the other (clinical swinging flash light test) until both pupils respond equally.[23] The density of pupil defect corresponds to degree of filter required to balance pupillary response and it is normally quantified by "log unit". The relationship between the induced RAPD and the measured RAPD, here in this case, by the pupillometer is of fundamental importance.

8.2 OBJECTIVE

The first objective of this thesis was to assess the ability of the P3000D pupillometer in recognising and distinguishing different neutral density filters with known calibrated filter values. By studying the raw pupillometric output data for each filter set, the aim was to determine the variables that influence the measured pupillometric RAPD, pRAPD.

8.3 METHODS

The following questions were addressed:

(A) Is the built of the instrument adequate for the assigned pRAPD measurement? Is the instrument registering the correct amount of light?

(B) Is the intensity of light provided with the instrument suitable for the experiment? In other words, does the instrument provide a correct level of light and able to detect a subtle pRAPD?

(C) How does the test perform on the normal subjects?

Subjects:

Heathy volunteers were tested for this part of the study. They were defined as subjects with no history of retina or optic nerve diseases, trauma or surgery to the eyes, and had a spectacle corrected visual acuity of 6/9 or better in either eye.

Instrument and device:

A Procyon digital infrared binocular pupillometer was used to study the pupil response and Kodak No 96 'Wratten' Neutral Density Filters (NDF) were used to attenuate the light transmission.

8.3.1 Is the instrument registering the correct amount of light?

Objective:

To determine how pupillometer registers the effect of attenuated stimulus light by calibrated neutral density filters with known filter values.

Methods:

In order to determine how Procyon P3000 registers the amount of light attenuation by calibrated neutral density filters, the absolute values of left and right channels illumination was first matched to less than 1% difference using a light meter. The NDFs were then slotted in one channel and readings were recorded.

Pupillary constrictions to direct light responses were used to calculate the pRAPD .

$pRAPD = (A_{small}/A_{large}) \times 100\%$

A_{small} = smaller amplitude of pupillary constriction on direct light stimulation

A_{large} = larger amplitude of pupillary constriction on direct light stimulation

Results:

It was found that a disproportionately large amount of 2.3 log units (<1% light transmission) was required to produce a pRAPD of 18%.

Findings:

The original P2000 series has been reported in previous papers to successfully distinguish between very small NDF's,[217] and Lankaranian & Spaeth reported it successfully identified glaucoma patients with RAPDs.[212] The design of the stimulus channels in the new P3000 is different to the P2000. That prompted us to explore further the reason for this weakness in picking up the attenuation difference. We suspected that there might be a cross-contamination between the left and right stimulus channels.

8.3.1.1 Subsequent Experiment: Is there a cross-contamination between the left and right stimulus channels?

Objective:

The results of the preliminary experiment have shown that the P3000 required disproportionately dense NDF (2.3 log units) in front of one eye to produce a pRAPD of 18%. The objective of this experiment was to determine if the eye positioned to the channel without any light stimulation was subject to the light used to stimulate the fellow eye through the corresponding channel.

Method:

A piece of card was put in place of a NDF into the right stimulus channel while the pupillometer stimulus was set to the left channel only. The pupillary light reaction was recorded from both recording channels. Therefore any light reaction recorded through the right recording channel would indicate the light leakage between the stimulus channels. A light meter was also used to measure any detectable light coming from the right channel in the above mentioned set up.

Results:

A 1.3 mm of pupillary constriction was elicited in the right eye, even when the intensity of the stimulus was turned down to 1 lux or lower in the left stimulus channel, and even though the lux meter registered that the light reaching the 'non-stimulated' right eye was less than 1/50th of the light reaching the 'stimulated' left eye.

Interpretations:

This confirmed leakage of light between the left and right light stimulus channels before reaching the eyes. A stimulus light as small as <0.02 lux can cause a reaction in the pupil. The result highlighted 2 important points:

(1) The importance of complete separation of the two light channels in an instrument such as pupillometer which measures the pupillary light response of an individual eye.

(2) The variability of the pupil responses to a clinical method of performing a swinging flash light test. This is because clinical swinging flash light test is done in open air with no partition to prevent light coming from the stimulus of the fellow eye. Therefore, depending on the amount of light leaking from the stimulus light to the fellow eye, which in turn depends on the technique of the examiner, and the variability of the amount of the background illumination, the amount of the resultant RAPD varies. Furthermore, many examiners use different light sources. The illuminated light source such as indirect head light beam may give more focused light to the eye being tested without much light contaminating to the other eye. Many people use pen torch in the clinics, which has no restriction on light being contaminated to the other eye.

RESULTANT CHANGE

Modification of the P3000D pupillometer

Procyon Instrument Limited was contacted for modification of the instrument to optimise its ability in detecting relative afferent pupillary defect. For the purposes of RAPD estimation, Procyon modified the P3000 to improve the isolation of stimulus channels by tight partition of the dividers between channels up to the level of the face rest and by using dark/black lining of the interior aspect of the light channels to eliminate any internal reflection. This has achieved zero percent leakage on further testing.

8.3.2 Does the instrument provide a correct level of light to be able to detect a subtle RAPD?

The pre-registered light level for the P3000 was 18 lux. It was important to know if this light intensity was suitable for segregating subtle differences in pupillary responses between the eyes.

Objective:

To answer the question of whether the dimmer stimulus light, less than 18 lux, would detect PLR differences between the eyes better than 18 lux, the inter-eye differences in pupillary responses (or pupillometric RAPDs) were further explored using a set of lower stimulus intensities.

Method:

First, the stimulus light was reduced to 1.0 lux from 18 lux. The unilateral afferent deficits were simulated by using NDFs of known attenuations in order of **1.0, 0.9, 0.8, 0.7 and 0.6 log units**. Seven alternating ON-OFF stimulus sequences were applied to the left or right eye in random order. The first acquisition was taken using 1.0 log unit of NDF in the right channel. The subject was asked to keep his head on the pupillometer while further acquisitions were taken using 0.9, 0.8, 0.7 and 0.6 log units NDFs respectively in the same channel. Five acquisitions were taken for each filter value. Pupillary reaction to direct light stimulus was used to measure the pRAPD, using Procyon ratiometric method as below.

$$pRAPD = (A_{small}/A_{large}) \times 100\%$$

A_{small} = smaller amplitude of pupillary constriction on direct light stimulation

A_{large} = larger amplitude of pupillary constriction on direct light stimulation

The mean and 95% confidence levels were calculated for each filter level.

Results:

Figure 8.1 depicts the relation of known NDF values used and the resultant pRAPDs in percentage.

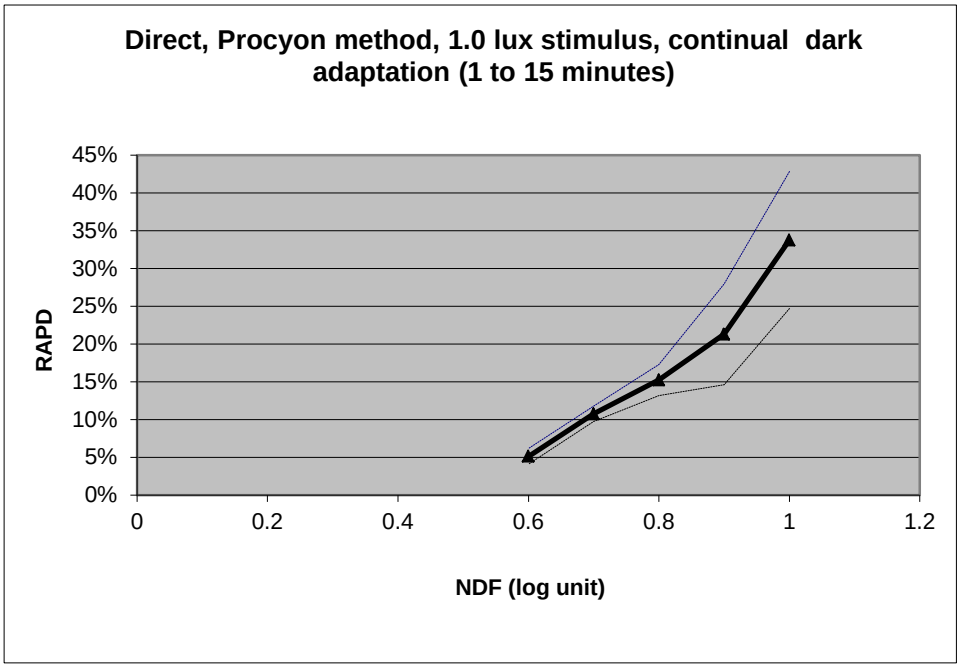


Figure 8.1: RAPD measurement on 1, 0.9, 0.8, 0.7, and 0.6 log unit NDFs using 1.0 lux stimulus, continual dark adaptation with 5 repeats on each filter. The dotted lines represent the upper and lower confidence levels of the pupillometric readings.

Observations:

(i) It was found that the pupillometer separated the attenuations induced by the NDF between 0.6 and 1.0 log units.

(ii) More repeatable results were seen with increase in duration of dark adaptation. The confidence interval was remarkably smaller with longer duration of dark adaptation towards the final test with 0.6 log units filter.

Interpretation:

(i) Although a good separation of attenuation of the light by the NDFs between 0.6 and 1.0 log units are apparent in the above diagram, the pRAPD reading for 0.6 log units is very close to zero on the Y axis. This suggests that if filters are < 0.6 log units, detectability of the inter-eye differences may not be strong enough. This finding is in agreement with that of Tatsumi and colleague[158] who in their study determined the clinically detectable RAPD to be 0.6 log units using neutral density filters on better eyes on glaucoma patients. The reliability of quantifying a smaller RAPD such as 0.3 log units was said to be poor in their study due to limitations of the clinical swinging flash light method of measuring RAPD. Correlating 0.6 log

units of RAPD to the differences in retinal nerve fibre layer thickness around the optic discs measured by optical coherence tomography (OCT), they estimated 27% reduction in the RNFL thickness in the more affected eyes compared with the less affected eyes. The association of this structural changes to that functional changes has R^2 of 0.557 ($p<0.0001$). Intuitively, one would expect this association to be stronger if more sophisticated instrument such as a pupillometer is used to detect a RAPD. Of note a difference as small as 13% in neural input between the two eyes produces a detectable pRAPD,[155] section 3.2.8.

With the view to further increase the ability of the pupillometer in discriminating the smaller inter-eye differences in afferent conduction, an experiment using further reduction in the intensity of the stimulus light was carried out as outlined below (section 8.3.2.1).

(ii) Dark adaptation reduces the pupillomotor threshold. During dark adaptation the retinal sensitivity threshold falls rapidly.[307] The pupil becomes more sensitive to light with dark adaptation. With continued adaptation to darkness as well as the repeated stimuli, it appears that the retina began to adapt to this new state of adaptation and light sequence. With continual adaptation to this new state, more stable results are obtained.

8.3.2.1 Subsequent experiment

Objective

This study was conducted to address the hypothesis that a stimulus light of even < 1 lux would increase the accuracy in detecting RAPD by the pupillometer.

Method

Further experimentation with further reduction in stimulus intensity was carried out. Seven healthy volunteers were recruited. A detailed history was taken and the Snellen visual acuity was recorded before the experiment. Un-dilated fundus examination was performed at the end of the experiment to avoid bleaching of the retina with the microscope light. If any iris, retinal or optic nerve pathology (section 7.2.5) was detected, the subjects were excluded from the study.

Stimulus intensities of **0.1 lux, 0.3 lux and 1 lux** were used. This was tested with 0.6 log units NDF placed in the right stimulus channel. The subject was allowed to dark adapt for 30 seconds between each test. The pRAPD measurement was repeated 5 times for each test-stimulus-intensity as in the previous experiment.

Results:

The pRAPD results using 0.1 lux of stimulus light were observed to have more noise in the traces, due to the amplitudes being much smaller, figure 8.2. However, the power of separation of the pupillary light response in the filtered and non-filtered eye was largest for the dimmest stimulus light of 0.1 lux.

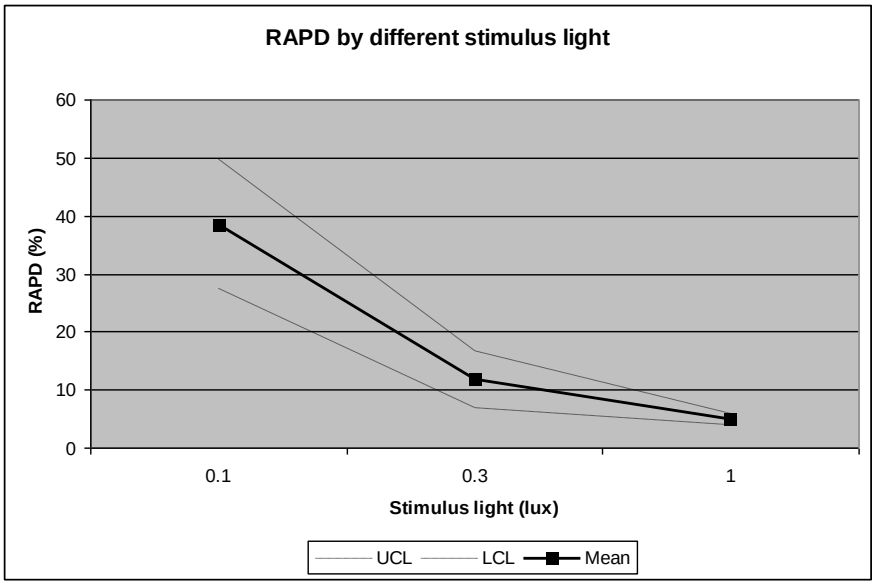


Figure 8.2. pRAPD by stimulus lights of 3 different intensities (0.1 lux, 0.3 lux and 1.0 lux) using 0.6 log units NDF, 30 sec dark adaptation with 5 repeats. Dotted lines represent the upper and lower confidence levels of the results.

Interpretations:

The dimmer stimulus light was associated with better detectability of the pRAPD, but had larger variability (larger confidence levels on the left compared to smaller confidence level to the right, figure 8.2). Therefore, it was settled for 0.3 lux, a trade-off between the noise in measurement and the separation detectable by the pupillometry. The stimulus intensity of 0.3 lux was chosen for further tests.

8.3.2.1.1 Further subsequent test

In order to confirm that the 0.3 lux stimulus with 30 seconds of dark adaptation further increases the instruments ability to segregate smaller NDFs, a series of tests with the NDFs between 0 and 1 log unit (0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, and 1 log units) were carried out.

Results:

The filter densities and corresponding pRAPDs were plotted.

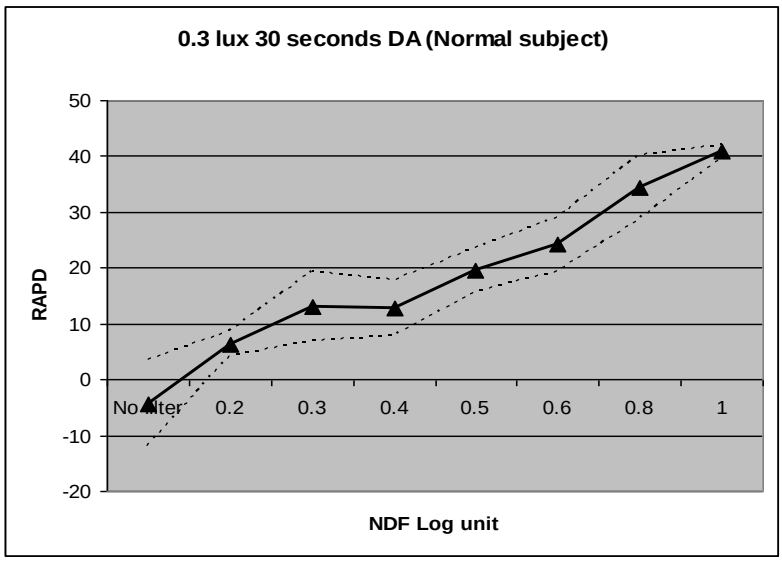


Figure 8.3. Test of simulated pRAPDs(%) with 0, 0.2, 0.3, 0.4, 0.5, 0.6,, 0.8, and 1.0 NDFs using 0.3 lux of stimulating light after 30 seconds of dark adaptation.

Observations:

Encouraging results were obtained. The above diagram confirms that the instrument can detect a very small amount of RAPD close to zero. The stimulus intensity used was 0.3 lux with 30 seconds of prior dark adaptation between each measurement.

8.3.3 Calibration of the pupillometer by known calibrated neutral density filters. (How does the test perform on normal subjects?)

From the above experiments, the following were achieved.

- Modifications were made for the pupillometer for the complete separation of the light channels with 0% light leakage between them. After the modification, it was found that the pupillometer can register 0.6 log units equivalent RAPD.
- The pupillometer was not originally designed for testing RAPD and the light level set in the instrument was at 18 lux. It was questioned whether this level of light would be too strong to separate the subtle pupillomotor differences. Investigation of lower light levels for measurement of pRAPD resulted in 0.3 lux light intensity being chosen as the best compromise between the noise and the detectability of a pRAPD.

A NDF reduces the light incident on the retina and produces corresponding attenuated pupillary light response. However, it is yet to identify if the pupillometer/subject combination distinguishes between different NDFs. This is a fundamental and important question to answer because a test of biological function may have a biological bias and variability for different levels of attenuation (or different levels of disease severity) that cannot be ignored. It needs to be resolved before any clinical work is initiated. Further experiment evaluates the pupillary test on the normal subjects using NDFs.

Objective

To investigate how pupillometry using the above parameters will perform on the normal subjects.

Method

Seven healthy volunteers were recruited. Detailed history was taken and the Snellen visual acuity recorded before the experiment. Undilated fundus examination was performed at the end of the experiment to avoid bleaching of the retinal with the microscope light as in previous experiments.

Pupils were tested for a pRAPD with no NDF, 0.3 log units, 0.6 log units and 0.9 log units NDFs introduced respectively before one eye using 0.3 lux stimulus intensity. Five acquisitions were taken for each filter. Before each acquisition they were dark-adapted for 30 seconds in the pupillometer; and between each acquisition the subjects were allowed to rest back from the pupillometer. The pRAPD was calculated as the

percentage of the ratio of larger pupillary constriction to smaller pupillary constriction minus absolute 1.

$$pRAPD = [(A_{large}/A_{small}) - 1] \times 100\%$$

A_{small} = smaller amplitude of pupillary constriction on direct light stimulation

A_{large} = larger amplitude of pupillary constriction on direct light stimulation

Results

Tests on healthy volunteers using NDFs between 0 and 1 log unit, in the increment of 0.3 log units, showed remarkable sensitivity to NDF increments, possibly able to distinguish between 0.1 log unit changes, figure 8.4. The average of 5 repetitions was taken and the 95% confidence levels plotted.

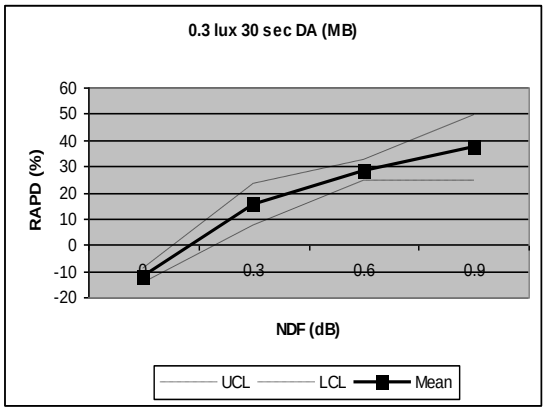


Figure 8.4a

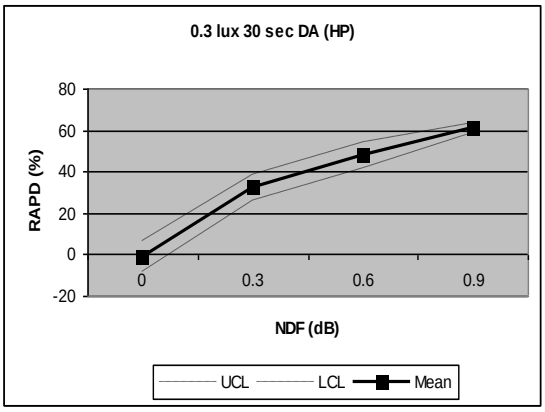


Figure 8.4b

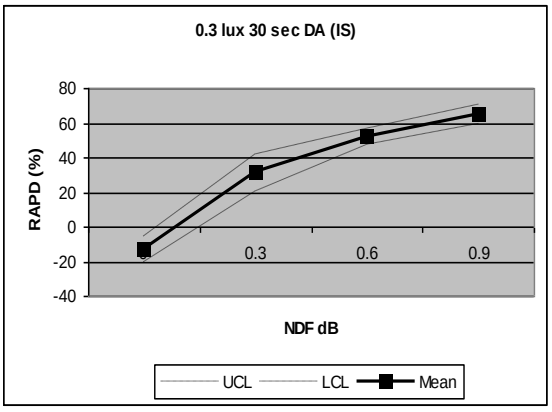


Figure 8.4c

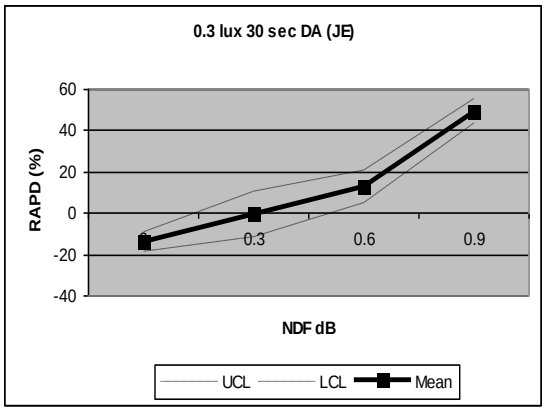


Figure 8.4d

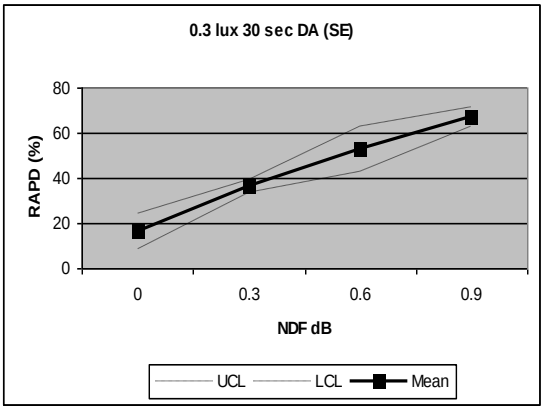


Figure 8.4e

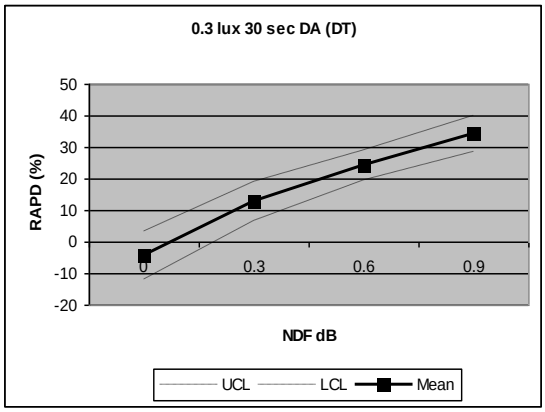


Figure 8.4f

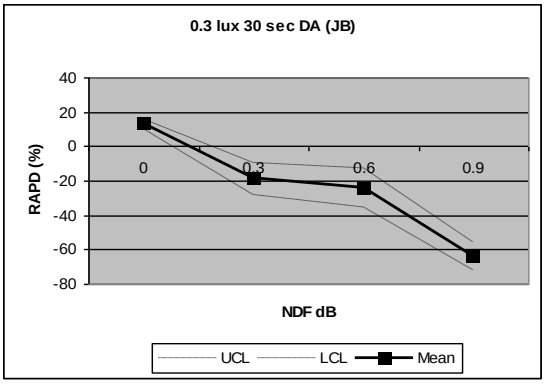


Figure 8.4g

Figures 8.4 (a to g). pRAPD measurement of healthy volunteers with no NDF, 0.3 log units, 0.6 log units and 0.9 log units NDFs inserted in one stimulus pathway. Subjects of figures 3a to 3f had the NDFs inserted to the right light channel. Subject of figure 3g had the NDFs inserted to the left light channel. Positive pRAPD represents pRAPD in the right eye and negative pRAPD represents pRAPD in the left eye. Dotted lines represent upper and lower confidence limits. Dark adaptation period = 30 seconds.

Interpretations

In this experiment, factors that can potentially influence the precision (e.g. corresponding amount of RAPD to the NDF used) and repeatability (e.g. noise level), other than the variables concerned, were minimised. Measurements were repeated 5 times. The subject was asked to come off the instrument between each acquisition and dark-adapted for a fixed duration of 30 seconds, in order to ascertain that each acquisition was preceded by the same amount of darkness adaptation and that there would be no issues of inadvertent retinal dark adaptation of the eye behind the filter.

Although the NDF were quantified in log units, the RAPD was described in terms of percentage proportion of pupillary constriction larger in the un-filtered eye than filtered

eye as shown in the above diagrams. It can still be seen that a good separation of NDF from un-filtered eye was achieved for each NDF using this stimulus setting.

8.4 DISCUSSION

Expressing the RAPD in log units after calibration with filters of accurately defined and measured attenuation values (0.3, 0.6, 0.9 log units) gives the measurement a meaning in terms of a 'standard'. This is no different to any measuring device – it needs be calibrated to a standard, when available, a national or an international standard. The main objective of this initial part of the study was to assess the ability of the P3000 pupillometer in recognising and distinguishing different neutral density filters. The instrument was calibrated first before any attempt was made to devise the method that estimates the amount of RAPD. Preliminary experiments included the study of the ratio of pupil constriction amplitude against the known filter value, the results of which lead to the modification of instrument. The different stimulus light intensities were also tested on this instrument to identify the stimulus that best suits the main study.

The most striking observation here was the effect of light leakage on the accuracy of the test, as even a slight amount of incomplete separation of stimulus light channels (<0.02 lux) can erroneously and largely reduce the sensitivity of the test. The results highlighted very important clinical information on testing this comparative test of afferent pathway dysfunction. For a very precise instrument which intends to measure the slightest differences in the afferent conduction of the optic nerves of the two eyes, any leakage in the stimulating light to the contralateral eye could lead to an inaccuracy in the measurement. This has given us some thought on the clinical swinging flash light test (SFLT) which is normally performed in the ambient lighting with no separation of the stimulus light to each eye, using various available light sources such as a pen torch. The results of SFLT performed clinically in this manner are deemed to be inaccurate for detecting subtle RAPDs. It also highlights the necessity of complete separation of light channels in an instrument that measures the relative difference in pupillary light response.

After addressing the issue of light leakage, and complete separation of the light channels, investigations were carried out to find a suitable light level for the pRAPD

test. With 1 lux of stimulus, and with 1 minute of dark adaption, it was found that this pupillometer could detect simulated afferent defects of 0.6, 0.7, 0.8, 0.9 and 1.0 log units.

This was an encouraging result. However, the magnitude of pRAPD recorded for the 0.6 log units was close to zero. This could potentially mean that a pRAPD lower than 0.6 log units may not be detected by this instrument. It is expected that an instrument with high precision should detect a subtle RAPD of less than 0.3 log units. This left the question of "how the detectability of subtle RAPD can be improved".

It was hypothesized that the detectability of a pRAPD depends on where the reference light is on the intensity-amplitude curve. As described in chapter 4 (figure 4.14), the relationship of the intensity and the amplitude of constriction are in the form of a sigmoid curve. This means to say that if a pair of intensities are too high or too low and are operating on the knees of the sigmoid curve, the differences of the corresponding amplitudes of constriction will be small as compared to a pair of lights of the same difference of intensities which are in the middle of the sigmoid curve. For the latter, the differences in the amplitude of constriction would be large – and hence a pRAPD. Figure 8.5 below describes the above hypothesis.

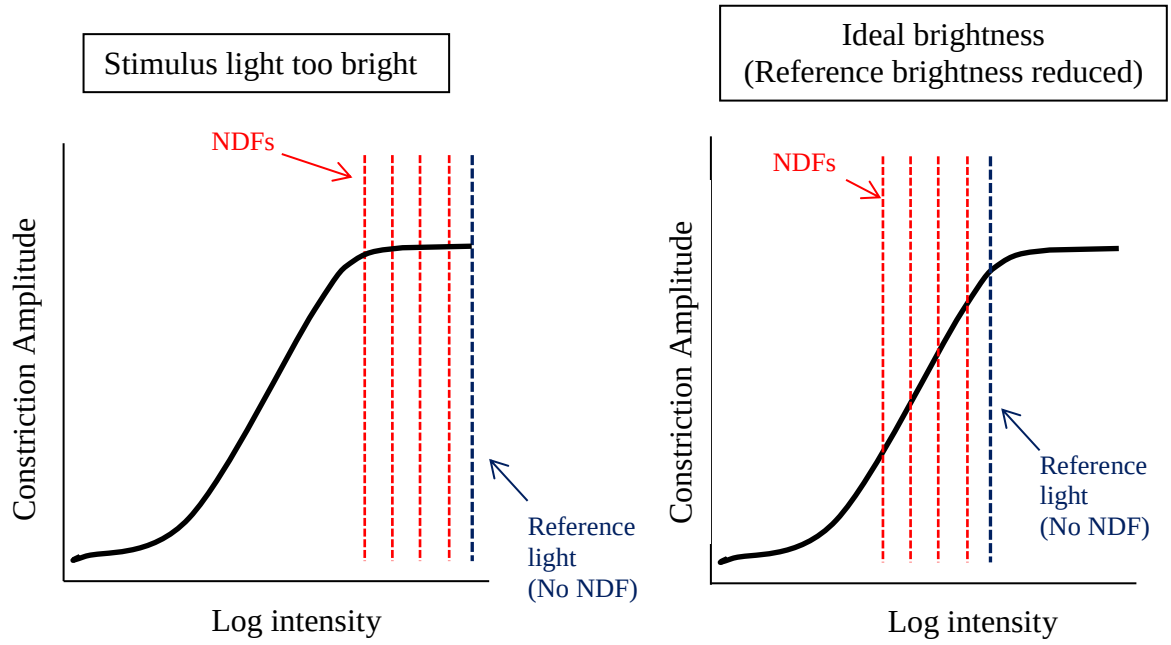


Figure 8.5. Relation of intensity-amplitude curve and the light levels. The amplitudes of the light levels operating on the knees of the sigmoid curve are less separated than those in the steep part of the graph.

Another explanation would be that there is stronger pupillomotor drive with stronger stimulus light. Therefore a good amount of pupil constriction can still be obtained even when the conduction afferent pathway is not so good. When the stimulus light is weak, however, there is not much pupillomotor drive and the conduction of signals relies largely on the quality of the afferent pathway. When the pathway is defective, the stimulation with dimmer stimulus light results in much smaller pupillary reaction than expected, making this deficit easier to detect. The repeated experiment with lower light intensities (0.1, 0.3, and 1.0 log units) indeed increased the detectability of the lower intensity response, figure 8.2. This agrees with the hypothesis proposed.

The very low light intensity responses, however, are noisier than the higher light intensity responses since the amplitudes of constriction are smaller in the lower light responses making the signal to noise ratio relatively smaller and the confidence interval larger. Higher intensity responses generally produce a more pronounced amplitude of pupillary constrictions thus larger signal to noise ratio with tighter confidence interval; however, the ability to separate subtle differences in the afferent pathway seems limited compared to that of the lower intensity responses. It thus appears that the ideal light level would be the one that is not too high to have limited ability to detect an afferent lesion but not too low to compromise the repeatability of the test. Among the three stimulus lights that were tested (0.1 lux, 0.3 lux and 1.0 lux), 0.3 lux was chosen as a trade-off between the noise and the detectability of small RAPDs.

In addition to the above experiments, one additional test was carried out using a shorter dark adaptation time. Instead of 1 minute of dark adaptation, a quick 20 seconds dark adaptation was done before testing with 0.5 log units NDF on a normal subject and repeated 3 times. This resulted in large amount of RAPD values by the ratiometric method described above (33%, 29% and 30%). The results are higher than the original value of 5% RAPD with 0.6 log units NDFs both using 1 lux of stimulus light. This has highlighted that, the reference light level is not the only factor that can be adjusted to maximise the detectability of RAPD. The duration of dark adaptation also plays a role.

Looking at the effects of dark adaptation on the intensity-amplitude curve, it can be seen that the sigmoid curve shifts to the left towards the lower intensity levels with longer

dark adaptation.[308] The Dark Adaptation curve changes most rapidly over the first few seconds.

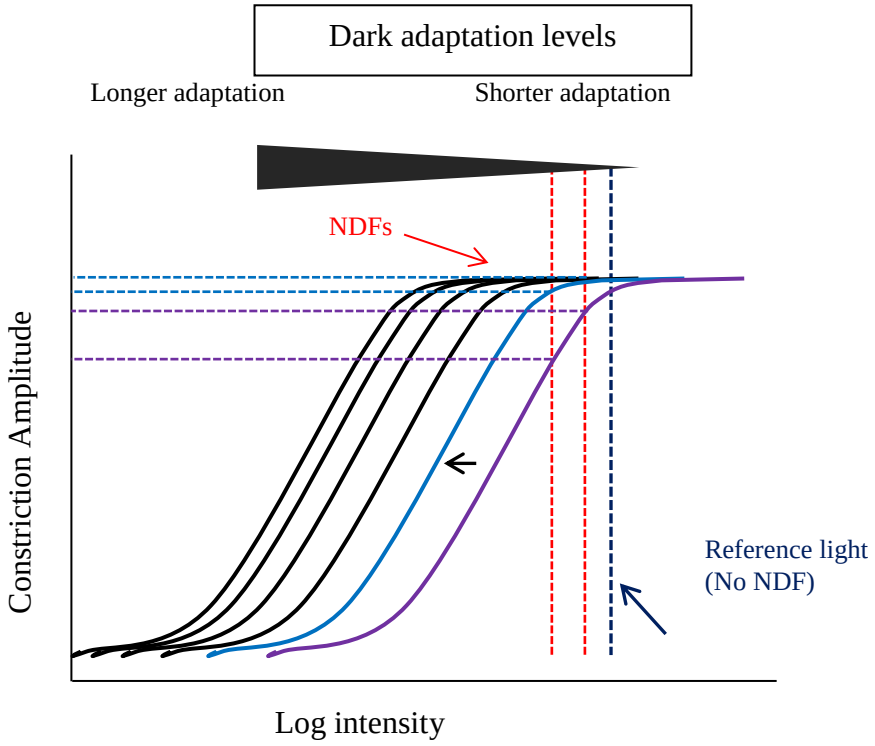


Figure 8.6. The diagram illustrating relation of dark adaptation on the log intensity amplitude curve. Red lines and arrow = light levels when NDFs are applied, dark-blue dotted line = reference light level with no NDF, black arrow = the direction of movement of the log intensity –amplitude curve with the dark adaptation. With dark adaptation the sigmoid curve (purple) moves to the left (blue). It can be seen that with longer duration of dark adaptation, the stimulus light attenuated by the NDF will fall on the flatter part of the knee (horizontal light-blue dotted lines) but with shorter adaptation, on the steeper middle part of the sigmoid curve (horizontal purple dotted lines).

In the above diagrams, neutral density filters represent surrogate markers of the afferent lesions. Thus, if these hypotheses are true, in this experimental setting with this specific instrument, the ability of the instrument in segregating the disease and non-disease can be further maximised by either reduction in duration of dark adaptation or the reduction in the light intensity level but with the compromise of reduction in SNR. The 0.3 lux level of stimulus light was chosen with 30 seconds dark adaptation period for the optimum results. This has proven the potential for the instrument to detect the RAPD at 0.1 log unit increment from 0 %.

The pupillary response to light is influenced by various intrinsic and extrinsic factors (physiology chapter 4) and thus biological variability of the RAPD as well as variability due to differences in test environment can be expected. This part of the study only concentrated on the effect of stimulus light intensity on the pupillometric output using P3000 pupillometer. The variability due to intrinsic biological variation and psycho-sensory distractions are not covered.

With the chosen stimulus and the duration of dark adaptation, the RAPD recording of various NDFs (0 NDF, 0.3 NDF, 0.6 NDF, and 0.9 NDF) were tested as a pilot study. This aims to investigate the ability of the *machine* to distinguish different light attenuation levels, or in clinical terms, the ability of the instrument in detecting different levels of severity of unilateral optic nerve disease. The preliminary results showed a good separation for all 7 subjects. The confidence levels were reasonable.

The next part of the study is to further test this ability of the pupillometer in a larger group of normal subjects and glaucoma patients.

8.5 CONCLUSIONS

From the above experiments, it was conclude that:

(a) Leakage of light between stimulus light channels could reduce the sensitivity of a pupillometer in testing RAPD.
(b) When choosing the optimum light level to detect a RAPD, it is useful to reference the intensity-amplitude curve.
(c) Dark adaptation reduces variability.
(d) Sensitive detection of small simulated RAPDs (< 0.3 NDF) was possible using P3000 pupillometer at much dimmer stimuli (0.3 lux and 0.1 lux).
(e) Dimmer stimuli (lower intensity) however are associated with higher level of noise (larger CI).
(f) Pupillometric output can be calibrated against the neutral density filter of known attenuations.

OPTIMISATION OF STIMULUS PARAMETERS

Chapter 9

Duration of dark adaptation

9.1 INTRODUCTION

Both rods and cones influence the pupil size (section 2.2.1, 4.3.4.1). When a stimulus light is switched off, the pupil enlarges after a transient widening and constriction.[309] Dark adaptation (DA) process begins immediately when the stimulus light is switched off. However, it takes about 10 minutes to dark adapt cones and 40 minutes to dark adapt rods completely. The effect of dark adaptation is that both visual and pupil thresholds get lower.[310] Retinal sensitivity increases and therefore with dark adaptation a dimmer stimulus light can be used for the same amount of response. The pupil light response amplitude increases with dark adaptation of both types of photoreceptors, mirroring the increase in perceptual sensitivity to light.[308] The pupil literature is divided with some research being carried out on dark-adapted eyes and some on light-adapted eyes.

As discussed in section 5.4.1, there are advantages and disadvantages with regards to DA prior to pupil testing. For research purposes, the advantages of dark adaptation are that (a) the initial pupil diameters are larger which means the constriction amplitudes are larger and less subject to noise, (b) the eyes under experiment are set to a level of retinal adaptation before the stimulus for a like for like comparison between and within subjects, and (c) the experiment can be carried out using less intense light levels.

For a clinical test performing a pupillometric relative afferent pupillary defect in this thesis, however, the emphases are different. The practical questions are asked of the pupillometric technology in clinical setting: does this test detect glaucoma? If so, how reliably does it detect glaucoma? And how useful is this test? For this practical test, it is not feasible for clinicians to spend 40 minutes to dark-adapt their patients before pupil testing in a routine clinic. However, the above-mentioned theoretical advantages of DA should not be ignored. In this section of the thesis, we set out to investigate the effects of DA on relevant parameters of PLR response.

9.2 OBJECTIVES

To address whether any amount of adaption is required for the reliable pupillometric test.

(1) How does dark adaptation (DA) affect pupil light response (PLR) amplitude?
(2) Does the length of time of DA matter?
(3) Does the dark adaptation affect responses to all stimulus intensities equally?
(4) How does DA affect the variability of PLR amplitude measured in this study?

9.3 METHODOLOGY

Inclusion and exclusion criteria

Definitions of healthy eyes, glaucoma patients and inclusion and exclusion criteria were the same as chapter 7 (sections 7.2.4, 7.2.5).

Methods

Interventional case study

All subjects were tested with and without dark adaptation. Duration of dark adaptation was pre-determined as 30 seconds, and 3 minutes. A total of 3 tests were instituted (test A = no DA, test B = 30 seconds DA, and test C = 3 minutes DA), table 9.1.

In order to avoid ordering effects, the order of the tests was chosen at random. A random table generated from Microsoft excel software was used to randomise the order of the test; test A = 1, test B = 2, test C = 3 (see random table in chapter 9).

Three stimulus (ON-OFF) sequences were applied for each randomly chosen test, but using 3 intensities of light in the same order: 0.04 lux (scotopic or SC), followed by 0.4 lux (low mesopic or LM) and 4 lux (high mesopic or HM) to standardise the effects of potential retinal bleaching. These 3 ordered sequences were repeated twice for each test.

Each test sequence included 7 pairs of alternating light dark stimulus to the eyes. The ON duration was set as 0.4 seconds and the OFF duration as 1.6 seconds.

Patients were allowed to rest between test sequences.

Test A (total time about 5 min) includes 2 repeats of the following sequences.

Sequence 1		Rest	Sequence 2		Rest	Sequence 3		Rest
No DA	Pupillometry using 0.04 lux stimulus light		No DA	Pupillometry using 0.4 lux stimulus light		No DA	Pupillometry using 4 lux stimulus light	

Test B (about 10 min) includes 2 repeats of the following sequences.

Sequence 1		Rest	Sequence 2		Rest	Sequence 3		Rest
30 sec DA	Pupillometry using 0.04 lux stimulus light		30 sec DA	Pupillometry using 0.4 lux stimulus light		30 sec DA	Pupillometry using 4 lux stimulus light	

Test C (about 24 min) includes 2 repeats of the following sequences.

Sequence 1		Rest	Sequence 2		Rest	Sequence 3		Rest
3 min DA	Pupillometry using 0.04 lux stimulus light		3 min DA	Pupillometry using 0.4 lux stimulus light		3 min DA	Pupillometry using 4 lux stimulus light	

Table 9.1. Test A represents a sequence of tests with no dark adaptation, B with 30 seconds of dark adaptation and test C 3 minutes of dark adaptation. “Rest” = rest away from the pupillometer to the ambient room light.

The outcome measures:

The pupil constriction amplitude (pupil light response, PLR, amplitude) represents the average of 2 repeats of 7 pairs of alternating light-dark stimuli. This was recorded for the left and right eye. Only the direct responses were used for this study.

9.4 RESULTS

Demographic

A total of 17 consecutive glaucoma patients (4 female, 13 male) and 5 normals (2 female, 3 male) were tested. The mean ages were (65 years for all subjects, 71 for glaucoma patients and 48 for the normal, minimum age was 40 for each group).

Analysis

For each light level, averaged constriction amplitude (direct response) was calculated for the left and right eye. Therefore there were a total of 18 outcome variables obtained, 6 for each light level (for both eyes with 3 levels of adaptation).

The constriction amplitudes of the left or right eyes of the normal and glaucoma subjects were compared using the student t-test. There was no statistical difference observed between normal and glaucoma subjects in each test category, (p values = 0.1 to 9). Therefore, the pooled data including both groups was used for future analysis.

(1) Question: Does dark adaptation affect PLR amplitude?

Mean difference in PLR measurement (+ 95% CI) compared with that observed after no dark adaptation:

		30 seconds DA		**3 minutes DA**	
		mm	%	mm	%
RE	HM	0.11 (0.05)	12.4 (5.0)	0.24 (0.07)	27.9 (9.4)
	LM	0.22 (0.07)	34.0 (9.4)	0.33 (0.15)	55.6 (22.5)
	SC	0.11 (0.06)	46.0 (23.7)	0.40 (0.15)	168.0 (75.9)
LE	HM	0.15 (0.06)	16.2 (6.1)	0.20 (0.17)	24.3 (15.0)
	LM	0.23 (0.06)	40.0 (13.9)	0.35 (0.18)	64.8 (25.2)
	SC	0.13 (0.05)	48.4 (22.2)	0.41 (0.15)	166.4 (75.4)

Significance testing of these changes in PLR amplitude following DA:

RE:	30 seconds			3 minutes		
	HM	LM	SC	HM	LM	SC
normality[#]	F	F	P	P	P	P
paired t	3.558	3.977	3.727	7.637	8.974	8.710
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

LE:	30 seconds			3 minutes		
	HM	LM	SC	HM	LM	SC
normality[#]	P	P	P	P	P	P
paired t	5.777	7.796	5.294	7.412	10.075	9.891
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[#]when normality test failed (F) signed ranks (Wilcoxon) test used instead

Tables 9.2. The test of significance of change in PLR amplitude with DA of 30 seconds and 3 minutes. HM = 4 lux, LM = 0.4 lux, SC = 0.04 lux.

(2) Question: Does the length of time dark adapting matter?

Comparing 30 seconds with 3 minutes dark adaptation:

	RE			**LE**		
	HM	LM	SC	HM	LM	SC
normality[#]	P	F	F	F	F	F
paired t	6.038	3.117	3.425	3.097	3.111	3.393
P value	<0.001	0.0002	<0.001	0.002	0.002	<0.001

Table 9.3. Significance testing between pupil responses to 30s and 3 min adaptation at 3 light levels. [#]when normality test failed (F) signed ranks (Wilcoxon) test used instead.

(3) Question: Does dark adaptation affect responses to all stimulus intensities equally?

Comparison of effect of 3 minutes DA on PLR amplitudes following HM stimuli compared with SC stimuli:

	RE		**LE**	
	mm	%	mm	%
normality[#]	P	F	P	P
paired t	4.403	4.015	4.547	4.391
P value	<0.001	<0.001	<0.001	<0.001

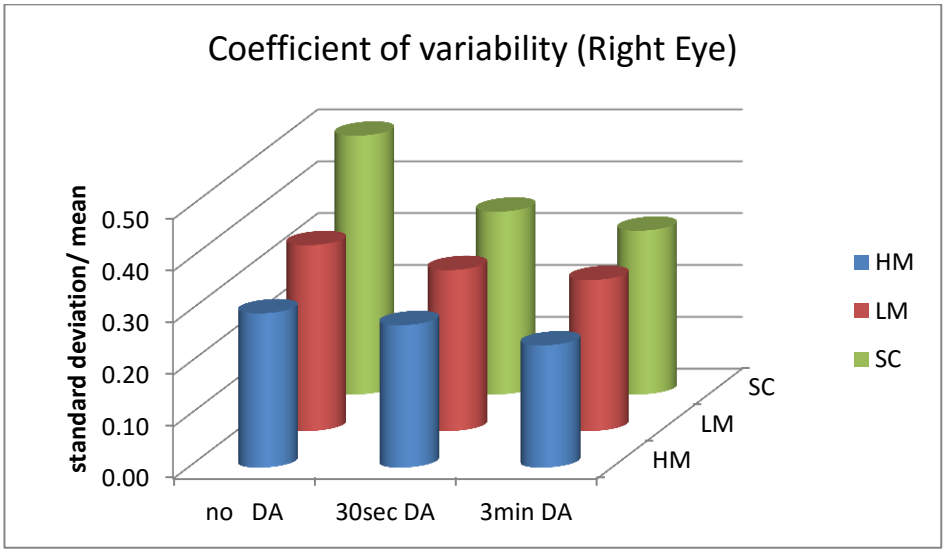
Table 9.4. Significance testing between HM and SC stimuli after 3 minutes of DA

[#]when normality test failed (F) signed Wilcoxon test used instead.

(4) Question: How does DA affect the variability of PLR amplitude measured in this study?

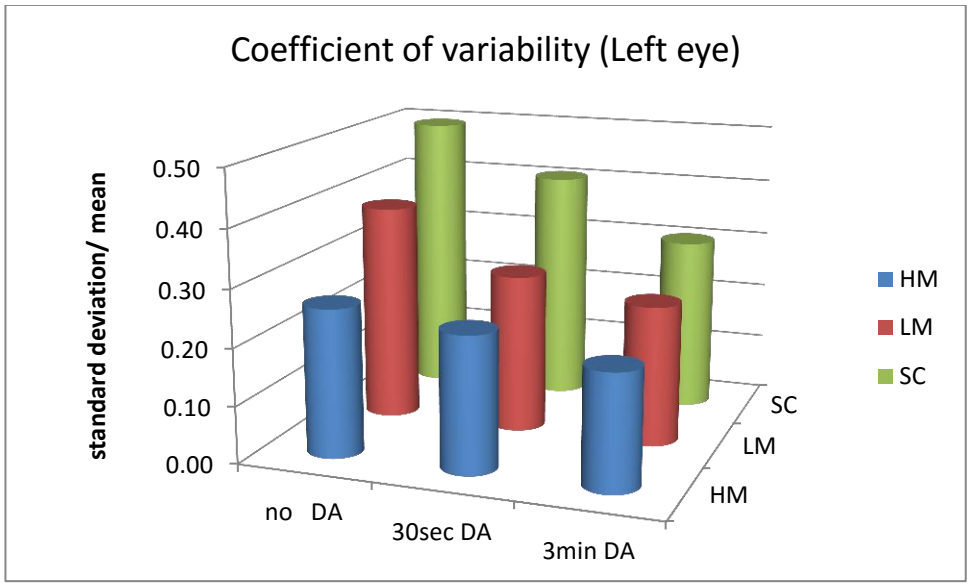
The coefficient of variability of the amplitude data were compared for each test condition across the subjects.

Coefficient of variability across the subjects			
Right	no DA	30sec DA	3min DA
HM	0.30	0.27	0.24
LM	0.36	0.31	0.29
SC	0.50	0.35	0.32



(a)

Coefficient of variability across the subjects			
Left	no DA	30sec DA	3min DA
HM	0.26	0.24	0.20
LM	0.38	0.28	0.25
SC	0.50	0.41	0.30



(b)

Table 9.5 coefficient of variability for the right eyes (a) and the left eye (b) demonstrating DA improving the repeatability of the test.

Observations

- Dark adaptation significantly increases PLR amplitude (table 9.2).
- The larger PLR amplitude is associated with longer duration of DA (table 9.3). The 3 minutes of DA has a significantly greater effect on amplitude than just 30 seconds of DA.
- Dark adaptation has greatest effect on SC stimulus conditions, least on HM. This is true for both amount of constriction amplitude (table 9.4) and the improvement in variability of the measured data (table 9.5). The percentage change in constriction amplitude is larger for dimmer light than stronger light for the same duration of DA. Also, the improvement in repeatability of the measured data is more pronounced for dimmer light than for stronger light stimuli.

9.5 DISCUSSIONS

Dark adaptation had effect on the amount of PLR amplitude which is in agreement with the findings in the literature. The increase in amplitude was proportional to the duration of dark adaptation. This effect seems largest for low intensity stimulus. Dark adaptation makes the retina more sensitive to stimulus light. This sensitivity is most pronounced with the dimmest stimulus in this study because the latter, in and of itself, has the least pupillomotor drive. It has more room for improvement. With higher intensities, the effect of DA on the PLR amplitude is less pronounced because comparatively higher intensity stimuli have enough pupillomotor drive to cause pupil to constrict without requiring much input from DA. This finding reinforces that fact that DA increases retinal sensitivity and that dimmer light can be used to obtain the same amount of PLR amplitude with prolonged DA prior to stimulus.

Although the intra-individual test-retest variability has not been tested on these cohorts, across the subjects, the variability of the test results were higher with no dark adaptation compared to 30 seconds and 3 minutes dark adaptation (table 9.5). DA thus reduces inter-subject variability of the test. It is recalled from the previous chapter (figure 8.1) that the DA is associated with reduction in within-subject variability also. Both of these results suggest that with DA more repeatable results can be obtained. These findings favour the requirement of DA prior to pupil testing. But how long do we need to DA?

In this study the durations of DA tested were $\leq$ 3 minutes. For flash light stimulus sequence (0.4 seconds of stimulation followed by 1.6 seconds of darkness), it is expected that the cone receptors are largely responsible for the pupillary constriction. It takes about 10 minutes for the cones to fully dark adapt. However, even with these short durations of DA, a significant increase in retinal sensitivity is noted since 3 minutes of DA resulted in significantly larger PLR amplitudes compared to 30 seconds of DA. Also, 30 seconds DA resulted in significantly higher retina sensitivity compared to no prior DA.

When the subjects were tested at ambient light level without prior dark adaptation, the total time taken for the test was about 5 minutes. With 30 seconds of DA, about 10

minutes and for 3 minutes of DA 24 minutes. Therefore, on balance, for a quick test of about 10 minutes with fairly repeatable results, 30 seconds of dark adaptation was chosen for further studies in this thesis.

The variability in this chapter is noted to be less with stronger stimulus compared to weaker stimulus. This is because the signal to noise ratio is much reduced with dim stimulus. Therefore it is not favourable to test non-dark-adapted pupils with dim stimulus of 0.04 lux.

Chapter 10

Stimulus Configuration

10.1 INTRODUCTION

The patterns of stimulus utilised in the literature for detecting RAPD are variable. A number of stimulus ON-OFF combinations that have been used for the estimation of RAPD (table 5.3).[115-117;119;212;215;226] They can, be broadly categorised into (1) *alternating stimulus* whereby each eye is stimulated alternately with or without a duration of pause in between, (2) *sequential stimulus* where the stimuli are presented sequentially to each eye. Most authors use alternating ON-OFF stimuli. 'ON-duration' here refers to the duration of stimulus and 'OFF-duration' refers to the inter-stimulus-interval. For all stimulus configurations, the morphology of the pupil response is different depending on *duration* (ON-duration) and *intensity* of the stimulus, and whether the duration of the darkness pause (OFF-duration) or the *inter-stimulus-interval* is long enough for the pupil to return to its original size (section5.4.2.1, figures 5.2,5.3).

The stimulus ON and OFF durations utilised in the literature have been reported as the most variable factor in pupillometric studies estimating RAPD. This variation is also due to the fact that various studies employed different instruments some of which are purpose-built pupillometers. The stimulus light source is often different and the test algorithms are also different depending on the purposes of the study. Cox TA (1989)[115] used 2 different stimuli where the duration of light stimulus was 3 seconds and 1 second. Kawasaki and Kardon (1995)[116] used 3 ON-OFF stimulus pairs: 2.8s-0.2s, 0.2s-2.8s, and 0.017s-0.983s; and found that 2.8s-0.2s pair was the best stimulus configuration in their studies using a Maxwellian pupillometer. They commented that their test variability was less when the darkness interval between the light stimuli was less than 1 second. Bergamin and Kardon in 2002[215] tested 0.2s-2.8s stimulus pair over a range of stimulus intensities and pupil images were captured using Maxwellian view purpose built pupillometer. Their study concluded that the ability to detect abnormal input asymmetry between the two eyes was best obtained by testing over a range of light intensities. In 2005, Lankaranian and Spaeth[212] considered RAPD measured with a commercially available digital infrared pupillometer P2000D as the gold standard and extrapolated the sensitivity and specificity of clinical swinging flash light test and the magnifier assisted swinging flash light test. In their study, they tested the eyes with 3

seconds ON duration and 1 second OFF duration to closely simulate clinical swinging flash light test.

Those authors who employed the stimulus ON duration of about 3 seconds and a short OFF duration, namely Cox[115], Thompson,[23] Kawasaki,[116] and Lankaranian,[212] intended to simulate the clinical swinging flash light test which is the most accepted test *clinically*. Their choice of this stimulus ON-OFF pattern was not targeted at obtaining the most repeatable results or to improve the test sensitivity and specificity. In order to optimise the stimulus parameters for estimating RAPD pupillographically, Kalaboukhova[209] in 2006 tested 5 pairs of ON-OFF stimuli and found that 0.5s-1.0s combination to be best suited for her instrument in detection of glaucoma. She chose a light intensity of 1000 cd/m^2. Using these stimulus parameters the author found the sensitivity and the specificity of measuring pRAPD in glaucoma patients to be 86.7% and 90%.[117]

There is no standardised stimulus configuration that is to be used for the estimate of pRAPD. The ideal stimulus ON-OFF configuration would be the one that produces most repeatable and reliable outcome parameters required for pRAPD estimation, and this will be specific to the test paradigm that is employed for the specific machine. For P3000 pupillometer, Procyon's own stimulus configuration (0.4s–1.6s ON-OFF configuration) was tested against 3 other stimulus configurations described in literature which were either commonly used or were reported as the best stimulus configurations for their pupillometric studies. These included 0.5s-1.0s, 3.0s-1s, and 2.8s-0.2s ON-OFF combinations adopted from the studies of Kalaboukhova,[117] Bergamin[215] and Kawasaki.[116] The comparison was made using Procyon P3000D pupillometer. From now on, these stimulus configurations will be termed as KALA, BERG, KAWA and Pro for Procyon's stimulus configuration in this chapter.

10.2 METHODOLOGY

Subjects

Healthy volunteers were recruited. The inclusion and the exclusion criteria were the same as those listed in the chapter 7 (section 7.2).

Method

The structure of method of measuring RAPD was modelled as closely as possible to the methods published in the literature given the limitations due to differences in stimulus parameters such as size, position and intensity of light stimulus, number of repeat stimuli, total duration for each stimulus, whether or not Maxwellian view was used, type of retinal adaptation done before the test, and different outcome measures among various studies. The summary of 4 stimulus configurations chosen is described in table 10.1.

Stimulus configurations	KALA	BERG	KAWA	Pro
no of pairs	4	4	4	4
ON (sec)	0.5	3	2.8	0.4
OFF (sec)	1	1	0.2	1.6
duration per pair (sec)	1.5	4	3	2
total duration (sec)	6	16	12	8
frames	150	400	300	200

Table 10.1. Summary of 4 ON-OFF stimulus pairs chose for a comparison

Pupillometry

The details of execution of pupillometry using P3000 were described in chapter 7. For this study the P3000 was programmed to stimulate each eye with predetermined stimulus configuration (duration of stimulus and pause combinations) as described above. A total of 5 sequences are registered for each test acquisition. The first recorded reflex was omitted. The recorded pupillograms were examined for noise (such as blinking and artefacts) and they were manually removed as described in chapter 7.

Test protocol

For each stimulus configuration, the stimulus ON-OFF sequence was repeated 5 times. Procyon incorporates the results of 3 light stimulus intensities namely high mesopic (HM) 4lux, low mesopic (LM) 0.4lux and scotopic (SC) 0.04lux for their RAPD estimate. These light levels were used for the other chosen stimulus configurations for a like for like comparison. The first test was with the 0.04lux (SC) stimulus followed by 0.4lux (LM) stimulus, and 4lux (HM) stimulus. This order was kept for all stimulus

configurations. The sequence was repeated twice. In order to overcome the problem of unequal retinal bleaching with repeated stimuli, 30 seconds of dark adaptation (DA) was incorporated between/before each acquisition. The test protocol is described in figure 10.1. A total of 6 acquisitions were executed for each stimulus configuration.

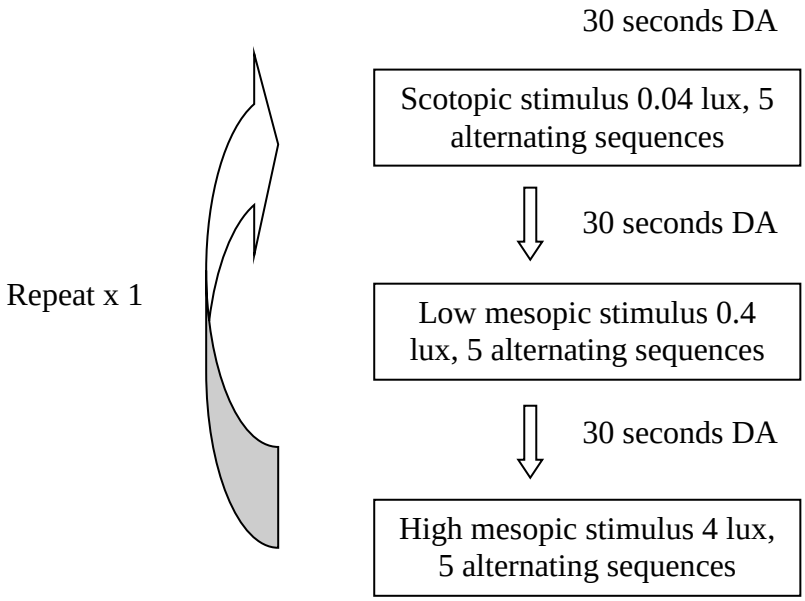


Figure 10.1. Test protocol

Ordering Effect

In order to eliminate the ordering effect a random table was used to decide the sequence at which each stimulus configuration (1= KALA, 2= BERG, 3= KAWA, 4= Pro) was chosen as described in chapter 7.

Outcome measures

The pupillary constriction amplitude was used as an outcome measure. P3000 records both direct and consensual pupillary light responses simultaneously. Therefore, a total of 4 sets of results of PLR amplitude could be obtained for each acquisition: (1) PLR amplitude of right eye direct response, (2) PLR amplitude of right eye consensual response, (3) PLR amplitude of left eye direct response, and (4) PLR amplitude of left eye consensual response. The averages of 4 sets of results (the result of the first alternating stimulus was discarded, see also section 7.3.4) per acquisition were calculated for each of the stimulus intensity. These sets were repeated 1 more time

under 3 intensities levels (HM, LM, Sco) as described above in figure 10.1. The results of the 2 repeats were averaged.

Statistics

Student t-test was employed to test the significance of differences between different light intensities. The coefficient of variation for the repeated measurements was obtained for each stimulus configuration for a comparison.

12.3 RESULTS

Demographic

A total of 8 normal subjects (4 male and 4 female) were tested. The average age was 47 (range, 25 to 72).

Analysis

(1) Question: Which stimulus configuration is more subject to noise?

The number of pupillograms that were discarded for noise and artefacts are summarised in table 10.3. The noise level is relatively low for all stimulus patterns (8-17% discarded); among which the KAWA stimulus configuration was associated with 17% rate of noise in the recorded pupillogram.

Pupillograms	KALA	BERG	KAWA	PRO
% recorded	89	89	83	92
% discarded	11	11	17	8

Table 10.3 Percentage of used and discarded pupillograms for each stimulus configuration is described.

(2) Question: Which stimulus configuration produces significant pupil constriction that are discernible between test light levels?

The mean of the two repeats of the 4 outcome measures were compared between 4 chosen configurations. Although the same set of intensities of stimulus light was used for the same subjects, the BERG stimulus configuration (3 sec ON, 1 sec OFF) gave the largest amplitude of constriction, followed by that of the Pro configuration (0.4 sec ON, 1.6 sec OFF), the KALA configuration (0.5 sec ON, 1 sec OFF) and the KAWA configuration (2.8 sec ON, 0.2 sec OFF). The amplitude of pupillary constriction attainable with KAWA configuration was significantly smaller than the others.

Mean	KAWA		KALA		Pro		BERG	
	Dir	Cons	Dir	Cons	Dir	Cons	Dir	Cons
HM	0.57	0.53	1.02	1.02	1.28	1.25	1.54	1.51
LM	0.56	0.54	0.94	0.91	1.16	1.14	1.35	1.32
Sco	0.52	0.50	0.75	0.71	0.89	0.89	1.01	1.00
All	0.55	0.52	0.90	0.88	1.11	1.09	1.30	1.28

Table 10.4. Mean PLR amplitude, Dir = direct, Cons = consensual

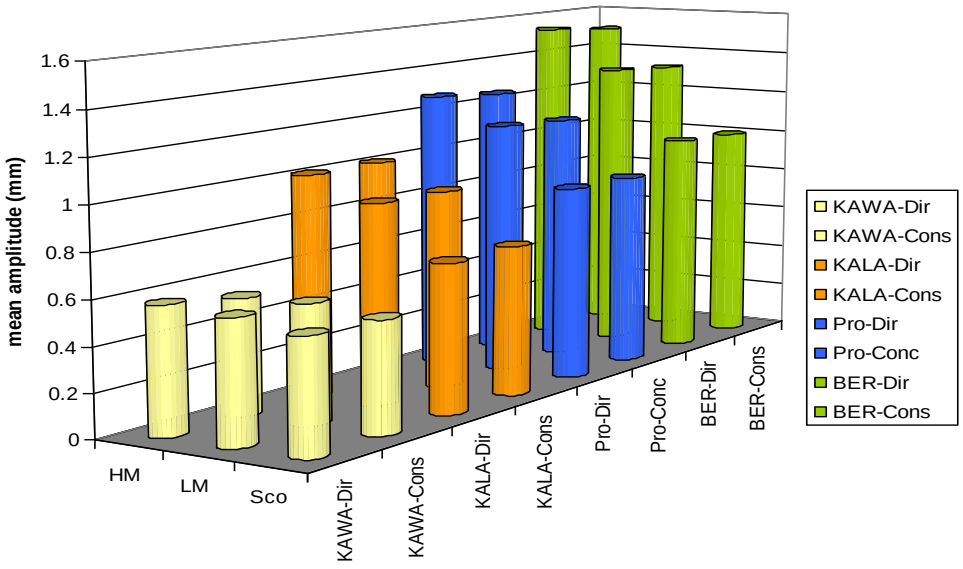


Figure 10.2. Mean amplitude comparison. HM = high mesopic (4 lux), LM = low mesopic (0.4 lux), Sco = scotopic (0.04 lux). Dir = direct light response, Cons = consensual light response.

Brighter stimulus produces larger PLR amplitudes.[311] The amount of constriction was significantly larger with brighter stimulus light for all configurations except again for

the KAWA configuration where similar amount of constriction was noted for any light level tested (table 10.5).

p values	KAWA		KALA		Pro		BERG	
	Dir	Cons	Dir	Cons	Dir	Cons	Dir	Cons
HM vs LM	0.79	0.88	0.40	0.29	0.24	0.27	0.17	0.17
LM vs Sco	0.58	0.63	0.06	0.04	0.01	0.02	0.02	0.02
HM vs Sco	0.44	0.75	0.01	0.00	0.00	0.00	0.00	0.00

Table 10.5. Significance comparison between light levels.

(3) Question: Which configuration provides most repeatable results?

The mean amplitude of pupillary constriction and their standard deviations were calculated for each set of outcome parameters (left eye direct response, left eye consensual response, right eye direct response and right eye consensual response) for each subject. The standard deviations appeared to be unrelated to means, figure 10.3.

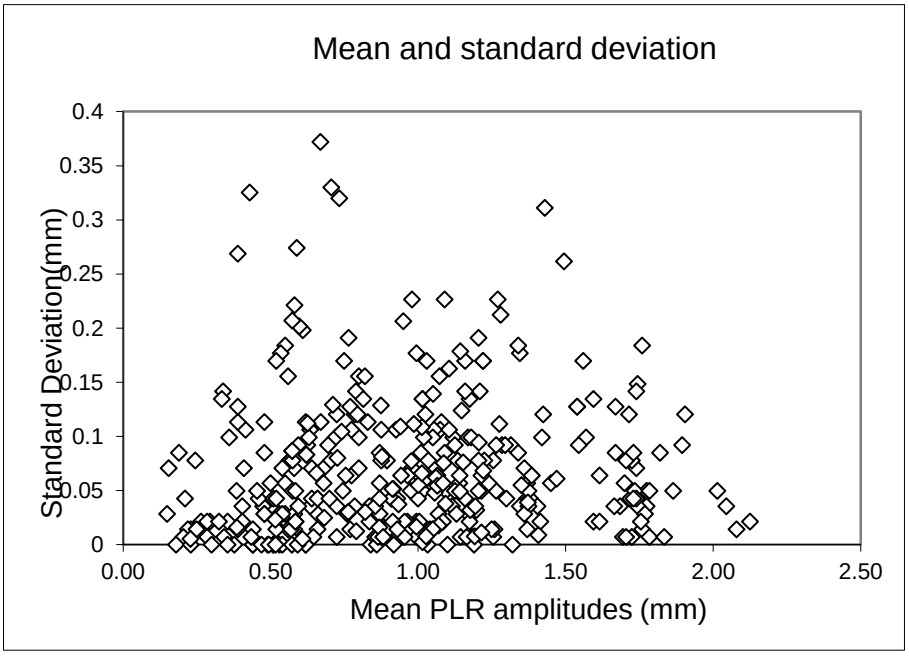
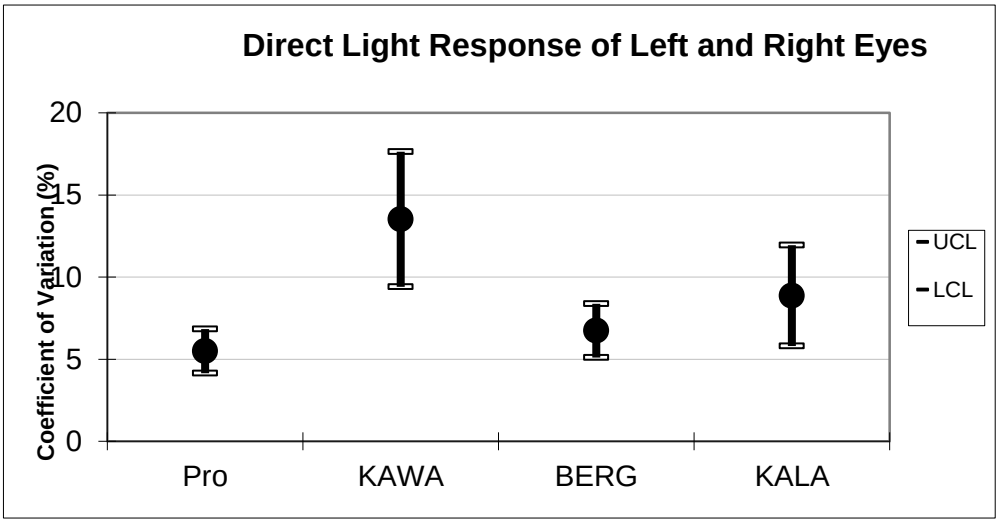


Figure 10.3. A plot of mean and standard deviation

The coefficient of variation (CV) was defined as the ratio of standard deviation by the mean times 100, and described in percentage. Coefficients were compared for 4 stimulus configurations, figure 10.4.

Direct light response

% CV	UCL	LCL	Mean
Pro	6.83	4.14	5.48
KAWA	17.64	9.41	13.53
BERG	8.37	5.09	6.73
KALA	11.93	5.81	8.87



Consensual light response

% CV	UCL	LCL	Mean
Pro	6.38	3.91	5.15
KAWA	14.24	7.10	10.67
BERG	7.81	4.80	6.30
KALA	14.49	7.14	10.81

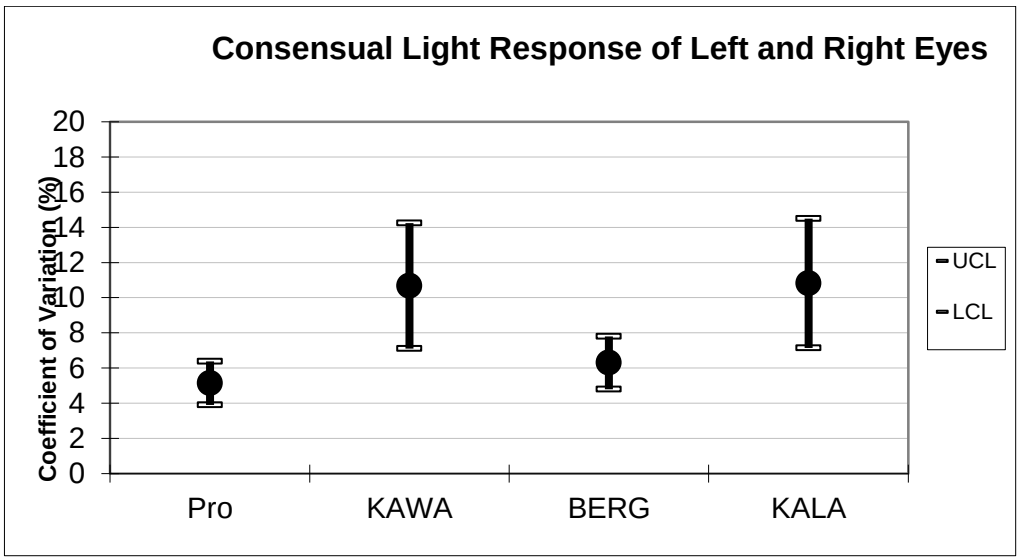


Figure 10.4. Mean and confidence levels of coefficients of direct (top) and consensual (bottom) light responses.

The coefficient of variation was large for the KAWA configuration for both direct and consensual responses and smallest for Pro configuration.

10.4 DISCUSSION

Pupillary response to light is under the influence of higher centre inhibition (section 4.3). Even when a comparison method is used between the eyes of the same individual, there is an intra-individual variability in the pupil response to light.[116] Although the amount of variability is small, it may contribute to the measurement error during the objective comparison of the PLR of the two eyes. According to Kuhlmann J,[219] the method of averaging the pupillograms and evaluating the averaged pupillogram has the advantage of removing noise but one has to be mindful of outlying values and artefacts that may distort the pupillogram considerably. In order to lessen this confounding effect, the alternating stimulus sequence was repeated 5 times and the outlying first pupillogram was removed before the average data was acquired to the analysis. The acquisition was further repeated for a more reliable data. Because the severity of glaucoma varies among the patients, it is also important that the RAPD test will be able to detect all grades of severity. Three light levels were used for each RAPD measurement.

A larger pupillary constriction can be brought about by a (1) brighter stimulus, (2) a longer duration of stimulation or (3) with longer inter-stimulus interval. In order to decide upon the suitable stimulus configuration for P3000, the stimulus configurations that have been used in the literature were chosen for a comparison with the one provided by Procyon. The criteria for the best configuration were that it provides a measurable pupillary constriction response which has high repeatability. Using the same pupillometer P3000D, four stimulus configurations pairs were evaluated for the outcome amplitude and tested for its repeatability on the same 8 volunteers. These were evaluated at 4 lux, 0.4 lux and 0.04 lux light levels provided by the Procyon pupillometer.

Noises and artefacts from the recorded data were removed (chapter 7.5), the number of reflexes used for each stimulus pairs were counted and the proportion of reflexes that were discarded due to poor tracing calculated. The KAWA configuration had the

smallest % of recorded data and Pro configuration had the highest % of recorded amplitude data.

The interesting finding was that in a situation where pupils were stimulated with alternating light, the amount of amplitude of pupillary constriction depended not only on the intensity of the stimulus[311] but also on the duration of stimulus and duration of darkness pause, and their combined ratio. The duration of stimulus for KAWA was 2.8 seconds and it produced the smallest amplitude. On the other hand, BERG configuration, which had very similar stimulus duration (3 seconds), produced the largest amplitude. The difference here was that the KAWA configuration had a darkness interval of only 0.2 seconds while the BERG had a 1 full second. Although the BERG configuration had the longest stimulus duration, KAWA configuration did not have the shortest stimulus duration. The short ISI did not allow the pupil to return to its original pupil size with resultant smaller initial pupil before constriction and subsequent smaller constriction. When small pupils are working in nonlinear range increasing light intensity has little effect on further increase of the constriction amplitude. With the Pro configuration (0.4 sec ON, 1.6 sec OFF) although the duration of stimulus was short it had relatively larger amount of time for the pupils to reach the baseline before constriction with the next stimulus and therefore the constriction amplitude was relatively large. The duration of stimulus for the KALA configuration was similar to that of Pro configuration (0.5 sec vs 0.4 sec); however, the ISI was shorter (1 sec vs 1.6 sec) with resultant smaller amplitude than that produced by the Pro configuration. With BERG configuration the pupil was stimulated for 3 whole seconds producing the largest amount of constriction. The pause duration of 1 second also allowed redilation to a reasonable pupil size before subsequent stimulation. Therefore, this stimulus configuration was associated with largest amplitude of pupillary constriction among others.

In addition to the amount of constriction amplitude, it is required that the data is less variable and more repeatable and reproducible in order to measure the afferent activity accurately. The repeatability was tested in terms of the standard deviation (SD) and coefficient of variability (CV). The SD is known to be dependent on the magnitude of the average or the mean. The scatter plot of the mean value against the SD, however,

showed that the standard deviation was unrelated to the mean. All mean coefficients (CV) were less than 20%. The repeatability is reasonably good for all configurations because the test protocol incorporated 5 repeated sequences for each acquisition which were repeated twice for 3 intensity levels. Averaging all these results give less variable more repeatable measurements. The Pro configuration (0.4 sec ON, 1.6 sec OFF) was associated with the most favourable repeatability of PLR amplitudes, for both direct [5.48% (4.14-6.83%)] and consensual [5.15% (3.91-6.38%)] responses. The KAWA configuration (2.8 sec ON, 0.2 sec OFF) had large coefficient for both direct [13.53% (9.4 –17.64%)] and consensual [10.67, (7.10- 14.24 %)] responses, figure 10.4.

It is important to note that the overall quality of the test depends on all factors that compose the test as whole rather than an individual component. Therefore, one cannot assume that a particular stimulus parameter independently represents the best parameter for detecting pupillographic RAPD. The results of this study prove the validity of the use of Pro configuration with Procyon P3000 pupillometer undertaking the protocol drawn out for this particular instrument and setting.

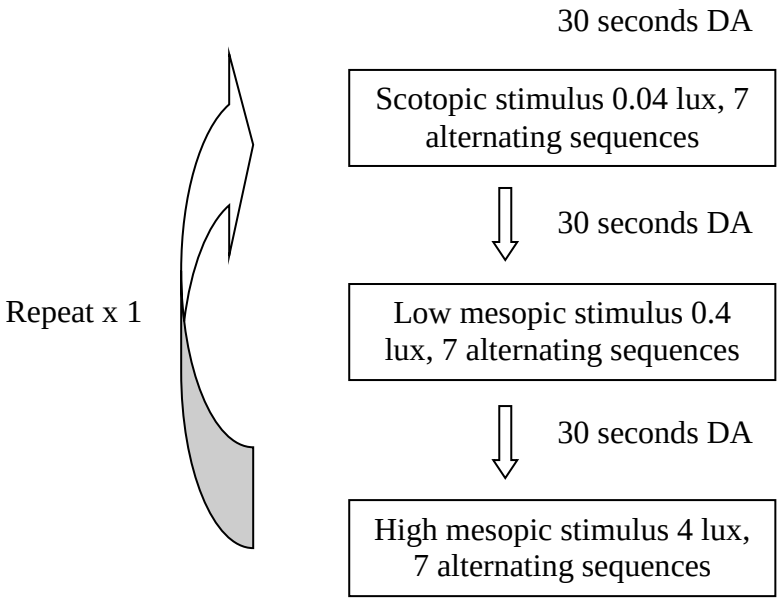
Chapter Link Comment

For subjects with a relative afferent pupillary defect, stimulation of one eye generates a consistently lower pupillary light response than that of stimulation of the other eye for a range of stimulus intensities. Therefore, the extent of the disorder can be quantified not only by the differences in the two measured data points but also can be quantified by determining the shift between the two profiles along the direction of the stimulus intensity axis. This represents the light intensity scaling factor required to bring the two profiles to coincidence, equivalent to the neutral density filter magnitude obtained in the swinging flashlight test. Based on the literature, it can be seen that some authors have adopted the use of graphical methods whereby a number of stimulus intensities were employed to tease out the relative afferent defect since there are theoretical advantages to using more than one test light level in the estimation of RAPD as discussed in section 5.4.

At this stage of my thesis, the developments of the final parameters for determining pRAPD were taken over by Procyon. This involved a change from quantifying differences in two measured data points of the two eyes elicited by a single light intensity to a determination of the difference in the range of pupil light responses (described by the area under the regression line) over a range of stimulus intensities (0.04 lux, 0.4 lux and 4 lux). The details of the formula are proprietary to Procyon and the final study involves the use of the proprietary formula in estimating pRAPD to which I am not privy.

The ***test protocol*** for estimating pRAPD was as follows:

The subject was tested with 0.4-1.6 ON-OFF stimulus configuration after 30 seconds of dark adaptation first with 0.04 lux, followed by 0.4 lux and 4 lux of stimulus lights. This was repeated once, figure below. Seven alternating sequences were applied for each acquisition.



Test protocol

The first pupillogram was discarded for each acquisition (chapter 7). From this protocol, 2 repeats of PLR amplitude data were obtained for the direct and the consensual responses of the left and right eye. The proprietary formula was then used to estimate the pRAPD using the direct pupillary response data ($pRAPD_{DIR}$), and the consensual pupillary response data ($pRPAD_{CONS}$). Anisocoria correction was performed (chapter 7) before the final pRAPD was calculated.

For all subsequent chapters the pRAPD was estimated by the above method.

OPTIMISATION OF OUTCOME PARAMETERS

Chapter 11

Calibration of the outcome

11.1 INTRODUCTION

When the pupillary responses of the eyes of a "healthy individual" are examined, two assumptions are made. The first assumption is that the individual is healthy based on our clinical judgement and available tests. The second assumption is that there are no differences in the afferent pathways of the two eyes, i.e. if the relative afferent pupillary differences are measured we expect the RAPD result to be zero. However, we know that there are physiological differences in the afferent visual path ways of a "healthy subject" and there are inter-subject variations to it.[118;203] Authors have reported the amount of physiological baseline RAPDs measured in the "healthy subjects" in their studies.[118;203] Generally, up to 0.3 log unit of RAPD is expected in a healthy individual, however, the upper limit of the physiological RAPD is yet to be identified.

In an ideal world, if one assumes that there is no physiological RAPD, using an instrument that measures extremely accurately, a healthy subject will have 0 log unit of RAPD. And if a known calibrated NDF of 0.3 log units is introduced to the left eye and the RAPD is measured, exactly 0.3 log units will be measured in the left eye. If 0.6 log unit of NDF is introduced in front of the right eye, 0.6 log unit of RAPD will be measured in the right eye. For any amount of filter put in front of the eye, the exact amount will be detected by the extremely accurate test. If we put this onto the correlation chart, where X axis represents the amount of known filter and the Y axis represents the measured result in the same unit, the correlation of the two for a given population, R^2, will be 1 (figure 11.1). The regression line will represent the formula: $y = mx + c$, where m (the slope) = 1 and c (the intercept) = 0. Therefore, $y = x$.

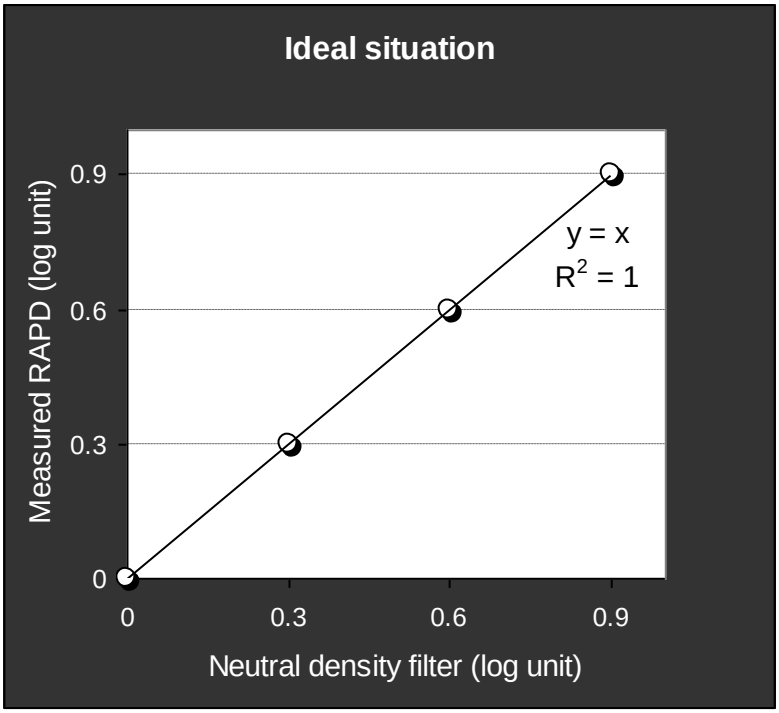


Figure 11.1 An ideal situation where the measured RAPD is equivalent to the amount of attenuation by the neutral density filters.

Clearly this is not the case for any existing test that measures a RAPD. When we measure the RAPD of a presumably healthy subject, we can get a value for the RAPD, which is the net result of underlying physiological RAPD and the intrinsic error of the test conducted.

In order to reduce the intrinsic error of the test to improve the test accuracy, a few preliminary standards are first set. Here, all subjects are tested in a standard test environment and are dark adapted for the same amount of time prior to each test acquisition. The pupillometry instrument produces fixed stimulus light for each of the intensities and measures the pupil diameter by the built-in pupil fit software. This standardisation of test algorithm minimises the error produced by the variation of the test itself. Therefore, if there is an error, it is, in all probability, due to the intrinsic error of the test. Using the concept of x log units filter producing corresponding x log units increment in the measured value, and giving allowances for the baseline RAPD, the results measured by the pupillometer can calibrated against the known filters.

11.2 OBJECTIVE

(1) The accuracy of the test to be optimised by calibrating the results against known neutral density filters.

(2) The range of baseline physiological RAPD of the study population to be determined.

(3)The diagnostic accuracy to be tested

11.3 METHODS

Subjects

Healthy subjects were recruited as per criteria described in chapter 7.

RAPD measurement by digital infrared pupillometry

Method of pupillometry is described in chapter 7. The stimulus configuration used was 0.4s-1.6s ON-OFF configuration. The test protocol is described in the diagram below, figure 11.2.

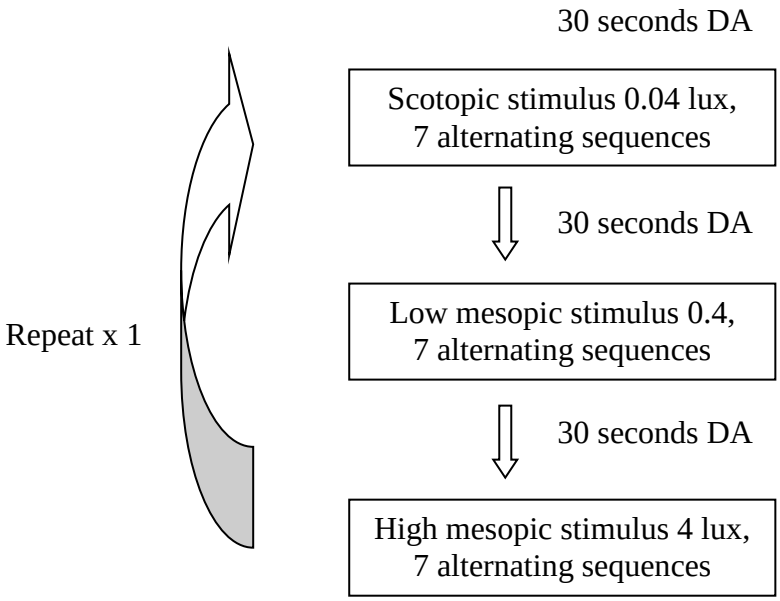


Figure 11.2. Test protocol

Data processing and correction for anisocoria were made as per method chapter 7. The pupillographic RAPD (pRAPD) was calculated using Procyon's proprietary method. This method utilised the amplitude of pupillary constriction of all 3 light stimuli and the RAPD was described in terms of the differences between the eyes scaled with the intensity levels and it was described in log units.

(1) Calibration

For each subject, the test protocol was repeated with no NDF, and with 0.3, 0.6 and 0.9 NDFs placed in the stimulus pathway of *a randomly chosen* eye. This aims to reduce the bias induced by the laterality of the eye. The filters were introduced in the same order for all subjects.

A number of methods can be used to combine the direct and consensual pupillary responses. In the literature some of the authors have used the average of the direct response and the consensual response per eye stimulation, as the average output of the each eye stimulus with the view to minimise contraction anisocoria (chapter 5, table 5.9). Since a commercially available pupillometer was used for this thesis whereby the pRAPD output was provided in the form of $pRAPD_{DIR}$ and $pRAPD_{CONS}$, the above method was not applicable for the final pRAPD estimate. The $pRAPD_{DIR}$ alone may be used as final pRAPD. As discussed in chapter 5, there is no evidence regarding which method is the best. Utilising a total output, ie the combination of direct and consensual responses per stimulation, however has theoretical advantages in averaging out the confounding effect of contraction anisocoria. Therefore, it was decided that the combined results of $pRAPD_{DIR}$ and $pRAPD_{CONS}$ would be used for the final pRAPD estimate.

The $pRAPD_{DIR}$ and the $pRAPD_{CONS}$ can be combined by the best proportion method whereby the results were combined by means of alpha or a constant. This constant value was derived from testing the ability of the algorithm to differentiate the results obtained by 0 log units NDF and 0.3 log units NDF in normal subjects. The amount of alpha, *"**α**"*, should be the value that gives the most optimum combined results of $pRAPD_{DIR}$ and $pRAPD_{CONS}$. The most optimum combined result is the pRAPD result that best discriminates the diseased and non-diseased population. This was measured by AUC of the receiver operative characteristic (ROC) curve, a graph of false positive (1-specificity) and true positive rates obtained as the decision threshold varied. In order to determine the optimised*"**α**"* for a given model, ROC curves were constructed using alphas ranging from 0 to 1 in increments of 0.1. The alpha (***α)*** that gave the largest AUC was chosen as the optimum alpha for the given model.

The mean pRAPD output was plotted as the ordinate (in log unit) against filter values (in log unit) as the abscissa. The regression line was constructed and the regression equation examined to calculate the calibration factors. However, because the eyes were chosen at random, the RAPD output would represent either left or the right eye. The pRAPD representing the smaller response to light in the right eye was assigned as positive value and the left eye a negative value. Figure 11.3 represents 6 possible outcomes to the pRAPD values by placement of different NDFs. If the filter was placed in front of the right eye, the resultant pRAPDs with increasing filter value (lines of action or LOA) would be in the positive quadrant. However, if it was placed in front of the left eye, LOA would be deflected into the negative quadrant because negative quadrant represents left pRAPD values. In order to produce a single regression line, the left-right standardisation was made, keeping the results in one quadrant. This was achieved by the application of the following equation to the measured RAPD.

Standardized pRAPD = absolute (pRAPD at 0NDF) + absolute (pRAPD – pRAPD at 0NDF) (-1) ……***Calc Equation 1***

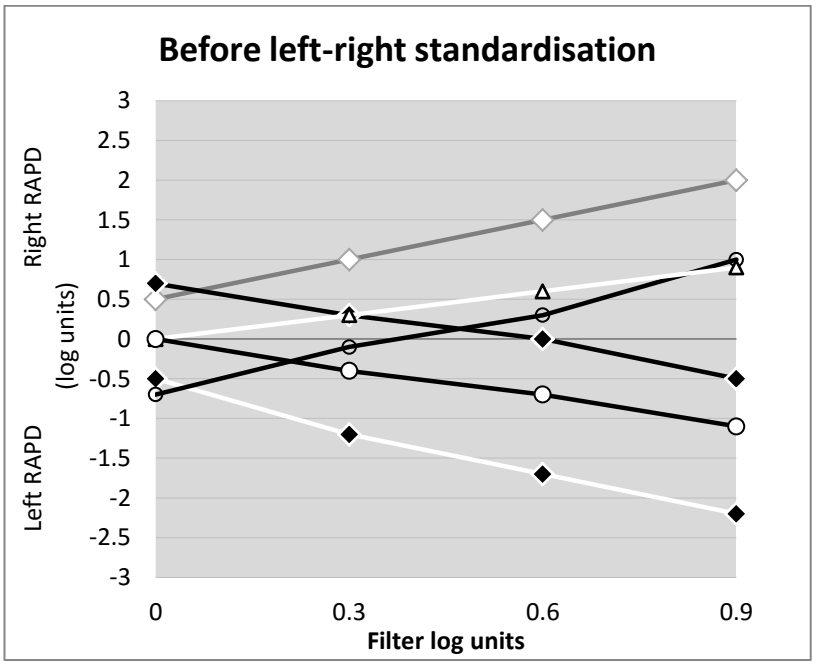


Figure 11.3. 6 possible lines of action by different neutral density filter placements. Right pRAPD values are assigned as positive values and left pRAPD values are assigned negative values on the ordinate. pRAPD values at 0NDF represent baseline pRAPDs.

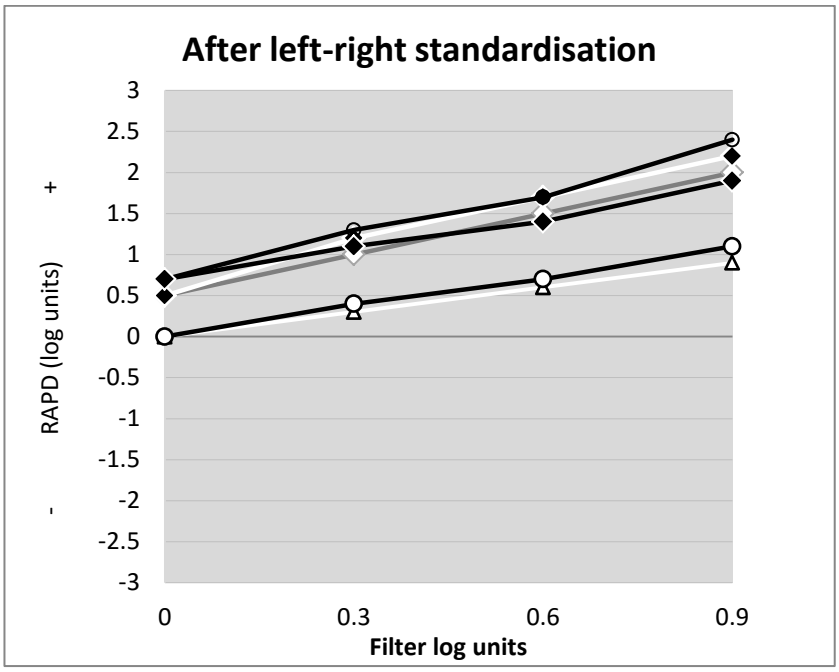


Figure 11.4 Lines of action after left-right standardization.

(2) Determination of the range of baseline physiological RAPD of the study population

The calibrated pRAPD values without a filter in place were used to estimate the ranges of baseline line, or physiological, pRAPDs for our cohort of healthy volunteers.

(3) Test of diagnostic accuracy

The baseline pRAPD (ie with 0NDF), and pRAPDs induced by 0.3, 0.6 and 0.9 log unit NDFs were tested for normality before any parametric tests were applied.

The Receiver Operator Characteristic (ROC) curves were constructed for sub-group comparison using the results of 0 cf 0.3, 0.3 cf 0.6, and 0.6 cf 0.9 log unit filters. This was done for both proprietary algorithm and 3 other ratiometric methods (section 5.4.4):

Method 1

$$RAPD_{M1} = 1 - \left[\frac{\text{average of direct \& consensual amplitudes (smaller)}}{\text{average of direct \& consensual amplitudes (larger)}} \right]$$

Method 2

$$RAPD_{M2} = 1 - \left(\frac{\text{average of normalised direct \& consensual amplitudes (smaller)}}{\text{average of normalised direct \& consensual amplitudes (larger)}} \right)$$

Method 3

$$RAPD_{M3} = \left(\frac{\text{average of direct \& consensual amplitudes (right eye)}}{\text{average of direct \& consensual amplitudes (left eye)}} \right) - 1$$

Normalisation of the pupillary constriction amplitude was by dividing it by the initial pupil diameter before constriction. The measurements of M1, M2 and M3 were also treated with the calibration factor before ROCs were constructed. The Area Under the ROC Curves (AUCs) and the sensitivity and the specificity in detecting the 0.3 log units of pRAPD induced by increasing NDFs were calculated for each method for a comparison.

11.4 RESULTS

Demographics

A total of 50 healthy subjects (17 male and 33 female) were included in the study. The mean age ± SD of the subjects was 45.15 ± 15.4 (range = 16 - 78 years).

(1) Calibration

Test Result Variable(s)	Area Under the Curve			Asymptotic 95% Confidence Interval	
	Area	Std. Error[a]	Asymptotic Sig.[b]	Lower Bound	Upper Bound
alpha0_0	.900	.031	.000	.840	.960
alpha0_1	.916	.028	.000	.861	.971
alpha0_2	.929	.025	.000	.880	.978
alpha0_3	.942	.022	.000	.898	.985
alpha0_4	.950	.020	.000	.911	.990
alpha0_5	.964	.016	.000	.933	.994
alpha0_6	.974	.013	.000	.949	.999
alpha0_7	.980	.010	.000	.960	1.000
alpha0_8	.986	.009	.000	.000	1.000
alpha0_9	.988	.008	.000	.000	1.000
alpha1_0	.986	.009	.000	.000	1.000

Table 11.1 describes the AUC obtained for each alpha variable.

Alpha of 0.9 was chosen to combine $pRAPD_{DIR}$ and $pRAPD_{CONS}$ since this was associated with the largest AUC, table 11.1.

Each subject had four un-calibrated combined pRAPD measurements (0, 0.3, 0.6 and 0.9 NDF). The lateralised combined pRAPD outputs are displayed in figure 11.5. One outlier in the 0.9NDF group was noted. After left-right standardization, the regression line was drawn figure 11.6. The calibration factors deduced from this equation were slope (m) = 1.5439 and intercept (c) = 0.1178, figure 11.7.

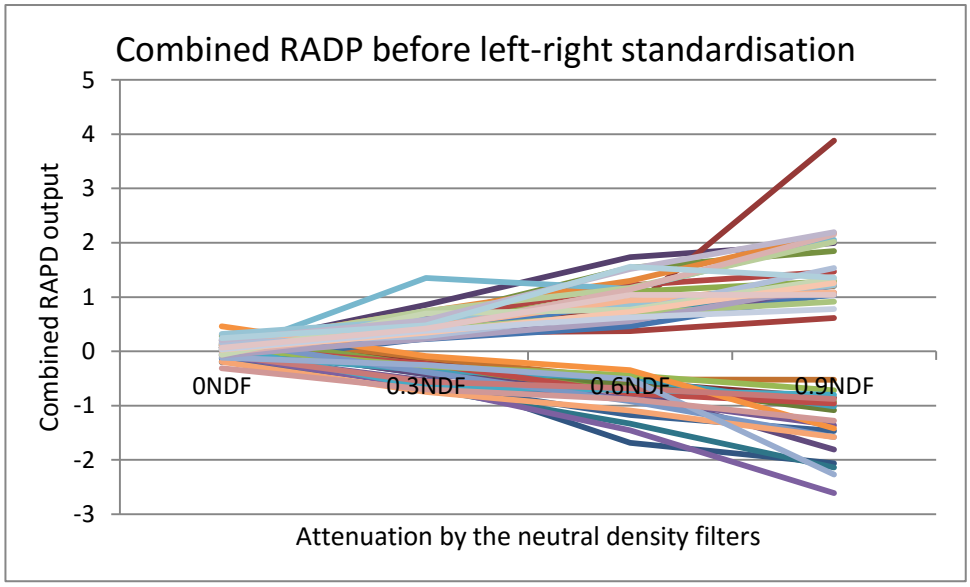


Figure 11.5 A plot of NDF against the combined pRAPD. The right pRAPD assigned as a positive value and the left a negative value.

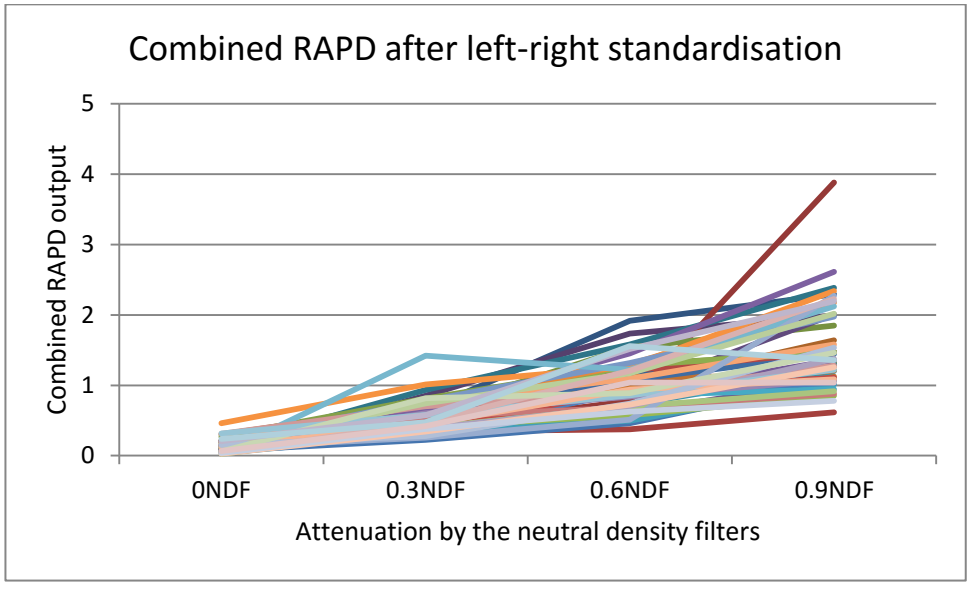


Figure 11.6 All RAPDs were plotted in one quadrant after the left-right standardisation.

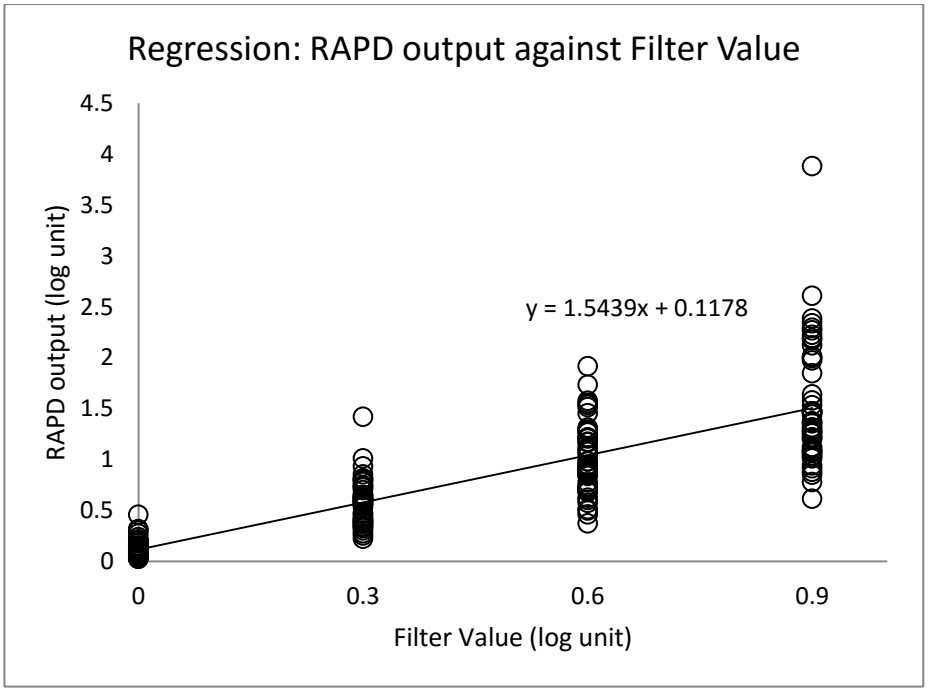


Figure 11.7 Regression line using the let-right standardized data. The equation shows that the correction factor: slope (m) = 1.5439 and intercept (c) = 0.1178.

(2) Determination of the range of baseline physiological pRAPD of the study population

Calibrated pRAPDs were derived from the equation: calibrated pRAPD = (raw pRAPD – 0.1178) / 1.5439. The range of normal values (no filter application) before calibration and after calibration were (0.02 to 0.46 log units) and (0 to 0.22 log units). The dispersion of the normal range, in comparison with the results reported by Wilhelm and colleagues who tested 102 healthy volunteers, is summarised in table 11.2.

pRAPD groups (log units)	**Procyon pRAPD $_{raw}$**	**Procyon pRAPD$_{calibrated}$**	**pRAPD** Wilhelm et al
RAPD < 0.08	20 (40%)	45 (90%)	53 (52%)
0.08 ≤ RAPD < 0.22	28 (48%)	4 (8%)	43 (42%)
0.22 ≤ RAPD < 0.39	5 (10%)	1 (2%)	6 (6%)
RAPD ≥ 0.39	1 (2%)	0 (0%)	0 (0%)
Total:	50 (100%)	50 (100%)	102 (100%)

Table 11.2. Summary of normal ranges estimated by the current method and that of Wilhelm et al.[118]

(3) Test of diagnostic accuracy. The sensitivity and specificity for distinguishing between filter groups

Results from normality testing indicated that the pRAPDs after calibration were not normally distributed. Box-Cox transformation, ($y = [(x+2)^{-2} -1] / -2$), was applied to render the data normally distributed for all filter populations.

For the ratiometric methods (M1, M2 and M3) the direct and consensual pRAPDs were averaged in each case. The sensitivity, specificity and the AUCs for all methods are summarised in table 11.3.

AUC (asymptomatic 95% CI)	Proprietary	Method 1	Method 2	Method 3
0.0 vs 0.3 NDF (unilateral simulated disease)				
4 lux	0.99 (0.97 - 1.00)	0.90 (0.84- 0.96)	0.91 (0.85 - 0.96)	0.93 (0.89 - 0.98)
0.4 lux		0.91 (0.85 - 0.97)	0.91 (0.85 - 0.96)	0.92 (0.87 - 0.98)
0.04 lux		0.95 (0.90 - 0.99)	0.95 (0.90 - 0.99)	0.97 (0.95 – 1.00)
0.3 vs 0.6 NDF (unilateral simulated disease progression)				
4 lux	0.86 (0.79 - 0.93)	0.83 (0.75 - 0.91)	0.83 (0.75 - 0.91)	0.68 (0.57 - 0.78)
0.4 lux		0.73 (0.63 - 0.83)	0.72 (0.62 - 0.82)	0.66 (0.55 - 0.76)
0.04 lux		0.78 (0.69 - 0.87)	0.78 (0.69 - 0.88)	0.66 (0.55 - 0.77)
0.6 vs 0.9 NDF (unilateral simulated disease progression)				
4 lux	0.79 (0.70 - 0.88)	0.65 (0.54 - 0.76)	0.65 (0.54 - 0.75)	0.58 (0.47 - 0.69)
0.4 lux		0.71 (0.60 - 0.81)	0.69 (0.59 - 0.80)	0.61 (0.50 - 0.72)
0.04 lux		0.64 (0.53 - 0.74)	0.63 (0.52 - 0.74)	0.58 (0.47 - 0.70)

Table 11.3: summary of the AUCs of 4 methods tested.

The AUCs were the highest for the proprietary algorithm, which utilised the results of all 3 stimulus light intensities, compared with any of the alternative ratiometric methods evaluated at any light level.

The area under the ROC curve for 0 cf 0.3, 0.3 cf 0.6, and 0.6 cf 0.9 log unit filters were 0.99 (95% CI = 0.95 – 1.00), 0.86 (95% CI = 0.79 – 0.93) and 0.79 (95% CI = 0.70 –

0.88) respectively. At the cut-point of 0.3 log unit, the proprietary method has 94% sensitivity and 94% specificity in differentiating 0 NDF and 0.3 NDF groups.

It can be seen that the ratiometric methods (M1, M2, M3) worked best at the stimulus intensity of 0.04 lux for distinguishing between 0 and 0.3 log units filter conditions, table 11.3. Comparative ROCs for results of ratiometric methods at 0.04 lux and that of Procyon algorithm is displayed in figure 11.8.

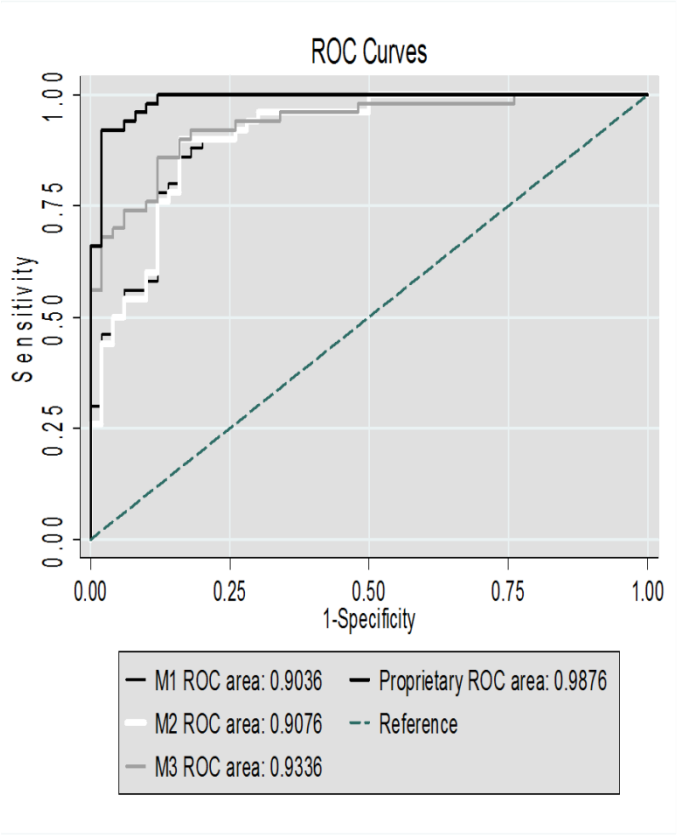


Figure 11.8 Summary ROCS of 4 tested methods.

Some overlap between the distributions of response to the neutral density filters was noted. In order to assess the probability that a given pRAPD score belongs to a particular filter response population, the z-score probability can be calculated to assess the disease severity, appendix (B).

11.5 DISCUSSION

To optimise the pupillometer's diagnostic accuracy across the disease status we have calibrated it against a series of neutral density filters of known attenuation and evaluated the test performance in detecting pRAPDs of 0.3 log units induced by increasing filter densities. A common calibration equation was deduced across all filter values. In order to minimise the test variability, each subject was dark-adapted for 30 seconds prior to each test, and all tests were performed in the same room with the same testing environment.

The normal subjects were measured for pRAPD response in the absence of any filter (0 log unit) and then in the presence of 3 known filters (0.3, 0.6, 0.9 log units) mimicking alternate stages of disease advancement. The regression coefficient and intercept described the absolute scaling bias of the pRAPD measurement across all filters. Application of the regression equation to each raw value then centred each filter population close to known filter value in a pattern of best fit from all filters. The calibrated value without the filter in place was used to estimate the ranges of the baseline or physiological pRAPD for this cohort of volunteers.

This method of standardization tries to simulate a situation where the filters are put in front of the *right* eyes of the subjects who have baseline physiological pRAPDs either in the left or the right eyes. It is known that a normal person can have a range of physiological pRAPD either in the left or the right eye. The laterality can flip between the eyes for the same subject. This method attempts to correct a systematic error induced by the machine. The baseline pRAPD will have all positive values, and therefore, when regression line is drawn, the intercept will have a larger value. In this situation, the calibration factor will not only calibrate the machine's systematic error but also take away the subject's baseline pRAPD. In the ideal situation, when x = y, and c = 0 in the formula y= mx + c, it assumes that subject has no physiological RAPD.

The filter values used were very small and in effect should not produce measurable effect of retinal dark adaptation. The measurements were also sequenced in a way that the first measurement was with no filter followed by 0.3NDF, 0.6NDF and 0.9NDF.

The subject came off the instrument between each acquisition into the ambient room light for a few seconds, and 30 seconds of dark adaptation was carried out before each acquisition for each subject.

Two other studies[116;118] have previously described the limits of pRAPD variation within a normal population. An isolated pRAPD in the range of 0.3 log units that is not associated with any other clinical or historical finding is considered to be physiological. With this cut-off there remain some exceptional cases or outliers where the normal pRAPD was found to be between 0.3 and 0.4 log units.[116;118] These studies did not further categorise RAPD severity. In this study it was seen that it was useful to have an assessment of the likelihood of any given measurement being 'just beyond normality' (0.3 log units group), 'mildly beyond normality' (0.6 log units group), or 'considerably beyond normality' (0.9 log units group), also see Z probability (These are described in appendix B).

Wilhelm and colleagues discussed in detail the distribution of 102 normal subjects' pRAPDs. There was a pRAPD of < 0.08 log units in 53 subjects, between 0.08 and 0.22 log units in 43 subjects, and between 0.23 and 0.39 log units in 6 subjects, table 11.2. The greater density of pRAPDs were observed in the 'less than 0.08 log units' category, and lower density in the higher bands in this cohort.

The sensitivity and the specificity of the Procyon pupillometer are high in distinguishing unilaterally placed 0.3 log units NDF, 94% (binomial 95% confidence interval (CI) = 86% to 99%) with the AUC of 0.99 (95%CI = 0.95 to 1.00). This was higher than that from other methods employed to calculate pRAPD. It is important to note that this does not necessarily mean that this algorithm is better than other algorithms, but that it performs best on this machine for which it was developed. Just as the pupillometers developed and tested by other groups in the literature[118;180;209;212;312] are also optimised to take into account differences in stimulus size, intensity and duration.

Because the limits of physiological pRAPD lie in the region of 0.3 log units this ability to distinguish at this level of filter offers clear indication of clinical application.

Interestingly, the sensitivity and the specificity to a 0.3 log units difference in filters decreased with increasing filter values, table 11.3. Increasing filter values would simulate bilateral asymmetrical cases. These would also have the asymmetry, according to the filter values, of 0.3 log units like those for the unilateral simulated cases. The stimulus intensities utilised in this experiment were in the range between 0.04 and 4 lux. It is not clear why the diagnostic ability is slightly reduced when the simulated disease severity was bigger but with similar amount of asymmetry, and whether this could be improved with different levels of stimulus intensities.

For the ratiometric methods the 0.04 lux stimulus intensity tends to give a better performance than the higher intensities (0.4 and 4.0 lux), table 11.3. One speculation would be that the higher light level in these case falls at or near the saturation level of the pupil drive system (i.e. at the knee of the intensity-amplitude curve). But with the proprietary algorithm it has the advantage of incorporating a range of light levels across the graph and thus associated with higher sensitivity and specificity.

In summary, pupillometric measurement of RAPD can be calibrated against neutral density filters of known attenuation. The range for normal physiological pRAPD is between 0 and 0.22 log units after calibration. Before calibration the range is between 0.02 and 0.46 log units. Changes in simulated pRAPD of only 0.3 log units can be detected with good sensitivity and specificity using a commercially available pupillometer. The results suggest that it is best for detecting disease but potentially useful for advancement of disease in addition.

ASSESSMENT OF REPEATABILITY OF THE TEST

Chapter 12

Repeatability Studies

12.1 IMMEDIATE REPEATABILITY STUDY

12.1.1 OBJECTIVE

To determine the test-retest variability of pRAPD on immediate repeats.

12.1.2 METHODS

The first (R1) and second (R2) measurements (each with 0.04 lux, 0.4 lux and 4 lux) were taken in a session without longer pause than usual in between. There was 30 seconds of DA before each test acquisition. The measurements were taken as independently of one another as possible: the observer was masked to the outcomes of the first test, and care was taken to make sure that the second measurement would have the same pre-acquisition dark adaptation period, the dark adaptation time of 30 seconds being fixed throughout.

One potentially important factor is that patients are in general more comfortable with test repeats than the first one since there is always a novelty effect. In order to lessen this potential bias of higher centre influence, the subjects were recruited from those who came for diurnal variation (DV) study. The immediate repeat test was not performed the first thing on the day but at any time after the 9:00 o'clock measurement of the DV study. By this time the subjects were familiar with the test and they were more comfortable.

Subjects: Normal and glaucoma patients were recruited. The inclusion and exclusion criteria were the same as described in general methodology chapter.

Outcome measures: (1) Direct PLR constriction amplitude and (2) pRAPD measured by the proprietary method.

Coefficient of test-retest variability of pRAPD was defined as 1.96 x standard error of the difference between the first and second pRAPD results. The change in magnitude of pRAPD (the absolute change, regardless of more or less reading) was utilised for

calculation of standard error (SE). The limit of agreement was the 95% range of the difference between the first and second pRAPD results. The upper limit = mean + SE and the lower limit = mean – SE.

12.1.3 RESULTS

Eleven normals (mean age 49 ± 14 years) and 12 glaucoma patients (mean age 72 ± 12 years) were recruited.

1. Amplitude analysis for immediate repeatability

Using **all** subjects (N=23), difference between R1 & R2 measurements (R1-R2):

	4 lux		0.4 lux		0.04 lux	
	RE	LE	RE	LE	RE	LE
mean	-0.03	-0.04	-0.09	-0.08	-0.14	-0.11
paired t	1.348	*1.235	*3.007	3.235	4.525	*3.216
p	0.193	0.224	**0.003**	**0.0004**	**<0.0001**	**0.001**

Table 12.1. Amplitude difference between the first and second tests. [*normality test failed, Wilcoxon Signed Rank test used instead, z-statistic quoted]

Comment:

The difference in constriction amplitude between R1 and R2 ranged from 0.03 to 0.14 mm for all light intensities used. This test-retest difference was smallest with the highest intensity (4 lux). The difference became significant with lower intensities.

4 lux stimuli: R2 measurements tended to be fractionally larger than R1 but this difference amounted to only a 2% (RE) or 3% (LE) change in amplitude and was not statistically significant.

Conclusion: There was good repeatability and no significant association with the order of testing.

0.4 lux and 0.04 lux stimuli: R2 measurements tended to be consistently larger than R1, which in the case of the tests with 0.04 lux amounted to a 24% (RE) or 18% (LE) average increase in amplitude which was significant.

Conclusion: There was poor repeatability and significant potentiation of the reflex on repeated testing. It may be because physiological variability has more effect on the results when the very dim lights are used with smaller pupillary constrictions.

Is the repeatability similar in glaucoma patients compared with normal controls?

The amplitudes of pupillary constriction were compared. Regardless of eye, R1-R2 differences for the two cohorts were:

	4 lux		0.4 lux		0.04 lux	
	G	N	G	N	G	N
mean	0.00	-0.06	-0.02	-0.13	-0.06	-0.17
statistic	*MWT		unpaired t = 3.406		*MWT	
p	**0.003**		**0.002**		0.052	

Table 12.2. Amplitude difference between the first and second tests. [*equal variance test failed so Man-Whitney Rank Sum used instead of t-test]

Comment:

- The tendency for R2 measurements to be larger than R1 was much greater among the normal controls than the glaucoma patients.

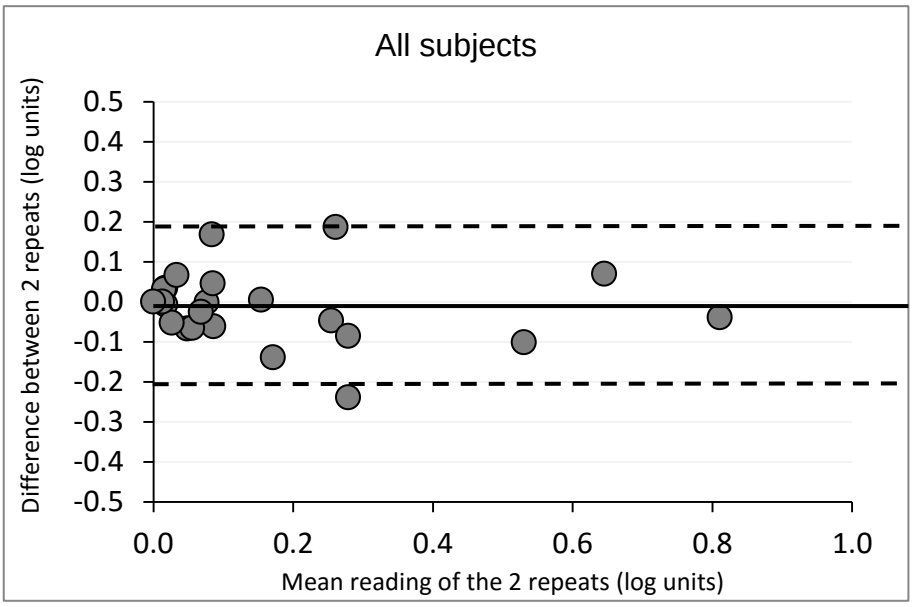
2. pRAPD analysis for immediate repeatability

The coefficient of test-retest variability for all subjects was 0.005 (normal = 0.013, glaucoma = 0.009). The 95% limits of agreement of the 2 repeated pRAPDs were between -0.006 to -0.043 log units for the normal subjects, between 0.009 and -0.018 log units for the glaucoma patients. For all subjects, the 95% limits of agreement lie between 0.00 to -0.018 log units. Negative value signifies the second test having higher value than the first.

The mean magnitude of change in pRAPD (regardless of the first being higher or lower than the second reading) was 0.067 log units for all, 0.073 for normals and 0.061 for

glaucoma patients. The difference between the first and second pRAPD results were statistically insignificant for all subjects (p = 0.67), for normals (p = 0.43) and for glaucoma patients (p = 0.97).

Bland Altman plots & analysis



Figures. 12.1 Bland Altman plot, repeatability of pRAPD. The upper and lower dotted lines represent the upper and lower limit of confidence level (95% limit = 2SD). The middle line represents the mean pRAPD level.

Comments:

- Results of dim light stimulus (0.04 lux) showed larger amplitudes of constriction with second repeat compared with the first. Agreement worsened and test-retest variance increased as the stimulus intensity was lower. This was expected to be due to poor signal to noise ratio (SNR) with dimmer lights. The measurement results with higher stimulus (4 lux) were highly repeatable. There is a tendency of the second test having marginally higher value than the first repeat. It is difficult to say if this is due to the higher centre input or the systematic error in the test set-up. Regardless, the difference is small and insignificant.
- The proprietary algorithm incorporated the results of all light levels for pRAPD measurement. The coefficient of test-retest variability of the final pRAPD was only 0.014 which signified a good agreement between the first and second tests. When Bland Altman plots was constructed for this, apart from one outlier the

difference between the first and the second repeats were within the 95% limit, figure 12.1.

- The magnitude of change in the final pRAPD between the test and retest was 0.07 log units for the normals. Glaucoma patients did not seem to have much variation either as their change in magnitude was 0.06 log units.

12.2 DIURNAL VARIATION OF pRAPD

12.2.1 INTRODUCTION

The pupil may be affected by a number of influences that vary during the day. One of the concerns about using the PLR to provide information about the optic nerve is that this brainstem reflex may be subject to many other influences, both central and peripheral, which could confound the measurements.

A normal subject can also have a small degree of relative difference in afferent input[116] but this is characteristically small and not always clinically detectable. In the above experiment, the pRAPD measurement for normal subjects was between 0 and 0.39 log units, median value 0.04 log units. As with other measures of biological functions such as intra-ocular pressure, blood pressure, ocular blood flow, spontaneous eye-blink rate, peak flow rate, or serological biomarkers, a degree of diurnal variability in the RAPD of healthy subjects was suspected.

However we know less about *central* influences and in particular how these may vary during the day and affect measurements of the PLR. For example, it is known that level of arousal (which clearly varies during the day) has a profound effect on the pupil: as subjects become sleepy their pupils miose and show low-frequency oscillations. There have been a small number of studies investigating variation in pupil parameters at different times of day;[13-17] all of these studies looked only at young healthy subjects, and all concluded that there is a small but significant effect of time of day on pupil measurements. However there is poor agreement over the nature of this influence: in some studies the pupil size was larger in the morning,[313] in others larger in the afternoon, and where tested the PLR amplitude was affected by time of day in some studies but not others.[314]

In clinical practice it is clearly going to be important to establish whether time of day affects pupil measurements in glaucoma patients.

Kawasaki and colleagues estimated the immediate short-term fluctuation of pRAPD to be approximately 0.1 log units based on 95% CI of their test specific repeated stimuli on a group of normal subjects.[116] The same authors[119] reported the long term variability of

relative afferent pupillary defect (*RAPD*), using a computerised infrared pupillometer. This study tested seventeen healthy subjects four times over 3 years and reported that median change in RAPD between any 2 sessions was less than 0.08 log units (25^{th} to 75^{th} percentile being 0.04 to 0.15 log units).

12.2.2 OBJECTIVE

To investigate the influence of time of day on pRAPD measurements.

12.2.3 METHODS

The inclusion and the exclusion criteria and pupillometry were the same as those described in chapter 7. All participants were advised to have a normal sleep/wake cycles before the study date, in other words, none of them were night workers or had just flown back from some different time zones. Participants were refrained from taking coffee during the study period (9 am to 5 pm).

All experiments were conducted in the same quiet room. Subjects were dark-adapted for 30 seconds before each recording, then two acquisitions were carried out at each of the three stimulus intensities (0.04, 0.4 and 4 lux). These tests were repeated at five different times during the day, namely 9AM, 11AM, 1PM, 3PM AND 5PM.

The time-of-day variability within an individual was described as the coefficient of variability of measurements across the working hours of the day. The frequency of zenith (highest) and nadir (lowest) pRAPD measurements was calculated for morning (9AM and 11AM testing) and afternoon (3PM and 5PM) testing, and chi-squared statistic used to evaluate the significance of any differences found.

12.2.4 RESULTS

In total, 28 healthy subjects were recruited (20 female) with median age 42 years (range 20 to 73); estimates of their pRAPD ranged from 0 to 0.53 log units (median 0.11, standard deviation 0.11, 92% estimates < 0.30). In the glaucoma cohort 23 patients

were recruited (9 female) with median age 76 years (range 53 to 88); estimates of their pRAPD ranged from 0 to 1.47 log units (median 0.31, standard deviation 0.36, 52% estimates >0.30). The pRAPD estimates of these cohorts were significantly different (Mann Whitney signed rank test: U statistic = 4081; p =0.001).

The mean change in magnitude of pRAPD between any two sessions (greater or less) within working hours was 0.09 log units for the normal subjects. This was comparable to that of Kawasaki who reported the median change in pRAPD between any 2 sessions over 3 years of their normal subjects to be 0.08 log units or the short term variability to be 0.1 log units. For glaucoma patients in our cohort, the change in magnitude of pRAPD between any 2 sessions within working hours was 0.11 log units. The slightly larger change in magnitude for glaucoma patients was suspected to be due to heteroscedasticity of this biological measurement. The difference between the normal and glaucoma group was insignificant (p=0.08)

Influence of disease status (or mean pRAPD), age and gender on time-of-day within-subject variability of pRAPD estimates

Age and mean

Glaucoma subjects also had larger pRAPD values compared to that of normals. It is thus important to investigate if these have any influence on the measured variability. The multivariate analysis was performed to test the potential influence of age or mean pRAPD on the variability. The detail analysis is described in Appendix C.

The results were as follows:

- On analysis, the results of 21.8% of the variability were mostly attributable to mean pRAPD values. Age contributed little. Increase in age by 1 year was only associated with 0.00017 log units increased variability in pRAPD. A change of 0.1 log unit in the mean pRAPD was associated with 0.24 log units increase in variability of pRAPD.
- Although our cohorts of control and glaucoma groups represented different age groups, there was no statistically significant association found between age and variability.

Influence of disease status on dispersion of pRAPD measurements

The estimates of pRAPD were on average significantly higher among glaucoma patients than healthy controls. As with most biological data, our measurement of time-of-day variability of pRAPD had a small amount (R-sq = 0.28) of heteroscedasticity in relation to its mean. This can be corrected by testing coefficient of variability, CV, which takes into account the magnitude of change in pRAPD. The CV for normal subjects was 2.49 (SD 6.51, range 0.13 to 35.26) and for glaucoma patients was 1.10 (SD 2.39, range 0.07 to 11.23), a difference that did not reach statistical significance (p = 0.36).

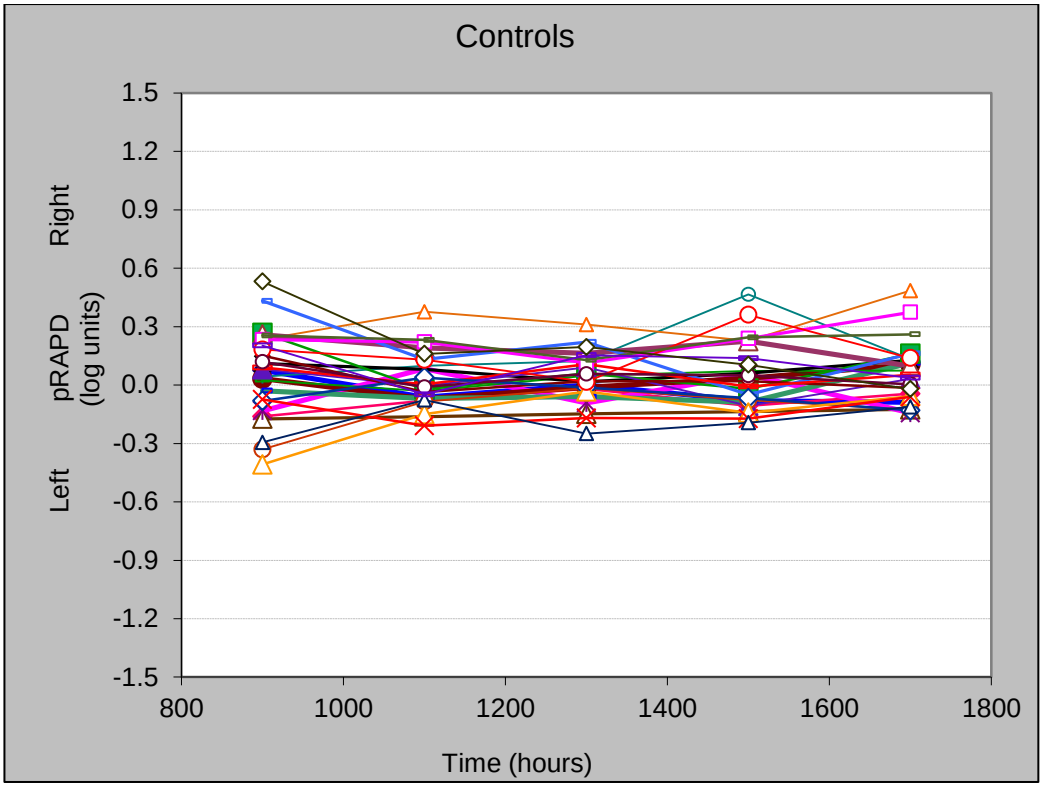
Gender

No significant gender effect on pRAPD variability was apparent in either cohort: among normal subjects, $R^2 = 0.01$, p = 0.64; among glaucoma patients, $R^2 = 0.09$, p = 0.17; for all subjects, $R^2 = 0.01$, p = 0.44.

Influence of time of day on pRAPD measurements

The dispersion of pRAPD estimates for each subject across the five time points in the day are shown in Figure 12.2. For both healthy controls (Fig.12.2A) and glaucoma patients (Fig12.2.B) some variation was seen in the pRAPD estimates, but in general the subjects maintained a similar rank order at all times and no consistent pattern emerged to suggest a diurnal influence on this measurement. Pair wise significance test showed no significant difference between the hours (table 12.3). It was seen in table 12.3(c) that although pRAPD values for the normal and glaucoma cohorts were significantly different, their fluctuations across mean were not significantly different.

The impression of the variability was tested by the frequency with which the highest (zenith) and lowest (nadir) pRAPD estimates were observed in either the morning clinic (9AM & 11 AM time points) or the afternoon clinic (3PM & 5PM time points). The results of this analysis are displayed in Figure 12.3 and shown as a 2x2 contingency table in table 12.4. These data showed that the chance of measuring a high or low estimate of pRAPD was fairly similar in morning and afternoon clinics and the chi-squared statistic was not significant.



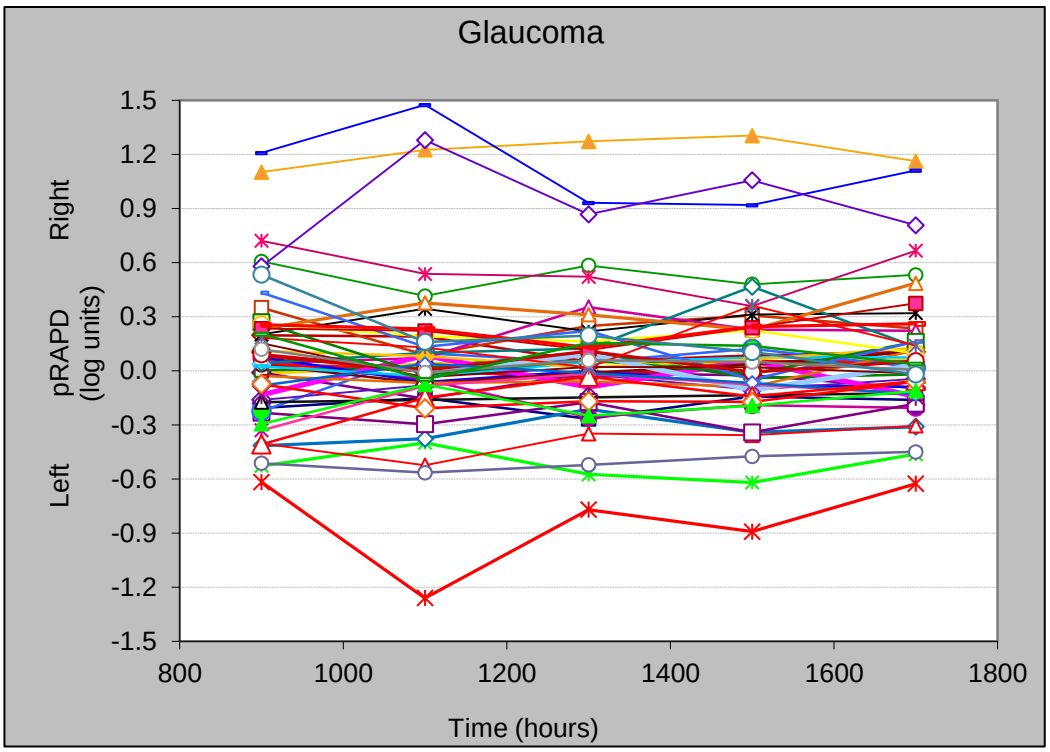


Figure12.2 A and 1B: Estimates of pRAPD in healthy controls (A) and glaucoma patients (B) at five different times of day.

Table 12.3(a)

Glaucoma	0900 vs 1100	0900 vs 1300	0900 vs 1500	0900 vs 1700
		1100 vs 1300	1100 vs 1500	1100 vs 1700
			1300 vs 1500	1300 vs 1700
				1500 vs 1700
p values	0.84	0.64	0.47	0.40
p values		0.71	0.24	0.19
p values			0.29	0.25
p values				0.88

Table 12.3(b)

Normal	0900 vs 1100	0900 vs 1300	0900 vs 1500	0900 vs 1700
		1100 vs 1300	1100 vs 1500	1100 vs 1700
			1300 vs 1500	1300 vs 1700
				1500 vs 1700
p values	0.36	0.75	0.38	0.65
p values		0.44	0.11	0.28
p values			0.17	0.49
p values				0.91

Table 12.3(c)

Glaucoma vs Normal	900	1100	1300	1500	1700
diff from the mean (p values)	0.99	0.31	0.90	0.35	0.74
absolute RAPD (p values)	0.00	0.01	0.00	0.01	0.01

Table 12.3 (a) inter-time significant tests for glaucoma, (b) normal, (c) bottom (between glaucoma and normal). Test hours: 900. 1100, 1300, 1500 and 1700.

Frequency	Controls		Glaucoma	
	Zenith	Nadir	Zenith	Nadir
AM (9am or 11 am)	15	12	14	13
PM (3pm or 5pm)	13	16	8	9
Total	28	28	22	22
chi-squared	0.644		0.096	
P value	0.422		0.757	

Table 12.4. The frequency of observing zenith and nadir estimates of pRAPD in morning and afternoon clinics.

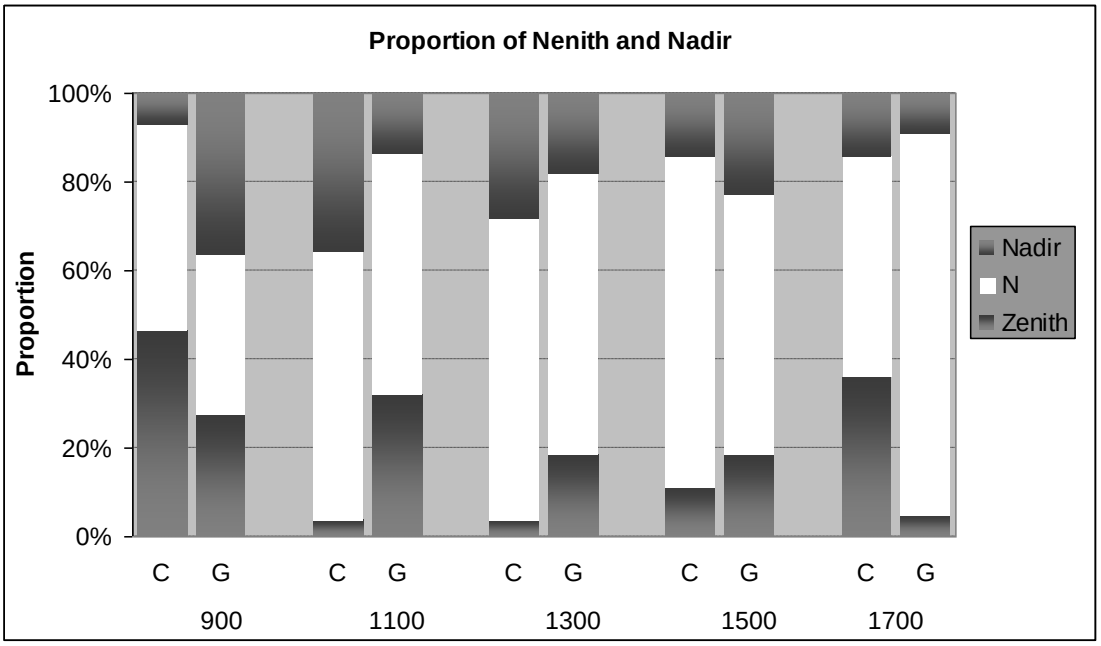


Figure 12.3: Proportion of control (C) and glaucoma subjects (G) showing highest (zenith) and lowest (nadir) pRAPD estimates at different times of day.

12.2.5 DISCUSSION

Patients who have or are suspected of having glaucoma may be booked for evaluation at any time within the working day, and so it is important to know whether the time of day affects our clinical measurements. For instance, it is well known that measurements of intraocular pressure vary significantly across the day, with the highest levels usually being recorded first thing in the morning.[18] The pupil is subject to several central influences that probably show diurnal variation, including state of arousal and autonomic 'equilibrium', and we hypothesised that pupil tests for glaucoma might

therefore be influenced by the time of day when they are performed. It was felt that it is important to investigate diurnal fluctuation in patients with established glaucoma as well as healthy subjects since functional measurements (such as perimetry) often show greater liability in damaged optic nerves.

With regards to the pupillometric test paradigms, a number of factors can induce measurement variability – for example higher variability of pRAPD estimates are associated with the utilisation of fewer number of stimulus pairs,[116] and less than optimum level of intensity[116;169;203](low signal to noise ratio). The instrument had been calibrated and the stimulus parameters optimised for the accurate and repeatable measurement of PLR.[315] The immediate repeatability of pRAPD was first tested. The magnitude of change in pRAPD over immediate repeat was low with a mean change in magnitude of pRAPD between any 2 tests was 0.07 log units for the normal subjects, which were statistically insignificant. Two of eleven subjects whose pRAPDs were close to zero (0.001 and 0.013 log units) change their side on immediate repeat. Although the test paradigm of this study was different from that of Kawasaki (1996)[119] and the temporal factors were different (immediate vs 3 year vs short term), similar amount of test-retest variability was noted: 0.07 log units in this study vs 0.08 in the long-term fluctuation and 0.1 for the short-term fluctuations by Kawasaki. Again for change in magnitude of pRAPD between any two sessions measured within working hours of the day were similar (normal subjects, mean = 0.09 log units) to those of immediate, short-term and long-term repeat measurements of pRAPD mentioned above. Therefore, variability of pRAPD over any period of time can be estimated to be $\leq$0.1 log units. The immediate repeatability is of similar magnitude to that of time-of-day variability implying negligible diurnal variation.

The results from the present study seem to show that pupillometric measures of RAPD do not vary significantly according to the time of day, both in healthy subjects and in patients with glaucoma. It may be that there is a small time-of-day effect which cannot be detected because of the size of this study. Another possibility is that by limiting the scope of our study to the working day we have missed significant fluctuations occurring outside working hours (we restricted our study to measurements between 9am and 5pm because the clinical relevance of any diurnal variation outside these hours is so small).

However the most compelling reason why pRAPD shows no apparent diurnal variation is probably because this is a *comparative* measure which will be *relatively* unaffected by any 'central' time-of-day influences that affect both pupils equally.

There was no statistically significant association found between age and dispersion or gender and dispersion despite age differences between control and glaucoma cohorts.

In visual field testing, many studies have shown that test-retest variability increases with defect severity[20], possibly due to the poor signal-to-noise ratio arising from stimulating only a few retinal ganglion cells using small perimetric stimuli. It may be that pRAPD measurements are more robust than psychophysical measurements of visual threshold – even when measured in patients with established glaucoma – because the light stimulus employed in pupillometry covers a wide central area and stimulates a much larger number of retinal ganglion cells, thus increasing the signal-to-noise ratio.

The important practical conclusion from this study is that pupillometric RAPD measurements are not influenced by time of day to a clinically significant extant. It is therefore valid to perform this pupil test on a patient with suspected glaucoma anytime within working hours, and when monitoring patients with established glaucoma it is fair to compare serial pRAPD measurements made sometimes in the morning clinic and sometimes in the afternoon clinic.

IV. ANALYSIS OF THE PUPIL CONSTRICTION AMPLITUDE DATA

Chapter 13

Analysis of pupil constriction amplitude data

13.1 INTRODUCTION

Pupillary constriction amplitude is a primary outcome measure from the pupillometer. It is important that the factors affecting pupillary constriction amplitude are appreciated before estimating the relative afferent pupillary defect. This can be done by investigating the association of the amplitude with other factors such as the intensity, pupil size, age, and visual field.

The data analysed was from the main study. Amplitude data was collected for both normal and glaucoma. Pupillometry was performed as per protocol described in the previous chapter 12 (the test protocol, link comments).

13.2 METHOD

The pupillary constriction amplitude (PLR amplitude) of 101 normal subjects and 117 glaucoma patients were analysed. Only the direct light response was utilised for the purposes of this study. The effect of age, laterality of the eye, the starting pupil diameter, the stimulus intensity, and the contraction anisocoria on the PLR amplitude were tested. The reliability of recordings was appraised by means of percentage of artefactual recordings that were discarded, or used. The results are as below.

13.3 RESULTS

(A) Normal subjects

1. Does stimulus intensity matter?

A plot was constructed for the intensities against the response amplitude.

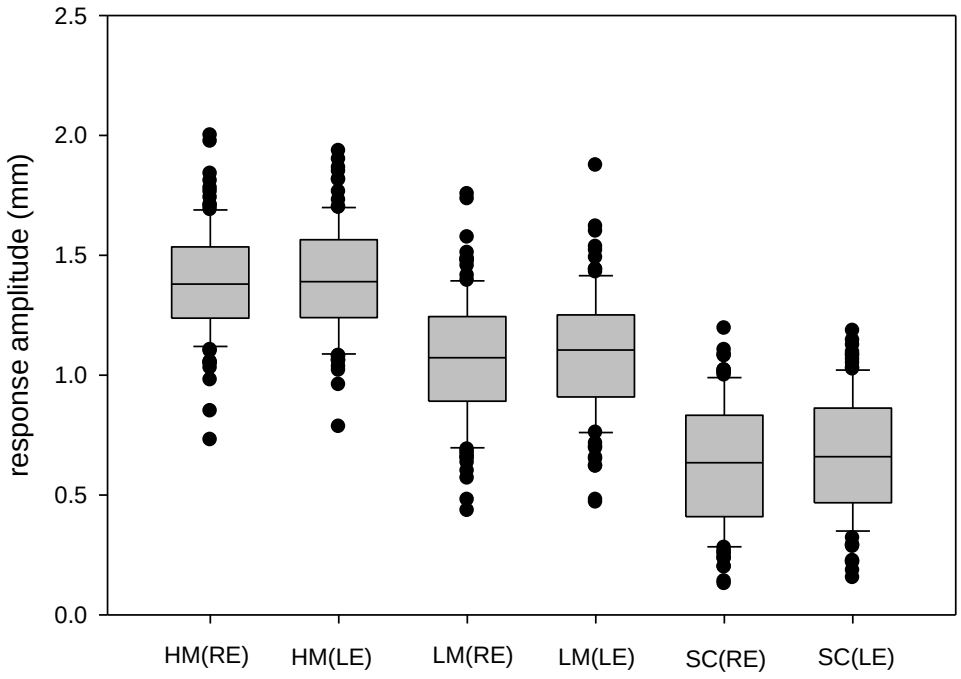


Figure 13.1. A plot of intensity against pupillary response amplitude. HM = 4 lux, LM = 0.4 lux, SC = 0.04 lux.

Comment:

Larger amplitude was associated with higher intensity. No notable difference was found between left and right eyes during each light level.

2. Does it matter which eye is tested?

The mean pupillary constriction amplitude for the right eyes and the left eyes were calculated and compared for each of the light intensities. Significance tests were performed.

	HM	LM	SC
RE mean	1.389	1.095	0.630
LE mean	1.399	1.118	0.662
t-statistic	-0.911	-1.940	-3.754
P (no difference)	0.365	0.055	**<0.001**

Table 13.1. Comparison of the response amplitudes obtained from the left eye stimulation and the right eye stimulation.

Comment:

It can be seen that there was a small amplitude difference (1-5%) between the right amplitudes and the left amplitudes - in the region of 1% for the 4 lux stimulus, 2% for the 0.4 lux stimulus and 5% difference for the 0.004 lux stimulus. The pupil constriction amplitude was always larger in the left eye than the right eye. This difference got more significant with lower intensity stimuli suggesting weaker signal to noise ratio with weaker stimulus intensity.

3. Does the starting size of the pupil matter?

The starting pre-stimulus pupil diameter is the diameter before individual stimulus or the diameter at the beginning of each pupillogram used in the test protocol. This is different from the "resting pupil diameter" which is the initial pupil diameter of the first pupillogram. The first pupillogram is discarded as described in the previous section (section 7.3). The means of the starting pupil diameter of the left and the right eye were calculated in the normal cohort. The relationship between the starting diameter and the pupil response amplitude was appraised.

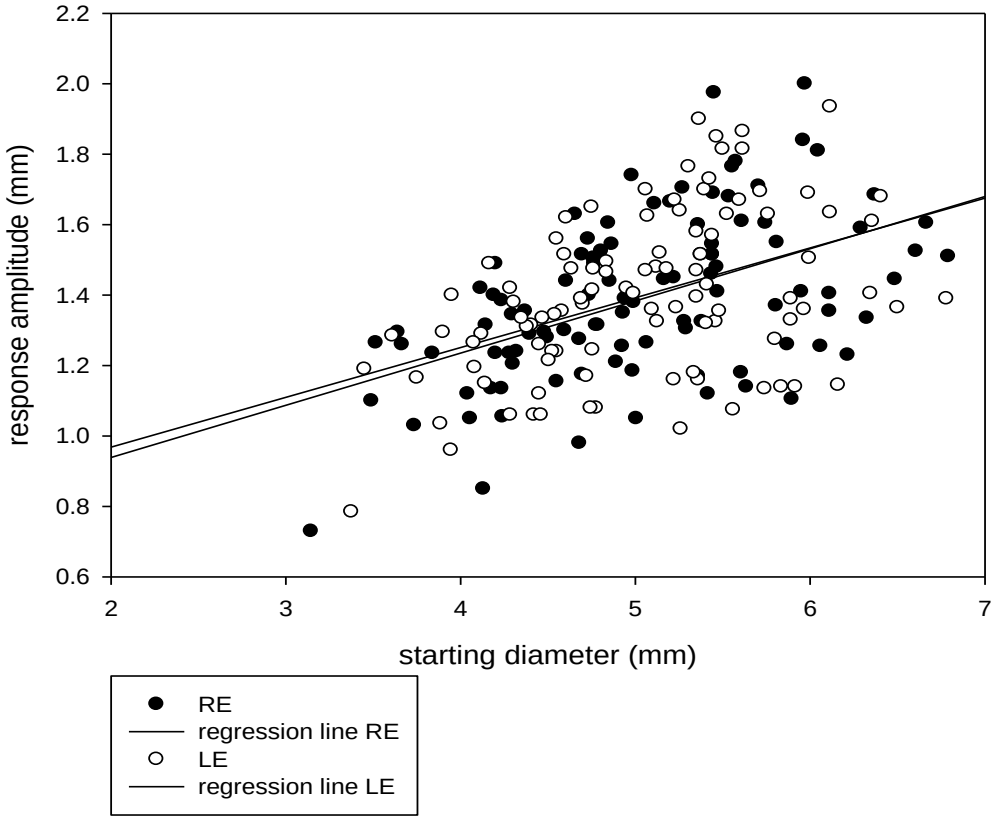


Figure 13.2. The plot of starting diameter and PLR amplitude.

Comment:

A significant positive correlation between response and starting pupil size for both left and right eye was noted (i.e. bigger pupils show bigger responses).

4. Does age matter?

The study population was not age matched. This was because of limited number of older healthy volunteers available at the time of study. It is important to know if age has effect on PLR and if the study results are confounded because of age differences. In the figure 13.3 below, the plot on the left shows the significant negative correlation ($p<0.001$) between age and PLR amplitude; i.e. older people have smaller pupil response. The plot on the right also confirms the significant negative relation between the age and the starting pre-stimulus pupil diameter. Given these results the two are likely to be related. Normalisation of the PLR amplitude can be achieved by dividing this with the starting pupil diameter. When this normalised data is plotted against age (figure 13.4), it can be seen that age effect on pupil response no longer exists. This confirms that it is not age but the size of the starting pupil diameter before constriction that matters. It is expected that a comparative test within an individual, such as RAPD test, is less affected by the pupil size effect. Furthermore, the test protocol incorporates a range of intensities for an optimum pupillary response.

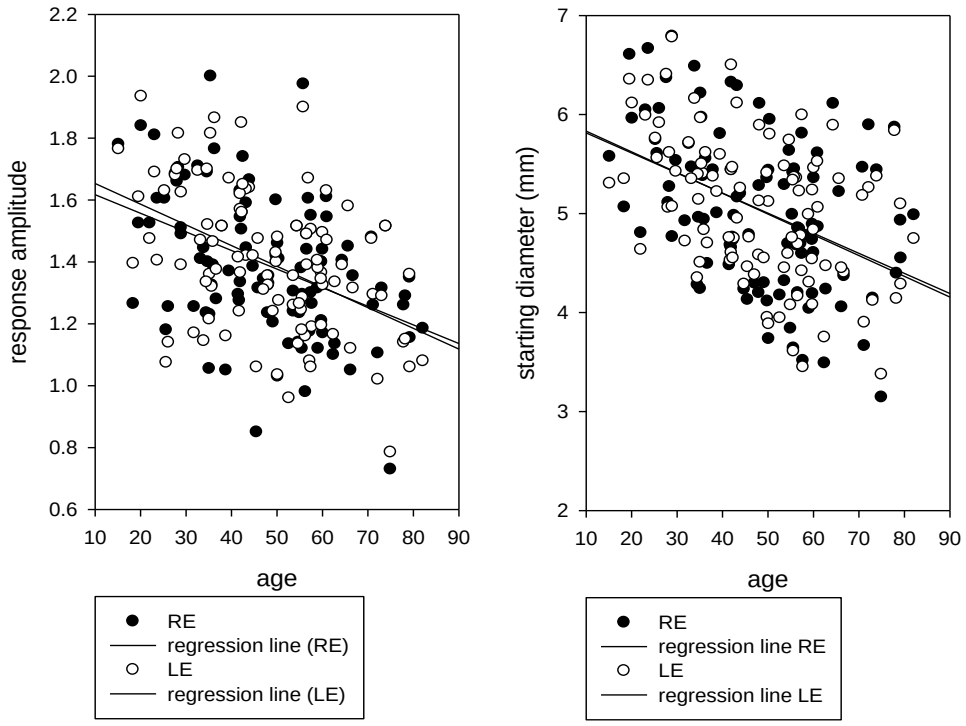


Figure 13.3. A plot of age and response amplitude, and age and starting diameter

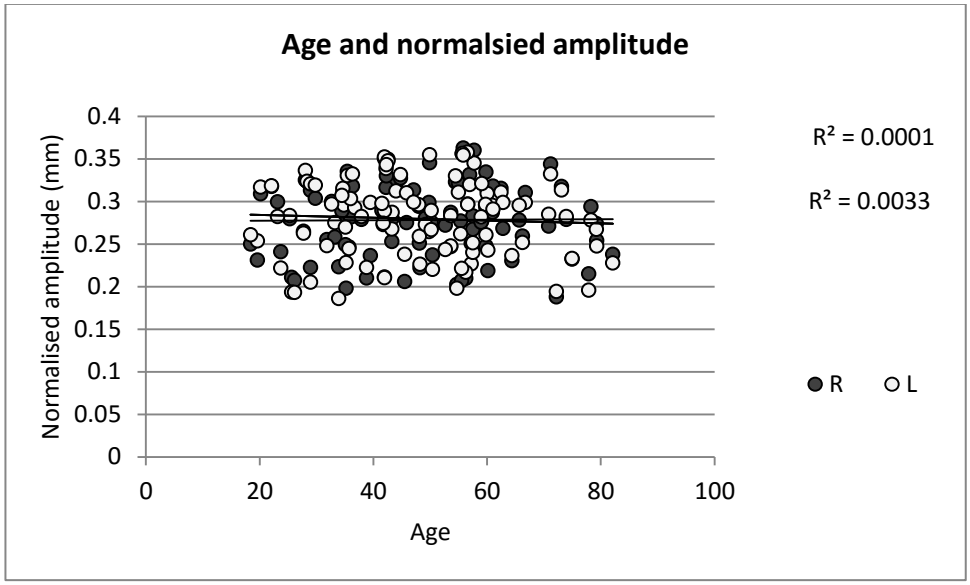


Figure 13.4. The pupil constriction amplitude is normalised with the initial pre-stimulus pupil diameter. This normalised data is plotted against age.

5. What proportion of data is artefact?

The average number of data points that were deleted after 'deglitching' and that were retained for analysis and compared between the light levels.

Proportion of all recordings usable for analysis:	HM	2307/2424	**95.2%**
	LM	2238/2424	**92.3%**
	SC	2192/2424	**90.4%**
	All	6737/7272	**92.6%**

Table 13.2. The percentage proportion of the recordings usable for analysis (normal subjects).

Comment: Overall the recording noise level is lower than 8%.

(B) Glaucoma patients

The glaucoma patients were not age-matched to the controls. Glaucoma patients were classified into perimetric glaucoma (PG), early perimetric glaucoma (EPG) and pre-perimetric glaucoma (PPG).The proportion of patients lying outside 'normal range' which is defined as PLR amplitude which are in *either* eye of patient being lower than 1.96sd below mean of normal cohort were calculated.

	lower limit of NR	PG	EPG	PPG
HM	0.95	46%	45%	36%
LM	0.57	24%	18%	14%
SC	0.14	5%	9%	7%

Table 13.3. The proportion of patients 1.96 SD below mean of the normal cohort. PG = perimetric glaucoma, EPG = early perimetric glaucoma, PPG = pre-perimetric glaucoma.

Comment:

This is an initial assessment of the validity of the data and it showed that reduction in constriction amplitude was higher with greater degree of perimetric defects in glaucoma patients. This pattern was consistent in the higher light levels. With low intensity of stimulus light (0.04 lux), however, the constriction amplitude of patients with early perimetric defects fell below the confidence level of normative data more than those with more severe visual field deficit. This may be due to the noise in the low intensity stimulus.

6. What is the starting size of pupil in the glaucoma group, compared to the normal group?

4 lux	Starting pupil diameter							
	R				L			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Normal	5.0	0.8	3.1	6.8	5.0	0.7	3.4	6.8
Glaucoma	4.3	0.8	3.0	7.0	4.3	0.7	2.6	6.9

0.4 lux	Starting pupil diameter							
	R				L			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Normal	5.5	0.8	3.4	7.4	5.5	0.8	3.7	7.2
Glaucoma	4.7	0.8	3.1	7.4	4.6	0.8	2.8	7.2

0.04 lux	Starting pupil diameter							
	R				L			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Normal	5.9	0.9	3.5	7.7	6.0	0.8	3.9	7.7
Glaucoma	5.0	0.9	3.4	7.8	5.0	0.9	2.9	7.5

Comment:

The mean starting pupil diameter ranged from 4.3 mm to 6 mm (the smallest 2.6 mm to the largest 7.8 mm) in both groups. This is within the commonly agreed mechanical working range of pupil above the average critical pupil size of 2.5 mm (section 4.3.1.2)

7. What proportion of data is artefact?

Proportion of all recordings usable for analysis:	HM	2643/2808	**94.1%**
	LM	2549/2808	**90.8%**
	SC	2438/2808	**86.8%**
	All	7630/8424	**90.6%**

Table 13.4. The percentage proportion of the recordings usable for analysis.

Comment:

The percentage of usable data was > 90% for all light intensities, 9.4% of data being discarded due to artefacts and poor fitting (section 7.4). The difference in the proportion of usable data of glaucoma patients compared to those of normals was marginal. Glaucoma patients could perform the pupillometry test as well as the normal subjects could do.

8. What is the effect of contraction anisocoria on amplitude?

Contraction anisocoria, although small, can confound the measurements of the PLR. (section 4.4) The effect of contraction anisocoria is particularly important when both direct and consensual responses are utilised for the measurement and analysis.

Direct and consensual responses of the left and the right stimulations were compared and the significance test was performed for both normal and glaucoma groups.

Paired t-test	mesopic Hi		mesopic Lo		scotopic		
stim:	RE	LE	RE	LE	RE	LE	
comparison:	RD vs LC	LD vs RC	RD vs LC	LD vs RC	RD vs LC	LD vs RC	
mean difference (mm)	0.04	0.05	0.03	0.04	-0.01	0.01	**Normal**
mean difference (%)	2.90	3.30	2.30	3.20	-0.80	2.30	
paired t statistic	5.22	*WSRT	3.65	5.37	*WSRT	2.38	
p value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.22	**0.02**	
mean difference (mm)	0.03	0.04	0.03	0.03	0.01	0.02	**Glaucoma**
mean difference (%)	3.10	3.60	3.40	3.70	1.00	3.30	
paired t statistic	3.11	3.40	3.13	3.26	0.70	2.40	
p value	**0.003**	**<0.001**	**0.002**	**0.002**	0.486	**0.018**	

Table 13.5. Significance test of contraction anisocoria between normal and glaucoma groups.

Comment:

1. Overall these data showed that the direct light response was significantly greater in amplitude than the consensual light response at all stimulus intensities regardless of whether tested in normals or glaucoma patients.
2. The scale of this difference ('contraction anisocoria') was very small - of the order of 2 to 4% only, and certainly not visible clinically. This is less than 6% described in the literature (section 4.4).
3. The scale and significance of this difference was the same for mesopic-Hi (4 lux) and for mesopic-Lo (0.4 lux) stimulus intensities
4. The scale and significance of this difference appeared a bit less when tested under scotopic conditions (0.04 lux), but the response amplitudes were much smaller (around 0.5 to 0.6 mm) and it may be that the signal was being lost in the noise.

13.3 CONCLUSION

The results of above analyses show that the associated noise level is very low with the amplitude data: - both left and right eye differences, direct and consensual differences were in the region of 1– 4%. These could represent the noise of the calculation. The percentage of data discarded was < 10%.

V. CLINICAL STUDIES

Chapter 14

Clinical Applications

14.1 PUPILLOMETRIC DATA AND VISUAL FIELD DATA

Amplitude and visual field association

Visual field measurement is one of the most commonly used functional tests in glaucoma. The results of visual field test influence the clinician's decision in glaucoma management. Previous authors have reported a relationship between visual field results and that of pupillometry (section 3.2.8). The correlation between pupil measurements and HFA 24-2 visual field tests was investigated.

What estimates of perimetric test should we use?

- One option is to use mean deviation (MD) – this gives an estimate of the average reduction in sensitivity across the entire central 48^0 visual field. In the example shown below this would be 6.62dB (or 0.66 log units).
    - Advantage: number readily available, and generally used/understood by practising ophthalmologists
    - Disadvantage: summarises function in area of retina over ten times larger than that assessed by the pupil test (1810 square degrees compared with 177 square degrees, respectively), reducing the correlation (especially for glaucoma which predominantly affects the peripheral field in early stages)

- Alternatively one could manually calculate the average reduction in sensitivity across just the central closest 18^0 around fixation corresponding to the area stimulated in the pupil tests (15^0). To do this, the mean value of the central 4 numbers of the 'total deviation' was calculated. In the example shown below these deficit values are [5dB, 3dB, 6dB, 6dB] giving a mean sensitivity loss within the central 15^0 = 5dB or 0.5 log units.

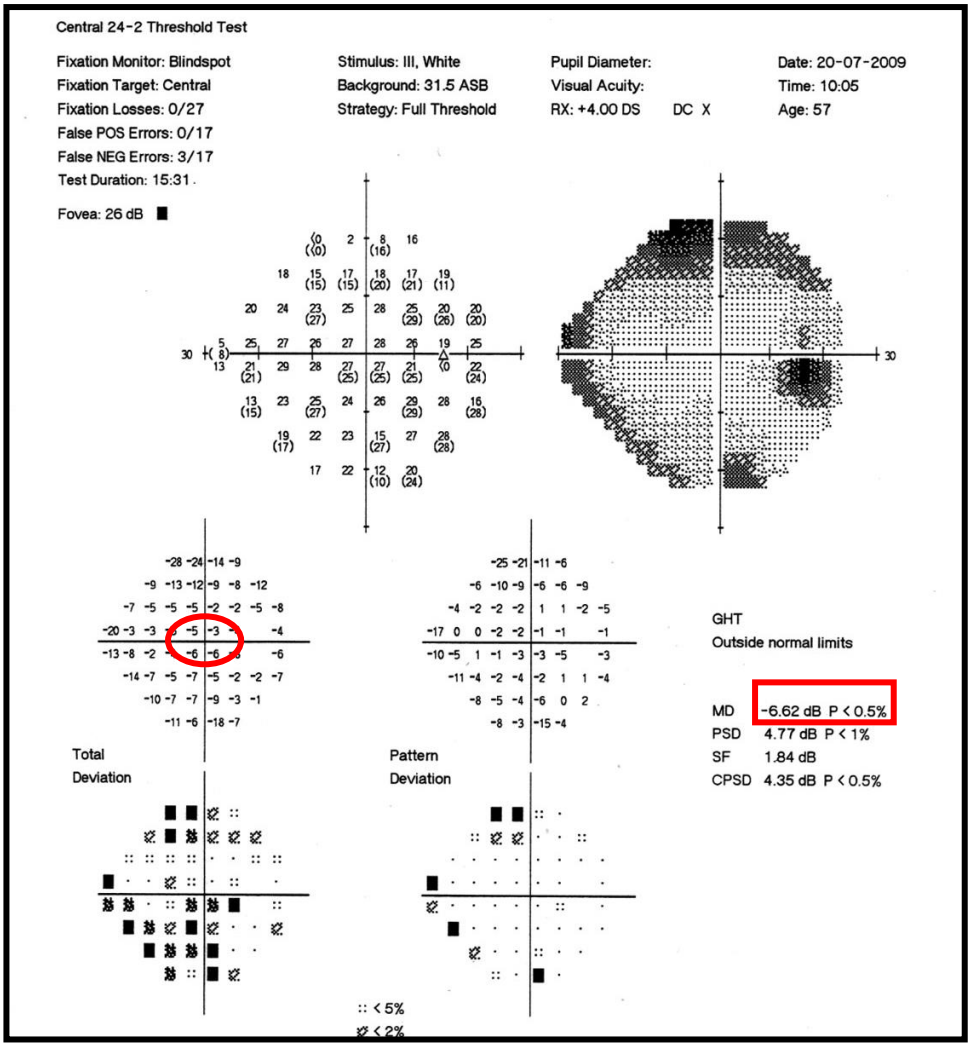


Figure 14.1 An example of a 24-2 Humphrey Visual Field test results

Comparison of MD with PLR amplitudes

Method

MD is defined as the *difference* between the observed visual sensitivity and that expected in normal subjects. For a similar comparison, the equivalent for the pupil test, namely 'PD' (pupil deviation) was calculated. This was defined by:

$$PD = Amp_e - Amp_o,$$

where the expected pupil response (Amp_e) is the mean PLR amplitude found in the normal control cohort, and the observed pupil response (Amp_o) is the PLR amplitude measured in that glaucoma patient.

Results

Using this approach, the results were:

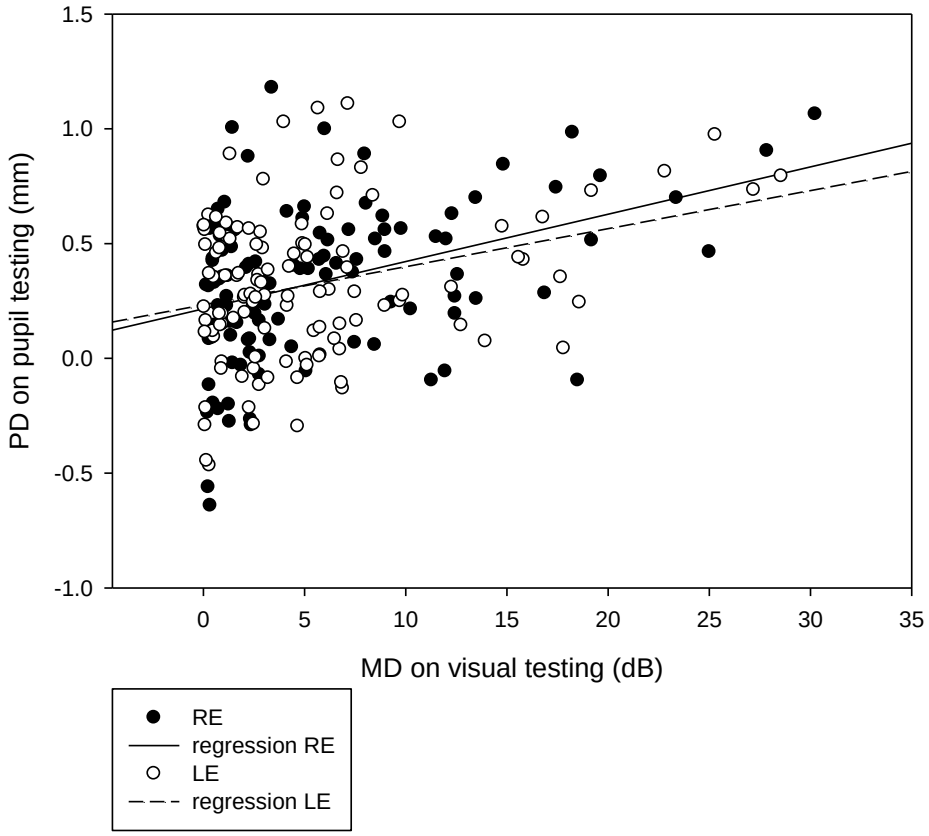
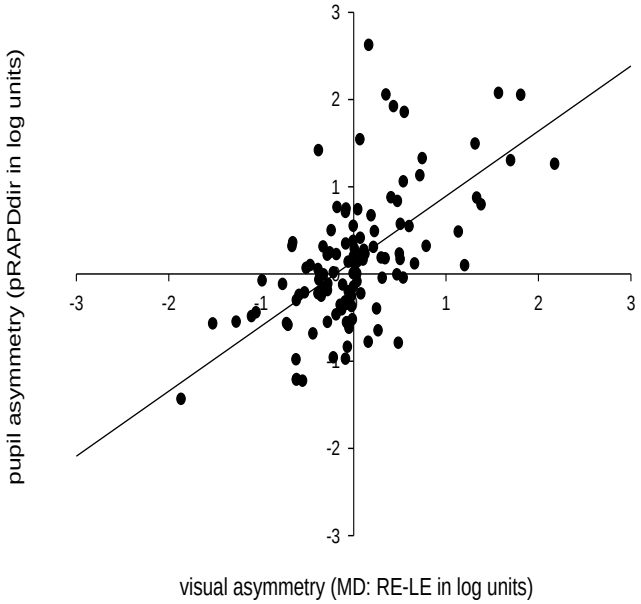


Figure 14.2. A plot of mean deviation and the pupil deviation

Comments:

- Despite the huge difference in retinal area assessed by the two different tests, and the lack of age-matching (or starting diameter matching) for the pupil measurements, there was a positive correlation between the amount of visual loss and degree of attenuation of the pupil response.
- The correlation coefficients for RE and LE were R = 0.391 and 0.307, which although small were significant ($P < 0.001$).
- This finding led up to consider comparing visual loss within the central 18^0 with that of the pupil deficit.

Comparison of MD with pRAPD estimates



Results

Figure14.3. A plot of visual asymmetry (difference between the right and the left visual field mean deviation) and the pupil asymmetry (pupillographic RAPD in log units) of glaucoma patients, n=113.

Comments:

- There was a significant positive correlation between visual asymmetry and pupil asymmetry ($R = 0.595$, $P < 0.001$). This is in keeping with the results of other authors in the literature (correlation R between 5 and 7, section 3.2.8).
- There was considerable scatter of the points around this regression line because these were derived estimates and variances summate: the variance of the coordinates of each point $V_{total} = [V_{MDinRE} + V_{MDinLE} + V_{PLRinRE} + V_{PLRinLE}]$. In addition, the visual estimate corresponds to function over a retinal area ten times larger than the pupil estimate. Naturally such 'noise' masks the relationship for small values of visual asymmetry (poor SNR if MD difference < 10dB or 1.0 log unit), and the true correlation between these parameters is best appreciated when there is much greater visual asymmetry (better SNR).
- As regards to the aim of the study (to assess the usefulness of pupil testing in detecting glaucoma), it doesn't matter that most early glaucoma patients have little visual asymmetry – this graph (above) merely serves as a proof of principle

that the pupil measurements bear *some* sensible relationship with visual test results. It remains to be seen using ROC plots whether pupil testing can identify diseased patients from within the asymptomatic (and presumed mostly healthy population)

Comparison of VD (visual asymmetry in central 18^0 field) with pRAPD

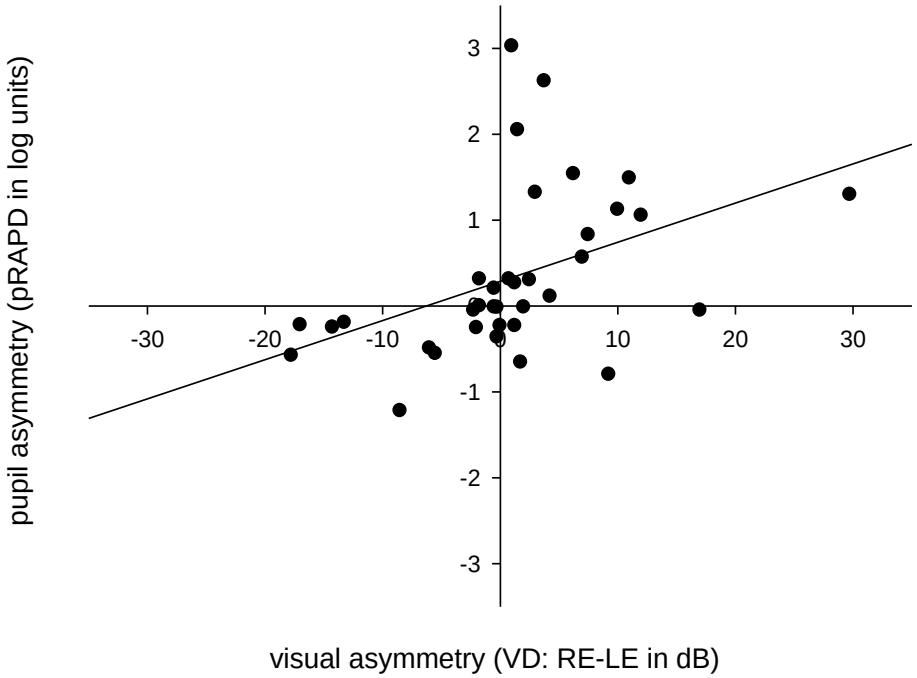


Figure14.4. A plot of visual asymmetry (difference between the right and the left visual field mean deviation) and the pupil asymmetry (pupillographic RAPD in log units) of 36 patients with glaucoma.

Comments:

- Also shows significant positive correlation between visual asymmetry and pupil asymmetry (R = 0.437, P = 0.008).
- At this stage the values for R and P seem less impressive than when using MD estimates of visual asymmetry, but comparison would be unfair as sample sizes are very different.

CONCLUSION

Should we expect the pupil test to give answers that correlate with the visual field test?

- PLR is driven by a different population of afferent fibres than those contributing to conscious perception of perimetric stimuli in VF test. There are fundamental differences in the nature of the two tests (type of stimulation, threshold vs suprathreshold, temporal multiple small foci stimulation vs a single large global area of stimulation, sensitivity profile, and anatomical differences in the visual and pupil pathways) as described in section 3.2.8. There are many examples clinically of 'pupillo-visual dissociation' (pupillary response to light poorly correlated with visual sensitivity), e.g. pupil 'sparing' in Leber's Hereditary Optic Neuropathy, or persistent RAPD after normalisation of VF in recovered optic neuritis. All of these points mean that the association between these two tests will always be modest.
- No conclusion can be made with regards to whether glaucoma has more effect on pupil afferent or visual afferent fibres, but this data makes an early and important contribution to this interesting topic.

14.2 GLAUCOMA DETECTION

14.2.1 INTRODUCTION

This section discusses the clinical applicability of the P3000 pupillometer for glaucoma detection. The diagnosis of glaucoma was made clinically by eliciting glaucomatous changes in the optic nerve, the nerve fibre layer and the associated visual field defects. Another group of glaucoma, pre-perimetric glaucoma signified the early disease stage where the diagnosis was made in the absence of reproducible visual field deficit. As discussed in the glaucoma chapter 6 the diagnosis of glaucoma is not straight forward in the early stages since there is an overlap of features of normal and diseased states. Nonetheless, a clinical diagnosis is the only available gold standard to which other diagnostic tests can be compared to appraise diagnostic capability. In the early stages of glaucoma, a single test is often not enough. More than one test is often required either in parallel or in serial. A test in essence is an aid to diagnosis. The devices do not diagnose glaucoma but the findings they provide alter the probability that a subject has glaucoma. The output from a device is merely a description of where the subject lies in relation to a normative data base. The clinician then determines the presence of glaucoma from a synthesis of all available data. The role of the pupillometer is intended to help clinician refine their impression gained from the clinical examination making the diagnosis more or less likely in cases of clinical uncertainty.

For case detection, however, the pupillometer, if not independently, may be used as a part of screening system for highlighting the suspicion of a diseased form the non-diseased population. Its ability to detect glaucoma will be higher if the sensitivity of the test compared with the gold standard is high. However, for a screening tool, a high false positive rate is undesirable. A highly specific test is required for case detection.

The objective of this part of the study is to determine the sensitivity and the specificity in detecting glaucoma by the Procyon P3000 pupillometer.

14.2.2 METHODS

Method comparison study

Subjects

Consecutive glaucoma patients and healthy volunteers were included in the study. The inclusion and exclusion criteria were the same as those described in the methods chapter, section 7.2.

Materials and methods

A full medical history was taken and Snellen's visual acuity and Goldmann applanation tonometry recorded. A full dilated fundus examination was performed by a single ophthalmologist (GTS). The Disc Damage Likelihood Scale (DDLS)[282] was recorded using rim to disc ratio measured by a 90D lens with a projected graticule. The disc scores range from 1 to 10, 1 being the indicator of the least change (rim:disc > 0.4) and 10 represents more than 270º rim loss (rim:disc = 0) for average size discs, table 6.2. The glaucoma patients underwent field testing with Humphrey SITA fast field analyser (program 24-2), standard automated perimetry (SAP). The diagnosis of glaucoma was a clinical diagnosis based on all available information as described in chapter 7. For all early cases the clinical records were re-reviewed independently by another glaucoma expert (IEM). Any patients for whom the diagnosis was in doubt were excluded from the study.

The test protocol was the same as that described in the link chapter. Each acquisition included stimulation of each eye alternately with a 15^{o} square stimulus for 0.4 seconds with 1.6 seconds of inter-stimulus-interval in between. This sequence was repeated seven times. A total of two acquisitions at three intensity levels: scotopic (0.04 lux), low mesopic (0.4 lux) and high mesopic (4 lux) were performed. The pupillary responses were recorded and any blink artefacts eliminated as described in chapter 7.

The proprietary algorithm was used to estimate pRAPD for direct and consensual responses. The pRAPDs were combined to estimate the final pRAPD which was described in the log units.

The sensitivity and the specificity in differentiating glaucoma patients form normal subjects were assessed using the Receiver Operative Characteristic (ROC) curves (chapter 7).

14.2.3 RESULTS

Demographic

Normal

Age	number	mean age	SD age	youngest	oldest
M	33	53	13.7	20	78
F	68	46	16.0	18	82
M+F	101	49	15.6	18	82

(a)

Glaucoma

Age	number	mean age	SD age	youngest	oldest
M	56	70	13.2	32	88
F	61	73	10.4	43	91
M+F	117	71	11.8	32	91

(b)

Tables 14.1 (a,b). Demographics of the normal and the patients with glaucoma

The normal subjects are younger in age than glaucoma patients ($p<0.5$). M:F is approximately 1:1 for the glaucoma group and 1:2 for the normal volunteers.

Glaucoma severity

Mean deviation (dB)	mean	SD	min	max
worse eye	-7.68	6.93	2.37	-30.24
better eye	-3.17	4.74	2.49	-28.56

(a)

MD in the worse eye	Percentage
>15 dB	16%
10-15 dB	10%
5-9 dB	39%
0-4 dB	35%

(b)

DDLS	mean	SD	min	max
worse eye	5.23	1.64	1.00	10.00
better eye	4.01	1.65	1.00	9.00

(c)

DDLS in the worse eye	%
9 to 10	6%
7 to 8	14%
5 to 6	49%
3 to 4	30%
1 to 2	2%

(d)

Tables 14.2 (1,b,c,d) Glaucoma severity measured by SAP mean deviation (MD) and disc damage likelihood scales (DDLS) of the worse eye.

Based on the visual field MD and DDLS scoring, a majority of the patients had early glaucoma. In 74% of patients the visual field mean deviation (MD) was <10 dB in the worse eye, and in 32% the DDLS score was <5 (rim/disc ratio < 0.1) in their worse eye. Only 16% had MD of > 15 dB and only 6% had DDLS score of 9 to 10.

Glaucoma asymmetry

Mean deviation	mean	SD	min	max
MD difference	4.51	4.55	0.00	21.80

(a)

MD difference	%
≥ 20	1%
$18 \leq \Delta MD < 20$	2%
$16 \leq \Delta MD < 18$	1%
$14 \leq \Delta MD < 16$	2%
$12 \leq \Delta MD < 14$	4%
$10 \leq \Delta MD < 12$	3%
$8 \leq \Delta MD < 10$	1%
$6 \leq \Delta MD < 8$	12%
$4 \leq \Delta MD < 6$	16%
$2 \leq \Delta MD < 4$	23%
< 2	35%

(b)

DDLS	mean	SD	min	max
DDLS difference	1.22	1.23	0	6

(c)

DDLS difference	%
10	0%
9	0%
8	0%
7	0%
6	1%
5	3%
4	3%
3	5%
2	17%
1	44%
0	28%

(d)

Tables 14.3 (a,b,c,d). Glaucoma asymmetry assessed by differences in SAP mean deviation and DDLS scoring of the two eyes.

Asymmetry, when assessed by the MD and DDLS scores, the large majority of patients had small asymmetry. Thirty five percent of the patients had MD difference of <2dB between the eyes and 28% had DDLS difference of 0.

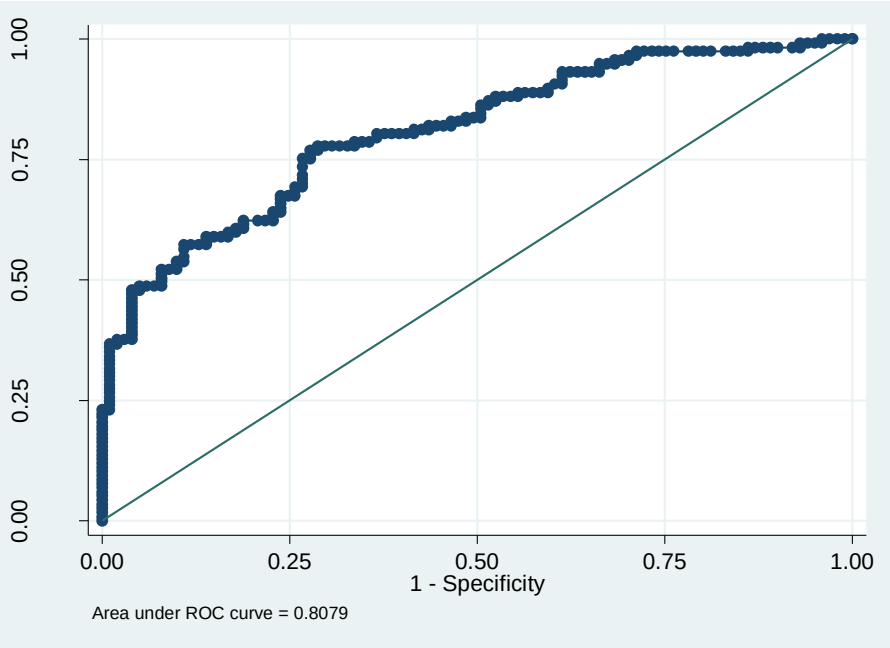
pRAPD results

pRAPD (log units)	mean	SD	min	max
Normals	0.047	0.09	0	0.44
Glaucoma	0.3	0.32	0	1.52

Table 14.4. pRAPD results of normals (total =101) and glaucoma patients (total =117).

The diagnostic ability of the test was further tested.

Diagnostic ability



ROC		-Asymptotic Normal--	
Obs	Area	Std. Err.	[95% Conf. Interval]
218	0.8079	0.0286	0.75177 0.86404

Figure. 14.5. The Receiver Operating Characteristic curve (101 normals and 117 glaucoma patients). AUC= 0.81 (asymptotic 95% CI 0.75 - 0.86).

The Area Under the receiver operative characteristic Curve (AUC) was constructed (section 7.5.2). An AUC of 1.0 represents perfect discrimination, whereas an AUC of 0.5 represents chance discrimination. The AUC was 0.81 (asymptotic 95% confidence interval (CI) = 0.75 - 0.86). Using the optimum cutpoint of 0.069 log units, the sensitivity of the test was 77% and the specificity 72%. The positive and the negative predictive values were 0.76 and 0.73. When the cutpoint was chosen at 0.064 log units, the balance of sensitivity and specificity was obtained at 74% and 73%. For a better specificity at 80-90% the sensitivity was 60-54% (cutpoint of 0.1-0.173 log units). For a better sensitivity at 80-90% the specificity was 58-40% (cutpoint of 0.03-0.004 log units).

When the sensitivity and specificity were 77% and 72%, the pRAPD of false positives and false negatives were:

pRAPD (log units)	mean	SD	min	max
false positives (FP)	0.17	0.08	0.07	0.44
false negatives (FN)	0.02	0.02	0	0.07

Table 14.6. pRAPD values of false positives and false negatives.

Visual field characteristics of TP and FN

The visual field characteristics of true positive (TP) and false negative (FN) glaucoma patients were as follows.

Visual field	FN	TP
Mean MD difference (dB)	3.05	4.98
SD	2.37	4.93
min	0.04	0.01
max	9.85	21.8
p values	0.008	

Table 14.7. Visual field comparison for the false negative (FN) and true positive (TP) patients.

The results suggested that disease detectability of pRAPD test is less when asymmetry is small based on visual field mean deviation results – smaller amount of mean deviation differences were found in the patients who were falsely diagnosed as not having glaucoma. This had lead us to explore further on testing diagnostic ability in groups with different level of asymmetry as below.

Degree of disease asymmetry and diagnostic ability of pRAPD test

The pRAPD is a *relative* test and therefore it is expected that the degree of asymmetry will have an effect on the diagnostic ability of the test. ROC curves were constructed for patient groups with high asymmetry ($\Delta MD > 10dB$), moderate asymmetry ($10dB \geq \Delta MD > 5dB$) and mild asymmetry ($5dB \geq \Delta MD > 0dB$) testing against normal subjects.

ROCs different degree of asymmetry	Obs.	AUC	Standard Error	Asymptomatic Normal [95% CI]	
5bB ≥ ΔMD > 0dB	174	0.7701	0.0364	0.699	0.841
10dB ≥ ΔMD > 5dB	128	0.8471	0.0443	0.760	0.934
ΔMD > 10dB	114	0.9733	0.0211	0.932	1.000

Table 14.8. Diagnostic ability assessed by the level of disease asymmetry.

It can be seen that as the disease asymmetry increases the diagnostic power of the pRAPD test also increases. At the lowest disease asymmetry of ≤ 5dB, the AUC is still high at 0.77 (asymptomatic 95% CI 0.7 – 0.8).

14.2.4 COMMENTS

- There was a significant age difference noted between the normal and the glaucoma group (P <0.05). As discussed in section 4.3, the less constriction amplitude in the older subjects than the younger subjects is mostly due to the older group having smaller starting pupil diameter than the younger group. Some adjustment can be done for this by normalising the pupil constriction amplitude with the initial pupil diameter, section 5.4.4.4. In this thesis, the final pRAPD was calculated by Procyon's proprietary algorithm which uses absolute constriction amplitudes but a range of stimulus intensities (0.04 to 4 lux) have been applied for a single pRAPD estimates giving allowances for both small and large pupils. The relative test also means the significance of initial pupil size difference being less significant in this case.
- The AUC for this cohort of 101 normals and 117 glaucoma patients was 0.81(asymptotic 95% CI 0.75 to 0.86) with a sensitivity and specificity of 77% and 72%. In the early part of the study, an analysis was done for a poster presentation using the data available: 58 normal subjects and 58 glaucoma patients (see Appendix E). The AUC, the sensitivity and the specificity of the P3000 RAPD test in detecting glaucoma were 0.92 (asymptotic 95% CI 0.87 – 0.97), 88%, 86%. It is interesting to note that the AUC was less with the larger sample size. It is possible that differences in the cohort (such as disease asymmetry differences) rather than the sample size, are accountable for this.

- Further analyses on the influence of disease severity and disease asymmetry on glaucoma diagnosis were done. Severity of glaucoma in the cohort of this thesis was not high. The majority of subjects had early stage of glaucoma based on perimetric and disc features. Nonetheless, the sensitivity and specificity of detecting glaucoma was at 77% and 72%. Asymmetry rather than severity thus seems to be an important factor in determining the diagnostic ability of the RAPD test.
- It was seen that the diagnostic ability of the disease increased with increases in the disease asymmetry, AUC of 0.97 for those with large asymmetry of ≥10dB vs 0.77 for those of ≤ 5dB (table 14.8). The asymmetric nature of glaucoma therefore makes the relative test a practical proposition for a clinical use. The parameter for measuring asymmetry in this study is only by means of a functional test (standard automated perimetry) and disc features. Asymmetry may be defined by other structural measurements or by functional measurements or both. Based on the perimetric method alone, the disease asymmetry was relatively small in this cohort (65% of the patients had SAP mean deviation asymmetry of ≤5dB, 24% between 5 and ≤10dB, and only 12% had mean deviation asymmetry of > 10dB). Despite this, the AUC was high at 0.81 for all glaucoma patients tested. A reasonable AUC of 0.77 was also obtained for the group with the smallest disease asymmetry as determined by perimetric test (ΔMD ≤5dB). This gives premises for pupillometric RAPD test for its use as a tool for detecting all stages of glaucoma which will have various amount of asymmetry and the detectability is expected to be higher with bigger asymmetry. The pupillometer also has other advantages including its ease of use, provision of objective, accurate and reproducible measurement results and being a commercially available item. It is expected that a test of neuronal reflex (pRAPD test) in addition to other available structural and functional test will enhance glaucoma case detection and help in the management of disease progression.

VI. OVERVIEW

Chapter 15

Thesis Overview

Overall objective of the study

In this thesis, the physiology of pupil response to light, methods of testing relative afferent pupillary defect both clinically and pupillographically are discussed. Stimulus parameters and outcome measures were optimised for a commercially available pupillometer P3000D and the diagnostic ability of this machine was appraised on normal and glaucoma cohorts. The overall aim of this thesis was to identify whether a pupillometer is capable of measuring the relative afferent pupillary defect accurately, and to assess its potential for glaucoma case finding in the suspected population. The thesis also aimed to identify the diagnostic ability of P300D pupillometer.

To date, there is no single test that detects glaucoma. It is considered that a sensitive and specific automatic test will aid the current methods of glaucoma case finding and diagnosis. Automated pupillometry was considered potentially suited for this purpose because it is relatively cheap, quick and easy to perform, and provides objective and reproducible measurement data. With the automated device, normative data can be collected and compared with that of a diseased population and serial measurements are possible in individuals over time. The observation is not influenced by examiner bias. Being a test of the neuronal reflex patient corporation is less critical. A non-clinical observer may be trained to assess the reliability of the data and operate the instrument. Importantly, the results of this automated pupillometry may be used as a brain stem reflex test, an adjunct to other structural and functional tests available for the diagnosis of early glaucomas.

Glaucoma is a bilateral disease and yet a comparative test is considered suitable to use for case finding purposes and monitoring changes and disease progression. This is because the damage involved in glaucoma is almost always asymmetrical. The modern automatic pupillometers allow detection of subtle RAPDs that are otherwise easily missed clinically. Early cases such as pre-perimetric glaucoma are common findings and up to 35% of the nerve fibres are expected to have damaged before any visual field defect can be elicited by the perimetry. Therefore, detection of early glaucoma is of great interest to the ophthalmologist in order to be able to control glaucoma damage before irreversible visual loss ensues. Even if for a small degree, the presence of RAPD in otherwise normal field but suspicious discs can be helpful for the clinician to make a

decision for treatment in early glaucoma. A goal of this thesis was to maximise the diagnostic potential of the device in glaucoma It was intended that the pupillometer would not only able to distinguish subjects with asymmetric glaucoma or advanced glaucoma which are easily detected by other available tests but also to be able to highlight those who are out of the normal range of RAPD but with equivocal clinical findings.

A commercially available pupillometer Procyon P3000 was chosen for this study. The stimulus configurations and the outcome measures were refined based on the evidence reported in the literature to optimise its ability to accurately estimate the RAPD for the purpose of detecting glaucoma.

Calibration of the pupillometer

Prior to any assessment of the pupillomotor response measured by P3000 it is important that the pupillometer is calibrated to the standards required for this task. The P2000 series has been used in the literature for the measurement of both light-adapted and dark-adapted pupil diameters[304;306;316] and the RAPD.[212] But P3000 has different set up and has not been used in the assessment of RAPD.

The pupillometer was first tested to see if it registered the intensity of light presented through its individual channels correctly. It was found that the non-stimulated channel was also registering light. The RAPD measured at this stage was inaccurate. This has led to the modification of the channels for a complete light separation. After the modification, more accurate results were read from the pupillometer. This highlighted the importance of complete separation of the light channels for an accurate RAPD measurement and also highlighted the potential errors with the clinical test which is performed in the open air without any arrangement for light separation.

Advantages of using neutral density filters for calibration

In this thesis, neutral density filters (NDFs) were used for calibration. The NDFs are well calibrated by their manufacturers. The value of the neutral density filter is distinguished by the optical density or by the filter factor. The Wratten 96 (Kodak)

NDFs were used for this thesis. The filter transmission factor is expressed as a ratio of two quantities with the same unit; it is thus a dimensionless unit. For example, the ratio of the transmitted luminous (5 lux) after the filter is inserted to the initial luminous (10 lux), will give the transmission factor of 0.5. The attenuation by the filter, in log unit, is the log of the transmission factor, which gives – 0.3 log units for the above example. This relation allows the transformation from any unit of luminous measurement to the log unit. Caution is needed to be made when NDFs are used. Prolonged use of thick NDFs can potentially dark-adapt the eye behind the filter. Therefore, it is important to use the filters for a short duration of time and to readapt both eyes before the next stimulus.

RAPD calculation

The proprietary algorithm was used to estimate the pupillographic RAPD (pRAPD). This method utilised the amplitude of pupillary constriction in response to 3 stimulus light levels namely 0.04 lux, 0.4 lux and 4 lux. The pupillary responses were corrected for anisocoria before RAPD calculation was made. The difference in the area under the regression curves representing intensity and response of the two eyes were thought to represent relative pupillomotor deficit more accurately than merely measuring the differences at single light intensity. Among the outcome measures available from the P3000 pupillometer, the pupillary constriction amplitude was chosen for the advantages discussed in sections 5.4.

Although anisocoria correction was performed for the amount of light reaching the retina no attempt was made for the correction of the difference in the mechanical contraction power of the iris muscles between the smaller and the larger pupil (chapter 4). This requires measurement of the iris motor muscles and was not suitable for the test protocol utilised in this thesis. It was, however, considered that the effect of this difference in the contractibility of the smaller and the larger pupil in the physiological anisocoria (which is typically < 2mm) is small and the resultant confounding effect would not be large enough to impose measureable effect on the accuracy of the RAPD estimate.

Optimising stimulus parameters for the pRAPD estimates

The next step of the thesis was to identify the most suitable stimulus paradigm for the accurate measurement of RAPD.

Dark adaptation

Unequal retinal bleaching is one of the most common confounding factors in the pupil testing. Observers looking at both pupils for comparative measures either light or dark adapt the pupils before their test in order to make sure that both retinas are equally bleached. Some authors light adapt the eyes[117] while others dark adapt prior to pupil testing[162;212] There is no theoretical reason for preferring any adaptive state for pupil testing. The choice of dark or light adaptation depends on the nature of the test. In light adapted pupils the starting pupil sizes are smaller and in dark adapted pupils the starting pupil sizes larger. Dark adaptation (DA) also increases the photoreceptor sensitivity to light. However, dark adaptation takes longer than light adaptation since a set amount of time needs to be allocated for DA whiles pupils can be light adapted simply by shining equal amount of light to each eye with a flash light. It takes about 10 minutes for cones to dark adapt and 40 minutes for rods. The duration of dark adaptation adopted by clinical observers performing pupillometric PLR tests varies between 30 seconds and 5 minutes, table 5.1. This thesis concerns the use of pupillometer in clinical setting where the test needs to be quick and efficient. The durations of dark adaptation tested in this thesis were ≤ 3 minutes because a longer adaptation would not be suitable. The results showed that dark adapting the eyes prior to pupil test reduce the variability of the pupil response, especially when stimulus lights of low intensity are used. But with higher intensities the effect of DA became less (chapter 9). The amplitude of pupillary constriction was significantly larger when the pupils were tested with prior dark adaptation of 3 minutes as compared to 30 seconds. However, this cost the total test time of 24 minutes with the current test algorithm, compared to 10 minutes for 30 seconds of adaptation. When no adaptation was performed, the test was subject to high variability in its measurement and unequal retinal bleaching. The reliability of the test was compromised. The dark adaptation of 30 seconds was chosen as a reasonable compromise between test variability and the total duration of the test.

Stimulus configuration

Pupillometric measurement of RAPD is not exactly the same as clinical SFLT. Although both test the relative difference of the afferent pupillomotor pathways, for the clinical SFLT longer duration of stimulus is presented allowing the phases of the pupil reaction to follow with the appreciation of "escape". But in the pupillometric studies, any of the features of the pupil dynamics can be compared. The most striking difference would be that in the clinical practice, because it is impossible to simultaneously observe both pupils by the observer, the duration of inter-stimulus-interval (ISI) is intended to be very short to allow for easier appreciation of the relative differences in the direct and the consensual responses.

In the pupil literature the stimulus configuration (duration of stimulus and the ISI) is variable among authors. Some authors in an attempt to replicate the clinical swinging flash light test stimulated the eyes for 3 seconds with very short duration of inter-stimulus-interval, while others used shorter stimulus duration and longer inter-stimulus-interval. Another group of authors sequentially presented the light to each eye individually instead of alternating the stimulus between the eyes. In this study, alternating sequence of 0.4s-1.6s ON-OFF combination was used. This configuration was initially chosen because it was similar to the stimulus used in the study of Kalaboukhova and Lindblom[209] who tested a number of stimulus ON-OFF combinations and regarded 0.5s–1.0s combination to be the best suited for detection of glaucoma in their study. With the current light levels (0.04 lux, 0.4 lux and 4 lux) and the study protocol specific to P3000, 0.4s-1.6s combination was compared with 0.5s-1.0s (KALA), 3.0s-1.0s (BERG) and 2.8s-0.2s (KAWA) combinations. It was found that the larger response amplitude was associated with (1) the brighter light level, (2) the longer ON-duration and (3) the longer ISI.

When the ISI was very short (e.g. 2.8s-0.2s, KAWA) the pupillary recovery was poor and the amplitude was also very small even though the ON duration was long. The difference in the light levels between 0.04 lux, 0.4 lux and 4 lux made little difference to the PLR response when the ISI was very short (< 1 seconds), figure 10.2. This is because with repeated stimulation without enough time to recover, summation of response happens and the iris muscles become less capable of responding to individual

stimulus. The human iris muscle takes this summation at low rate. It is also expected that the latency would be longer with this stimulus configuration. With short ISI queuing of the pupillomotor signal happens at the motor endplate despite being stimulated by the stimulus intensity which is bright enough to produce an action potential sufficient to reach the midbrain. The study data also highlighted the association between short ISI and high response variability (or poor repeatability), figure 10.4. By increasing the ISI, the amplitude became larger, the response variability was less and there was a stronger relation of the constriction amplitude with the stimulus intensities; e.g. 3.0s-1.0s (BERG) compared to 2.8s-0.2s (KAWA).

Shorter stimulus duration (ON-duration) was associated with smaller pupillary constriction amplitude for all light levels tested. But this was improved with longer ISI (e.g., 0.5s-1.0s KALA vs 0.4s-1.6s Pro). As with Kalaboukhova and co-authors, we found the stimulus configuration with short stimulus duration was associated with less variability (e.g. 0.5s-1.0s KALA). This was further improved with longer ISI (e.g., 0.4s-1.6s Pro).

The 3s-1s (BERG) combination was also associated with large pupillary constriction and less variable results. However, this configuration had more noise in terms of recordings of the pupillogram and was associated with higher rate of discarding of unwanted pupillograms before analysis, compared to the 0.04s-1.6s combination. Therefore, the 0.4s-1.6s combination was chosen for the study.

Optimisation of outcome parameters for the pRAPD estimates

Importance of calibrating the outcome measures

The measured pRAPD can be variable or inaccurate due to a number of factors including the variability in the test environment, the biological fluctuation in the PLR responses of the left and the right eye (variable physiological RAPD) as well as the machine's systematic error. This variability was tested but with standardised test environment using a light-calibrated machine.

As it is important that the RAPD assessment instruments need to be able to differentiate not only between diseased and non-diseased eyes (sensitivity and specificity) but also between varying stages of disease advancement (progression of the disease), the measured pRAPDs were calibrated across a range of filter densities. The direct comparability of 'abnormal' subjects represents a challenge because each simulated abnormal filter assessment has its specific measurement error and offers a unique calibration factor to centre the scores on the filter value. In a practical setting a subject's disease state is not known, thus, for disease assessment the direct use of RAPD machine across disease states requires a common reference calibration. In order to achieve a common calibration that would centre patient variability from the normal and 'filter populations' a linear regression calibration was used. Normal subjects were measured for RAPD response in the absence of any filter (0 log unit) and then in the presence of 3 known filters (0.3,0.6,0.9 log units) mimicking alternate stages of disease advancement.

When the filter values were plotted against the measured RAPD, a systematic error was noted represented by a shift from the ideal regression line: $y = x$, $R^2 = 1$, where the measured RAPDs equal the filter values. The regression line obtained was instead $y = mx + c$. The regression coefficient (m) and intercept (c) described the absolute scaling bias of the RAPD measurement across all filters. This displacement from the ideal line resulted in the calibration factors of $m = 1.54$ and $c = 0.1178$ applied to the "raw" pRAPD. Application of the regression equation to each raw value gave a better fit for all known filter values. The machine's systematic error as well as fluctuations related to the subjects' biological variation and the simulated densities of the disease by the NDF would all be attributable to this displacement.

Normative data

Normal healthy subjects with no apparent optic nerve disease may also have a small amount of physiological asymmetry,[115;118;119] the laterality of which can vary from time to time. This can sometimes be picked up as a RAPD especially when highly sensitive instruments are used to measure the relative difference. If the ranges of the amount of pRAPD for the normal subjects are identified the probability of a measured pRAPD being abnormal can be assessed. Normative data was collected from the healthy cohorts without any filter placed in the light channel and the calibration factor applied. The

normal values measured ranged from 0 to 0.22 log units after correction. The normal range of RAPD reported in the literature is from 0 to 0.3 log units. None of these authors mentioned any form of calibration in their studies. However, during left-right standardisation (chapter 11), some of the true physiological RAPDs may be corrected. If the standardisation equation (standardised pRAPD = pRAPD x (-1), ***Calc equation 2***) was used instead, for all left pRAPDs which were assigned as negative, instead of ***Calc equation 1*** described in chapter 11, the dispersion of the recorded pRAPD at the base-line level (no filter applied) would straddle across 0 log unit, figures below(15.1, 15.2). For this version of standardisation, the calibration factor would be m = 1.5, c = 0.008, and the normal range would be from 0.01 to 0.32 log units. The normal range of this version is more comparable with that reported in the literature. It may be that some of the physiological RAPD were corrected with ***Calc equation 1***. In either case it is seen that healthy eyes can have a small degree of RAPD. It is important to note that the value of normative data is read in reference to how the estimate has been derived as different methods of data handling accounts for different sets of normal ranges. If the RAPD is to be measured to differentiate normal and diseases eyes, the reference normative data should be the one that has been measured from the same instrument using the same algorithm.

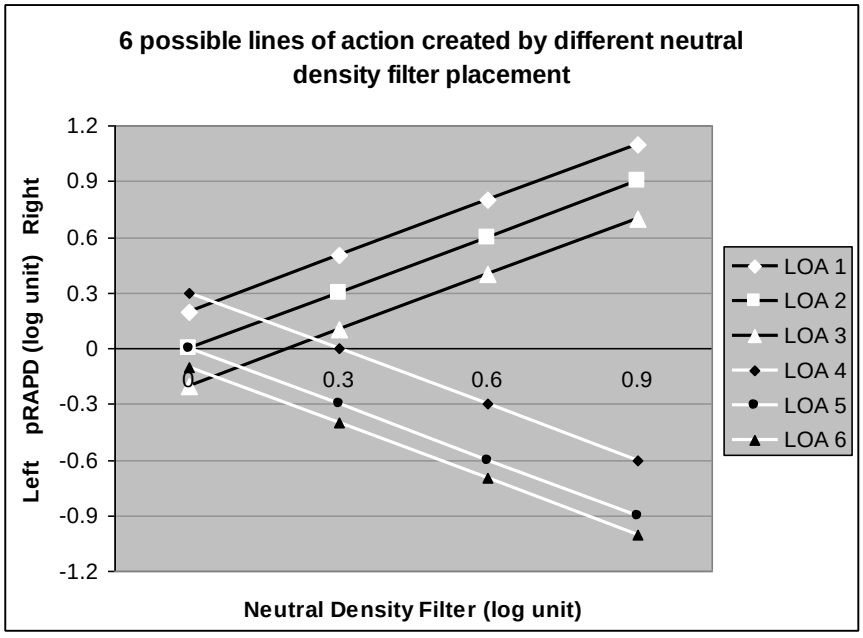


Figure 15.1. 6 possible lines of action by different neutral density filter placement. Right pRAPD values are assigned positive values and left pRAPD values are assigned negative values on the ordinate. Pupillographic RAPD values at 0NDF represent baseline pRAPDs.

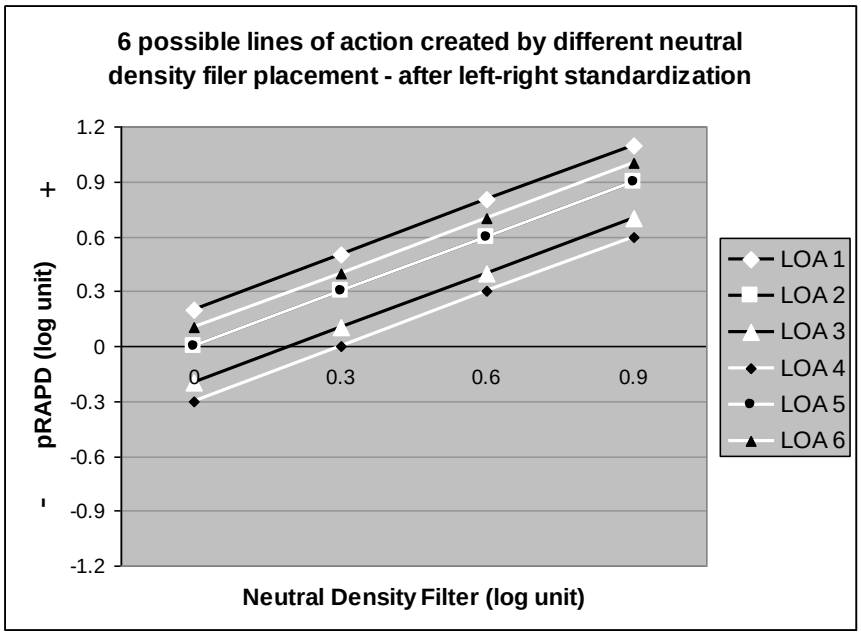


Figure 15.2 Lines of action after left-right standardization –application of ***Calc equation 2***.

The test paradigms including the stimulus and outcome parameters used in this thesis were titrated for the Procyon P3000 alone and therefore the stimulus parameters that were chosen above may not be suitable for other types of pupillometers which employ different designs and different light sources.

Repeatability

Repeatability studies of normal and patients with glaucoma

Immediate test-retest repeatability results showed a good repeatability of measurement for the RAPD test with P3000. On second immediate repeat, the mean magnitude of change in pRAPD from the first was 0.07 log units for the normals (0.06 log units for the glaucoma patients). This variation has no statistical significance. At individual light levels, however, due to less signal-to-noise ratio with dimmer stimulus light, the repeatability was low for stimulation with 0.04 lux compared to 0.4 and 4 lux stimuli. Because pRAPD estimates incorporated the results of all light levels, the results were less variable.

Diurnal variation of pRAPD

Two cohorts were studied, patients with glaucoma and (unrelated) healthy subjects. A small amount of test-test within-subject variability was noted in both groups: for normal subjects the change in magnitude of pRAPD between any 2 tests was 0.09 log units and glaucoma 0.1 log units, the difference between the two were not significant. The change in magnitude for an immediate repeat was 0.07 log units (0.06 for glaucoma group) as stated above. It was reported in the literature that change in magnitude over 3 years was 0.08 for normal subjects.[119] The change in magnitude of pRAPD over any period of time is therefore estimated to be <0.1 log units.

Despite there being a clear difference in the magnitude of pRAPD estimates between normal subjects and glaucoma patients (median value 0.11 vs 0.31 log units), there was only a small difference noted in the variability of these pRAPD estimates across the working day. In fact, if the heteroscedasticity in the data (i.e. the increase in measurement variance expected for larger pRAPD estimates) was taken into account, from the regression model it was estimated that this effect to be equivalent to an increase of 0.024 log units variance in pRAPD measurements for every 0.1 log unit increase in mean pRAPD. The variability estimates (CV) adjusted with respect to these different means were greater in the normal controls (2.49) than in the glaucoma patients (1.10), although this difference did not achieve statistical significance (p=0.36). In addition, among the glaucoma patients there was no correlation between the variability of the pRAPD measurements and the degree of asymmetry in disc appearances (DDLS; $R^2 = 0.015$, P = 0.58), visual field loss (mean deviation; $R^2 = 0.027$, P = 0.47), overall disease severity (as evidenced by the worse DDLS score: $R^2 = 0.01$, P = 0.65; or by the worse mean deviation value: R2 = 0.06, P = 0.30). This is contrast to other studies which have shown test-retest variability to increase with defect severity.[317]It may be that pRAPD measurements, based on an involuntary brain stem reflex, are more robust than psychophysical measurements of visual threshold. Another reason may be that the light stimulus employed in pupillometry covers a wide central area and stimulates a much larger number of retinal ganglion cells, thus increasing the signal-to-noise ratio.

The sample in this study included younger healthy participants than glaucoma patients. From the regression model it was estimated that only 0.00017 log units of change in

pRAPD to be associated with each year of life; this change is not significant. No significant time-of-day variability was also noted for different gender.

There was an equal chance of having a higher or lower pRAPD in the morning compared with the afternoon in both normal and glaucoma cohorts suggesting that there is no influence of time of day on pRAPD estimates between 9AM and 5PM. This provides a useful information for the clinician as the clinician can compare those results that are measured in the morning and in the afternoon clinics

Factors that affect pRAPD measurement

Stimulus intensity and the pupillary response amplitude for normals and patients with glaucoma

The amplitude of pupillary constriction was largest for the brightest stimulus (4 lux) followed by those for 0.4 lux and 0.04 lux stimuli. The repeatability of the data is higher with the brighter stimulus.

The left and the right eye pupillary response amplitude in normals

When the stimulus acquisition was applied during pupillometry, the first eye (left or right) to be tested was chosen at random. When the amplitude data was analysed for the eyes, there was no significant difference in the PLR amplitude of the left and the right responses at 4.0 lux (0.1%) and 0.4 lux (2%) intensities. Under the low intensity stimulus (0.04 lux), the left pupillary constriction amplitude was significantly higher compared to the right amplitude (5%). This is thought to be due to having lower signal to noise ratio and higher variability of responses with the lower luminous stimulation. Although it was not statistically significant for all intensity levels and the absolute difference was very small (0.01 to 0.03 mm), the amplitude was always larger in the left eye. The reason for this is not clear. This systematic error could be from having slight difference in the light set up in the left and right channels or difference in the measurement setup/device on each side. Procyon had already restructured the channels so that there is no light leakage in between. It is unlikely that this is related to light leakage. Nonetheless, the effect is very small as it is easily masked under higher light

conditions. The physical amount is also considered too small to have a measureable effect on the RAPD estimate.

Contraction anisocoria and the pupil response amplitude in normals and patients with glaucoma

Contraction anisocoria (direct pupillary response > consensual response), section 4.4, is one of the most common potential confounders in the measurement of PLR. Although the effect is usually small (6.1% of the amplitude), this effect needs to be looked at when both the direct and the consensual responses are used for the estimation of RAPD. But if only the direct pupillary responses are used for the RAPD estimate the physiological anisocoria is more relevant than the contraction anisocoria. The discrepancy in PLR amplitude due to physiological anisocoria has been corrected by the proprietary formula. The pupillary constriction amplitude data from this thesis showed that the direct light response was significantly greater in amplitude than the consensual response at all stimulus intensities regardless of whether tested in normals or glaucoma patients. The scale of this difference was very small (0.01 to 0.05 mm or 2 to 4%), an amount which would not be visible clinically. The scale and the significance of this difference was the same for both 0.4lux and 4lux stimulus intensities but they were smaller when tested under 0.04 lux illuminations. This may be due to having much smaller amplitudes (0.05 to 0.6 mm) with lower intensity stimulus and it may be that the signal is being lost in the noise. It is interesting to find that both the scale of the left-right response difference and the direct-consensual repose difference are in the region of 2 to 5%.

In this thesis, both the data from the direct and consensual responses were combined in an optimal ratio to reduce the effect of contraction anisocoria as well as to maximise the diagnostic ability of the test.

Pupil size and pupillary response amplitude

In keeping with what is documented in the literature, the data from this thesis also supports that the pupil response amplitude has a positive relationship with the starting pupil size. The bigger pupils showed bigger responsiveness, figure 13.2. The effect of iris colour on the pupil dynamics were not considered in this study. It has previously

been shown that within subject measures such as RAPD estimation are not affected by difference in iris colouration.

The proportion of useable data from the study

The pupil acquisition video and the pupillograms of the P3000 were carefully checked by the author to identify any unusual traces, glitches and noise and they were manually removed. The proportion of all recordings useable for analysis was appraised. The average proportions for both groups were above 90% except for glaucoma patients tested with scotopic stimulus where the proportion of useable data was 86.8%. This effect was considered to be due to low-intensity related low signal-to-noise ratio being more prevalent in the pupil tracing of smaller pupils. The mean proportion for normal cohort was 92.6% and for glaucoma cohort was 90.6%. The differences are marginal. Although glaucoma patients have different age the effect of age as well as the negative effect of glaucoma itself on pupil traceability seem negligible.

Pupil asymmetry and visual asymmetry

Many authors have attempted to correlate visual field asymmetry and pupil asymmetry (section 3.2.8). This is because the perimetry is one of the primary investigations that are performed in the diagnosis and management of glaucoma. When a new test is to evolve in glaucoma investigation, it is tested against the gold standard which incorporates the perimetric findings. Perimetric features, however, do not always reflect glaucoma damage: histological studies have shown that up to 25-35% of ganglion cell loss can proceed before perimetric loss. There are also issues of repeatability and reliability with the test. The studies that tested perimetric pupil asymmetry reported only moderate correlation between the two.[150] This modest correlation is due to the fundamental differences in the anatomy and the nature of the tests for each entity. In keeping with the findings of the other authors, the correlation coefficient for the pupil asymmetry and the Humphrey mean deviation asymmetry, in this study was 0.6 (section 14.1). It may be that comparing perimetric test of the central 15 degree may have a closer relation with the pupil. But more work is still needed to address this proposition.

Sensitivity and specificity of detecting glaucoma

Potential use of pRAPD in the clinical practice

The clinical application of the pupillometer on glaucoma patients was tested in a method comparison study. This included glaucoma patients of all grades, pre-perimetric to perimetric and both unilateral and bilateral glaucoma patients. Ocular hypertension patients and patients with secondary glaucoma were, excluded. The gold standard for this comparison was the clinical diagnosis based on available tests in the clinic which included optic disc and nerve fibre layer assessment, perimetric assessment, IOP check, CCT measurement, and risk assessment. Testing against this gold standard, the area under ROC curve (AUC) was 0.81 (asymptotic 95% CI = 0.75-0.86) with the optimum sensitivity and specificity of 77% and 72%.

The AUCs for perimetric devices in diagnosing glaucoma, reported in the literature, are in the region of 0.6 to 0.9.[318] For the structural assessment instruments such as CSLO, GDX nerve fibre layer analysers and OCT, the associated sensitivities and specificities are in the range between 60% and 96%.[252]

Kalaboukhova and colleagues tested pupillometric RAPD in glaucoma patients against normals with a custom-built pupillometer. Their reported AUC, sensitivity and specificity were 0.92, 86.7% and 90%. Compared to the cohort of this this thesis, the sample in their study was smaller (30 glaucoma and 30 normals vs 117 glaucoma and 101 normals) and included patients with pseudoexfoliative glaucoma, and pigment glaucoma. The patients from this study were older (71 vs 65 years old) and had less disease asymmetry (ΔMD = 4.4 vs 6.3dB). When the AUC was tested initially on the first 58 patients in this thesis comparing to the first 58 subjects from the normal cohort, the AUC and the sensitivity and specificity were 0.92 (Asymptotic 95% CI = 0.89 - 0.97), 88% and 86.2%, (see appendix E) which are comparable to that of Kalaboukhova's report.[117] Both studies show that the pupillometric tests have high sensitivity and specificity in distinguishing glaucoma patients from normal subjects.

Other factors may contribute to the reduction in the AUC with a larger sample. It is possible that the structural (optic disc) and functional (visual field) and pupillomotor reflex (neuronal reflex) assessments are measuring different (but correlated) aspects or

manifestations of the disease which are not identical but together represent the same disease condition. The differences between these entities may be more pronounced when a larger sample size is assessed. Also, a larger sample would capture a wider spectrum of physiological variability of PLR in the normal cohort.

The majority of patients in this study have early glaucoma judged by the visual field and the DDLS parameters. The asymmetry in early disease can be small. Disease asymmetry, rather than disease severity, is expected to affect the diagnostic accuracy of the relative afferent pupil test. There is a tendency for the sensitivity and specificity of available glaucoma tests to fall off at the extremes of disease severity. The sample included more patients with early glaucoma than those with late glaucoma, and yet pRAPD test discriminated early disease from normal with high sensitivity and specificity. The balanced sensitivity and the specificity were 77% and 72%. If one needs to use this as a community based glaucoma case detection, the cutpoint value can be adjusted to maximise the specificity, at a cost of sensitivity (for example: specificity of 90%, sensitivity of 54% with cutpoint of 0.173 log units). For the hospital eye services the sensitivity of the test may be increased at the expense of its specificity (for example: sensitivity of 90% specificity of 40% with cutpoint 0.004 log units).

The association between visual and pupil asymmetry was moderate but significant between normal and glaucoma patients. There was no significant association between the visual asymmetry and the pupil asymmetry of the false negative patients (mean pRAPD ± SD, range = 0.02 ± 0.02, 0 to 0.06 log unit). False negative patients however had a smaller mean deviation asymmetry and smaller DDLS asymmetry compared to the true positive patients. Further investigation on pupillometric diagnostic capability of patient groups of different degree of asymmetry based on SAP mean deviation, it was found that the AUC was the largest for those with the MD asymmetry of > 10dB (AUC = 0.97), compared to those with MD asymmetry between 5 and ≤10 dB (AUC =0.85) and those with MD asymmetry ≤ 5dB (AUC = 0.77). The pupillometric diagnostic ability increases with more asymmetrical disease. This is not surprising since RAPD is a test of relative asymmetry. But having small inter-eye differences in the disease manifestations thus can limit the diagnostic ability of the comparative test because the normal subjects can also have inter-eye asymmetry (physiological noise). The overlap

between the features of normal and glaucoma is also evident in other assessments such as optic disc appearance and NFL thickness. The question is whether or not pRAPD would also be able to isolate subjects with less asymmetric glaucoma. The majority of patients (65%) in this study had MD asymmetry of only ≤ 5dB. The AUC for this group is 0.77 [95% CI of 0.7 to 0.8]. Reassuringly, this result is comparable to many other diagnostic tests that are employed in the clinical practice including some of the perimetric tests.

There was a question of age difference in normal and glaucoma cohorts in this study. Glaucoma is an age-related disease and most patients with glaucoma are over the age of 60 years. The definition of glaucoma in the last few decades has been evolved from a rather isolated damage of optic nerve due to relative raised in the intra ocular pressure to an entity of general systemic condition: multifoci degenerative disease. In this study, the mean age of the normals and the glaucomas were significantly different. This was due to limited number of older "healthy" volunteers available at the time of study. Could this confound the study results? The pupil size decreases with age (0.04 mm/year).[219] When the relationship between age and pupil response amplitude was tested, a negative relationship ($p < 0.001$) was noted (figure 13.3a). Also there was a negative relationship between age and starting pupil diameter (figure 13.3b). These two are likely to be related given the above results. When the normalised response amplitude was plotted against age (figure 13.4), age effect on pupil response no longer existed, confirming the fact that it is not age but the size of the starting pupil diameter before constriction that affects the pupil response amplitude. Furthermore, analysis on relationship of age on dispersion/ diurnal variability (chapter 12, appendix C) also confirmed the little relation that exists between age and pupil response variability. The pupil size (which is often found smaller in the older population) has effect on the pupil movement characteristics. In this study the constriction amplitude was used to estimate the RAPD. The study employed the proprietary formula in estimating the pRAPD. The formula does not normalise the results by the starting pupil size, yet the sensitivity and the specificity profiles are high. This may be because the test protocol for estimating RAPD in this thesis incorporates a range of intensities for an optimum pupillary response giving allowances for both larger and smaller pupils. Also It has been reported that the pupillary constriction amplitude and latency time are much less age-dependent.[319]

According to Loewenfeld (chapter 4) the age-related effect on amplitude of pupillary constriction can be lessened with short or weak light stimulus because they do not require much of a mechanical work.[183] It may be that the stimulus configurations employed in this thesis were short and the two of the stimulus intensities 0.04 lux and 0.4 lux were relatively weak stimuli compared to those employed by other observers. The most promising factor is that the relative afferent pupillary defect is a relative test and therefore, unlike an absolute test, it is less affected by the mean differences in the starting pupil diameter for the two cohorts.

If age was confounding the results of RAPD measurement, it is important to elucidate whether it is negatively or positively confounding the measured results and test sensitivity. The results showed that age was related to having smaller starting pupil diameter and this resulted in having smaller pupillary constriction. Therefore, the pupillary constriction in the elderly subject is expected to be small. If an elderly subject suffers from glaucoma, it is intuitive to think that the difference in the pupillary response amplitudes between the two eyes would be smaller compared to a younger glaucoma patient who has similar afferent pupillomotor deficit but much more efficient efferent motor system contracting larger amounts for the same stimulus. If this was the case, having an older glaucoma and younger control group would have a negative effect on the test's sensitivity and specificity profiles. Would the sensitivity and the specificity profile be better if the normal and diseased groups are better age-matched? Until, a future study is done on two samples of the same age groups on this pupillometer using the same algorithm it cannot be certain.

The sample in this thesis aimed to reflect the real life glaucoma cases, the study population was confined to the Wiltshire area in the United Kingdom where the study took place. Also, the study only looked at patients with POAG and omitted secondary glaucoma groups and other conditions that may be prevalent in the glaucoma patients such as dense cataract and diabetes. With the current protocol, the positive and negative predictive values will therefore only be high when this test is performed in selected populations such as normal and POAGs. The study only included subjects with pristine pupils and excluded those with local pathologies or those on medications that affect or have diseases that limit the pupil movement. Glaucoma patients were not asked to stop

their medications because it would not be ethical to do so. Only those who were on sympathomimetic medications were omitted due to their proven effect on pupil size. Patients on beta blockers (timolol) were allowed to participate since there is no evidence of effect on this medication on pupil size or amplitude of constriction.[303] If the test needs to be performed in other populations, further evaluations need to be carried out in the relevant cohorts. Also, a larger multicentre study will be required to evaluate primary and secondary glaucomas. This thesis evaluates the automated relative afferent pupillary test by the pupil constriction amplitude available on the pupillogram. For future studies, more parameters from the pupillogram such as latency and velocity can be optimised in addition to the pupil constriction amplitude. Incorporating more parameters for the diagnosis may improve the robustness of the test paradigm. This is because while some parameters are more affected by confounders others are less affected, and therefore use of more than one parameter has theoretical advantage (chapter 4 and 5). Whilst this thesis mainly concentrates on glaucoma subjects with optic neuropathy, there is potential use of pupillometer for other optic nerve diseases since a relative afferent pupillary defect is a hallmark of optic neuropathies. Future studies may test the pupillometry on subjects with non-glaucomatous optic neuropathies. Pupillometry may also be useful in making differential diagnosis in complicated cases.

The pupillometric test is not yet suitable to "screen" glaucoma *independently*, however, the results of this thesis show that it has an important role in glaucoma case finding because of its ease of use, providing objective, accurate and reproducible measurements, and providing a good level of test sensitivity and specificity. For practical use of the P3000, an observer/technician may be trained to recognise the noise in the record due to blinks and poor fits of the circle on the pupil image etc. and remove them. It is easy to execute the test and a very little cooperation is required from the patient. Testing for an RAPD can be carried out within 10 minutes by a trained observer. No patient recruited in this study had any issue with positioning the face on the face rest and focusing on the target light during the test. Also, 30 seconds of dark adaption gives the patient a break before the start of the next test. However, for patients with ptosis, taping of the eyelid was required to make the pupil area visible for the PupilFits to overlap the best fit circle on the pupil. The majority of the data that were

discarded were due to blinks or due to poor fitting because of low eye lids. One other observation was that some patients felt sleepy during the test since there was not much involvement from their part. It may be advisable to make conversation with the patient during 30 seconds rest time to keep them in a calm and awake state. The proportion of pupillograms discarded were less than 10% for all subjects.

Glaucoma case detection is traditionally difficult. This is because the specificity of diagnosis of glaucoma on the basis of pressure is low. To obtain a meaningful visual field is time-consuming and expensive, interpretations are difficult, and, as mentioned above, 30-40% of the neurons in the optic nerve are damaged before visual-field defect becomes detectible. Some elderly glaucoma patients are unable to undertake the visual field test accurately. Detection and diagnosing on the basis of optic disc appearance may be the most sensitive and specific method, but requires imaging equipment which are expensive and not freely available. Nonetheless, a glaucoma diagnosis may not be made by a single test alone especially in the very early stage. Although not every author may agree,[320] glaucoma is considered as a bilateral disease with asymmetrical damage. Thus the use of an automated machine to detect slight differences is a sensible option to be considered in detecting a large percentage of patients with glaucoma. The input from the structural analysis, visual function analysis, IOP measurement, risk factor assessment, as well as pupillomotor reflex assessment may all play a role to give complete picture of the disease status for a clinician to make a management plan.

In conclusion, the pupillometry performed by the P3000 with optimised stimulus and outcome parameters may make a feasible adjunct during glaucoma case detection either in the clinic or in the community. If this is to be used for glaucoma detection in the community pupillometry may be used along with other screening tools such as risk factor assessment and optic nerve photographs. In the clinic setting, the pupillometry can be one of the serial/parallel tests that the physician perform to aid his diagnosis or management of early glaucoma cases.

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VIII. APPENDICES

Appendix (A)

Evaluation and Calibration of a binocular infrared pupillometer for measuring relative afferent pupillary defect (chapter 11) – publication at Journal of Neuro-Ophthalmology 2012.

Appendix (B)

Z-score probability

This probability was calculated on the basis of finding some overlap between the distributions of response to the neutral density filters used to simulate the RAPD. In order to assess the probability that a given RAPD score belongs to a particular filter response population, the z-score probability can be used to assess the disease severity. Left-handed z-score gives the proportion of subjects from a normal population that lie below a given point, the right handed z score the proportion above the point. If the given pRAPD value is below the mean of the filter group then the left-hand probability is used. If it is above the right hand probability is used. The z score probability with any particular filter can be standardised as a proportion of 100. The calculated z score probability with a particular filter is divided by the sum of the calculated z score probabilities for all of the filters tested. The standardised Z scores are shown in the table below. By expressing the results in this way, the clinician can assess the probability of any pRAPD belonging to a population centred on each filter value, figure below. For example, if the estimated pRAPD is 0.1 log unit, from the Z probability chart it can be seen that there is a 47:47 chance of being normal or have a an early disease asymmetry.

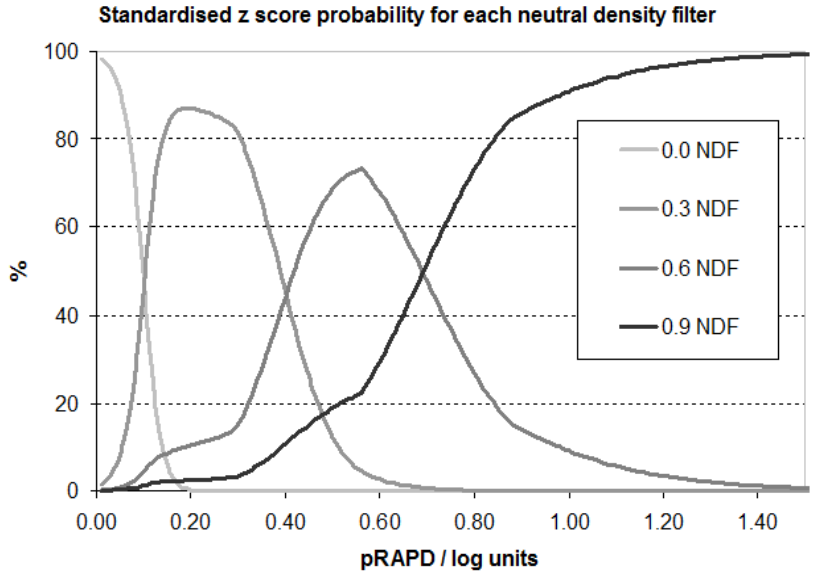


Figure. ***The standardised probability that the measured pRAPD comes from each of the four filter groups.***

Calibrated pRAPD reading	Z probability (%) in neutral density filter group				Total (%)
	0	0.3	0.6	0.9	
0.01	98.16	1.61	0.17	0.06	100
0.02	97.26	2.40	0.25	0.08	100
0.03	95.91	3.61	0.36	0.12	100
0.04	93.85	5.44	0.54	0.17	100
0.05	90.77	8.18	0.80	0.25	100
0.06	86.22	12.23	1.19	0.36	100
0.07	79.73	18.01	1.75	0.51	100
0.08	70.94	25.84	2.51	0.71	100
0.09	59.93	35.65	3.47	0.96	100
0.10	47.49	46.71	4.57	1.23	100
0.11	35.06	57.75	5.70	1.50	100
0.12	24.13	67.42	6.72	1.73	100
0.13	15.60	74.91	7.57	1.91	100
0.14	9.59	80.14	8.23	2.04	100
0.15	5.67	83.47	8.74	2.13	100
0.16	3.25	85.43	9.13	2.19	100
0.17	1.82	86.48	9.46	2.24	100
0.18	1.00	86.96	9.75	2.28	100
etc	*etc*	*etc*	*etc*	*etc*	

Table. The z probability that a calibrated pRAPD reading belongs to the population of pRAPD elicited by placing a particular neutral density filter (0, 0.3, 0.6 or 0.9 log units) in front of one eye chosen at random.

APPENDIX (C)

Dispersion study – analysis of association of age and mean on dispersion

The results of the age analysis showed that more than one factors could potentially confound the dispersion analysis. The age in the scatter plot showed no correlation to the amount of dispersion, however, the amount of mean may have some bearing on the dispersion or variance, figures 1 and 2.

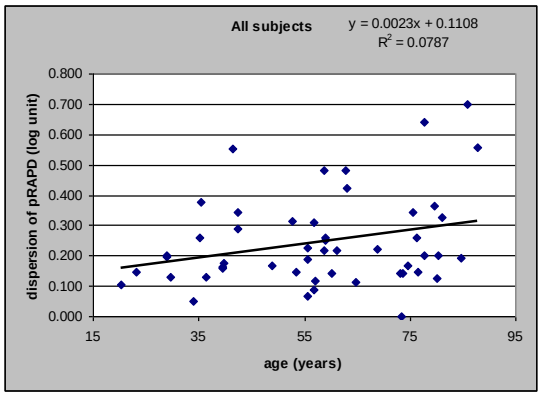


Figure 1. Scatter plot of dispersion of pRAPD vs age.

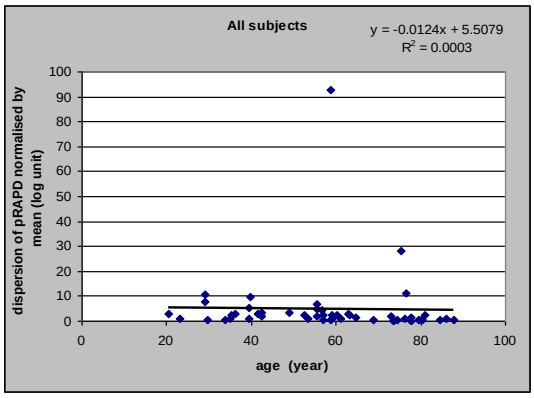


Figure 2. Scatter plot showing normalised dispersion of pRAPD by mean pRAPD plotted against age.

When scatter plot of variance vs mean was plotted there seemed to be a small amount of heteroscedasticity, figure 3. Heteroscedasticity if mild is not a problem for parametric conclusions but if large, the standard errors of the estimates can be biased. If the standard errors are biased we cannot use the usual t statistics or F statistics or LM statistics for drawing inferences.

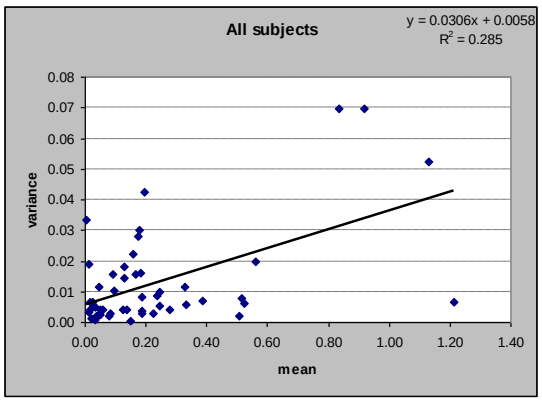


Figure 3. Scatter plot of variance (within subject dispersion) vs mean (amount of pRAPD) for all subjects.

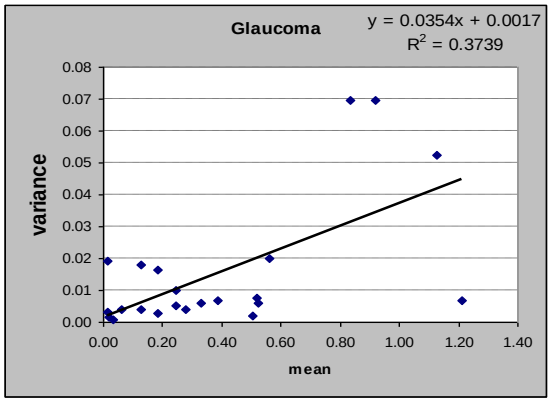


Figure 4. Scatter plot of variance (within subject dispersion) vs mean (amount of pRAPD) for glaucoma patients alone.

Is there an association between age or mean pRAPD with the degree of dispersion?

In order to quantify the possible association, multivariate analysis was performed to test the effects of predictor variables such as age and mean on outcome variable: dispersion of pRAPD. STATA 10 Data analysis and statistical software was used.

```
                Number of obs =      50

                W = Wilks' lambda      L = Lawley-Hotelling trace
                P = Pillai's trace     R = Roy's largest root

    Source |  Statistic     df   F(df1,    df2) =   F   Prob>F
-----------+-----------------------------------------------------
     Model | W   0.0120     46    46.0     3.0      5.37 0.0948 e
           | P   0.9880           46.0     3.0      5.37 0.0948 e
           | L  82.3185           46.0     3.0      5.37 0.0948 e
           | R  82.3185           46.0     3.0      5.37 0.0948 e
           |-----------------------------------------------------
  Residual |                3
-----------+-----------------------------------------------------
      mean | W   0.0163     28    28.0     3.0      6.47 0.0742 e
           | P   0.9837           28.0     3.0      6.47 0.0742 e
           | L  60.3503           28.0     3.0      6.47 0.0742 e
           | R  60.3503           28.0     3.0      6.47 0.0742 e
           |-----------------------------------------------------
       age | W   0.0542     18    18.0     3.0      2.91 0.2060 e
           | P   0.9458           18.0     3.0      2.91 0.2060 e
           | L  17.4528           18.0     3.0      2.91 0.2060 e
           | R  17.4528           18.0     3.0      2.91 0.2060 e
           |-----------------------------------------------------
  Residual |                3
-----------+-----------------------------------------------------
     Total |               49
-------------------------------------------------------------------
             e = exact, a = approximate, u = upper bound on F
```

Table 1. Multiple analysis of variance.

Table 1 describes the F-ratios and p-values for four multivariate criterion, including Wilks' lambda, Lawley-Hotelling trace, Pillai's trace, and Roy's largest root.

P values here for all the models is 0.0948, and is >0.05. This means to say that the coefficient of regression of dependent variable (dispersion) and independent variables (age and mean) was **not statistically significant** testing with multivariate criteria. We suspected that age had no significant effect on dispersion and this could explain the overall p value larger than the significant level.

The results of further testing of multivariate regression of individual variables are described in table 2. Tables 2a,b & c describe the results of multivariate regression of dispersion, mean and age. First part of

the table gives the number of observations, number of parameters, RMSE, R-squared, F-ratio, and p-value of the model concerned. The second part of the table contains the coefficients, their standard errors, test statistic (t), p-values, and 95% confidence interval, for each predictor variable in the model, grouped by outcome.

The "R-sq" value was 0.2177. This means to say that the predictor variables (age and mean) explained **21.8% of the variance in the outcome variable "dispersion"**.

The p value for mean was significant (0.003) but p value for age (0.888), table 2a, and was not statistically significant as predicted.

Further hypothesis testing, table 2 b and c, confirmed this conclusion. In table 2b, null hypothesis was that coefficient for mean in dispersion equation was 0. However, null hypothesis was rejected (p = 0.0033). Therefore, the overall effect of mean on dispersion is statistically significant. In table 2c, null hypothesis was that coefficient for age in dispersion equation was 0. Null hypothesis was accepted (p=0.888). There was no statistically significant overall effect of age on dispersion.

The results of this analysis estimated that change in 1 year of age was associated with 0.00017 log units change in the dispersion of pRAPD. This is hardly significant. On the other hand, change in 1 log unit of mean pRAPD was associated with change in 0.24 log units of dispersion.

Equation	Obs	Parms	RMSE	"R-sq"	F	P
dispersion	50	3	.1337565	0.2177	6.538016	0.0031

	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
dispersion						
mean	.2429019	.0784041	3.10	**0.003**	.0851732	.4006305
age	.0001677	.0011869	0.14	**0.888**	-.0022201	.0025554
_cons	.1810236	.0646514	2.80	0.007	.0509618	.3110854

Table 2a.

```
 [dispersion]mean       =  0

       F(1,47)          =  9.60
              Prob > F  =  0.0033
```

Table 2 b

```
 [dispersion]age        =  0

       F(1,47)          =  0.02
              Prob > F  =  0.8883
```

Table 2 c

Correction of heteroscedasticity and resultant association between dispersion and mean

When the form of the heteroscedasticity is clear it can be modeled. However, in most cases the source of heteroscedasticity is not well understood. In this case, the classic correction for heteroscedasticity is the HC0 estimator proposed by Huber (1967) [Huber, P.J. 1967. "The behavior of maximum likelihood estimates under non-standard conditions." *Proceeding of the Fifth Berkeley Symposium on Mathematical Statistics and Probability* 1: 221-233] and White (1980) [White, Halbert. 1980. "A heteroskedastic-consistent covariance matrix estimator and a direct test of heteroskedasticity." *Econometrica* 48:817-838]. But although this estimator is correct in large samples, it is no better than ordinary Least Squares (OLS) regression in small samples. MacKinnon and White (1985) discussed three improvements, HC1, HC2, and HC3.

Long and Ervin [Long, J.S and L.H. Ervin, 2000, "Using Heteroscedasticity Consistent Standard Errors in the Linear Regression Model." *The American Statistician* 54:217-224] suggested that HC3 correction is the best for small samples.

Tables 3a and 3b described the regression before and after the correction of heteroscedasticity. After HC3 correction (STATA 10 Data analysis and statistical software) change in mean pRAPD values was still associated with 1 log unit change in dispersion however the 95% confidence intervals were larger (-0.02 to 0.52 log units) and considered statistically insignificant (p = 0.07)

```
      Source |       SS       df       MS              Number of obs =      50
-------------+------------------------------           F(  1,    48) =   13.33
       Model |  .233583627     1  .233583627           Prob > F      =  0.0006
    Residual |  .841224346    48  .017525507           R-squared     =  0.2173
-------------+------------------------------           Adj R-squared =  0.2010
       Total |  1.07480797    49  .021934857           Root MSE      =  .13238

------------------------------------------------------------------------------
  dispersion |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        mean |   .2482386    .067996     3.65   0.001     .1115235    .3849538
       _cons |   .1894597   .0245082     7.73   0.000     .1401826    .2387367
```

Table 3a. Regression of dispersion and mean before HC3 correction.

```
                                                Number of obs =      50
                                                F(  1,    48) =    3.45
                                                Prob > F      =  0.0693
                                                R-squared     =  0.2173
                                                Root MSE      =  .13238

------------------------------------------------------------------------------
             |             Robust HC3
  dispersion |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        mean |   .2482386   .1336203     1.86   0.069    -.0204231    .5169004
       _cons |   .1894597   .0275008     6.89   0.000     .1341656    .2447537
```

Table 3b. Regression of dispersion and mean after HC3 correction.

Conclusions

It can be concluded that:

- Overall, 21.8% of the variance of dispersion was attributed by age and mean.
- Although our cohorts of control and glaucoma groups represented different age groups, there was no statistically significant association found between age and dispersion.
- Therefore, most of it is coming from the mean.
- As with most biological data, our measurement of dispersion of pRAPD had a small amount (R-sq = 0.28) of heteroscedasticity in relation to its mean. When this is corrected, there was no significant association found between change in amount of mean pRAPD and amount of dispersion.

Appendix (D)

Influence of time of day on pupillometric measurements of the relative afferent pupillary defect in normal subjects and glaucoma patients

Audrey Shwe-Tin, MRCOphth, Guy T Smith, BSc (Hons) FRCOphth, Fion Bremner, PhD FRCOphth, Ian E Murdoch, MSc MD FRCOphth

ABSTRACT

Background: There has been recent interest in using pupil tests to detect and monitor glaucoma. However the pupil may be affected by a number of influences that vary during the day. We investigate whether pupillometric measurements of the relative afferent pupillary defect (pRAPD) show diurnal variation.

Methods: A commercially available dynamic pupillometer (Procyon P3000) was used to measure the pRAPD in 28 healthy volunteers and 22 patients with glaucoma. These measurements were repeated every 2 hours between 9 am and 5 pm.

Results: The mean change in magnitude of pRAPD between any 2 measurements within the test period for normal and glaucoma cohorts were 0.09 and 0.1 log units respectively (p = 0.74). No consistent pattern of diurnal variation was evident in either cohort. The frequency percent of observing the highest estimates of pRAPD in either the morning or the afternoon clinics was 50:50 among healthy controls and 59:41 among patients with glaucoma. The frequency percent of observing the lowest estimates of pRAPD were also similar in morning and afternoon clinics (43:57 for healthy subjects, 50:50 for glaucoma patients).

Conclusion: The degree of variability of pRAPD across the working day is small and similar in glaucoma patients and healthy subjects. There is no evidence that time of day affects estimates of pRAPD in either cohort.

INTRODUCTION

Assessment of optic nerve function is an essential component in the evaluation of patients with glaucoma. Psychometric tests such as automated visual field analysis have variable reliability, particularly in elderly patients. There has been increasing interest recently in the possibility of using pupil measurements.[1-10]The *afferent* limb of the pupil light reflex (PLR) is conveyed in the optic nerve, hence glaucoma is likely to have a measurable effect on the amplitude of the PLR. As this reflex is not under voluntary control it is likely to provide an objective indicator of optic nerve function. There is, however, a wide variation in PLR amplitude in a healthy population. For this reason, the most commonly used pupil test in assessing glaucomatous optic neuropathy is the relative difference in PLR between the two eyes, also known as the relative afferent pupillary defect (RAPD). This can either be tested clinically by the swinging flash light test or using pupillometry.

Most pupillometry studies to date have been undertaken using custom-built pupillometers.[2-7;9;10] Commercial devices capable of making dynamic measurements of the pupillary response to light are now becoming available,[8;11] making pupil testing a practical proposition in routine clinical practice. Various stimulus protocols have been tried, including large-field illumination,[1;4;5;10] perimetric stimuli[2;6-9]and grating patterns.[3] In some studies the responses of the two eyes were compared,[1;4;5;11] relying on

asymmetry of optic nerve damage in early glaucoma. In others comparison is made between upper and lower field responses of the same eye[2;6;8]or between the responses to red and to blue light.[10] In screening for glaucoma,[2;4-8]these pupil tests are associated with good sensitivity and specificity (75-95%) and achieve areas under the receiver-operating characteristic (ROC) curve of not less than 85%.

One of the concerns about using the PLR to provide information about the optic nerve is that this brainstem reflex may be subject to many other influences, both central and peripheral, which could confound the measurements. There are many conditions affecting the *efferent* limb of the reflex (such as diabetes, Holmes-Adie syndrome or use of anti-muscarinic medications) which would preclude use of this test; it is also doubtful whether pupil testing is valid in some of the secondary glaucomas (e.g. pigmentary, pseudoexfoliative, neovascular) which may directly affect the iris. Less is known about *central* influences and in particular how these may vary during the day and affect measurements of the PLR. For example, it is known that level of arousal (which varies during the day) has a profound effect on the pupil: as subjects become sleepy their pupils miose and show low-frequency oscillations.[12] There have been a small number of studies investigating variation in pupil measurements at different times of day;[13-17]all of these studies looked only at young healthy subjects. There is poor agreement over their findings. Some studies found pupil size larger in the morning,[13-16] in others larger in the afternoon,[17] and ,where tested, the PLR amplitude was affected by time of day in some studies[14] but not others.[17]

If PLR is to be used in detection and monitoring of glaucoma patients it is important to establish whether time of day affects pupil measurements. This study assesses the variability of RAPD measurements over the course of a working day using a commercially available pupillometer. Two cohorts were studied, patients with glaucoma and unrelated healthy subjects.

METHODS

The study was approved by the National Research Ethics Service, UK; all subjects were aged 18 years or over and gave informed consent for the investigations performed.

Subjects

Glaucoma patients were recruited from the Great Western Hospital, Swindon, UK. The patients were included if they met standard diagnostic criteria for primary open angle glaucoma (characteristic morphometric changes of the optic disc in keeping with glaucoma[18] and corresponding visual field loss) and no other underlying optic nerve diseases that may contribute to these optic disc changes. Healthy controls were included if they had no past history of ophthalmic disease, trauma or surgery, normal corrected visual acuity (6/9 or better), normal intraocular pressure as measured by Goldmann applanation tonometry (8-21mmHg) and a normal slitlamp examination including fundoscopy. Healthy subjects with a family history of glaucoma were excluded. In both cohorts, potential participants were excluded if they had any other ophthalmic problems (e.g. amblyopia, strabismus, visually significant media opacities or retinal disease), neurological diseases, diabetes or psychiatric disorders, or if they were taking topical or systemic medications that could affect the pupil. Glaucoma patients continued their intra-ocular pressure lowering medications.

All participants were advised to have normal sleep/wake cycles before the study date (none were night workers or had just returned from different time zones). Participants refrained from taking coffee during the study period (9 am to 5 pm).

All subjects had their Snellen visual acuity recorded and their intraocular pressure measured with calibrated Goldmann applanation tonometer. The optic nerve rim-to-disc ratio was recorded using the Disc Damage Likelihood Scale (DDLS).[19]Automated threshold perimetry (Humphrey SITA 24-2 programme) was performed in the glaucoma patients.

Pupillometry and measurement of the RAPD

Pupil measurements were made using the Procyon P3000D^{TM} dynamic pupillometer (Procyon Ltd, UK). This device records both pupils simultaneously using standard infrared video techniques (spatial

resolution ± 0.05mm, frequency resolution 40ms), using a dim green target (<0.05°, <1mLux) at optical infinity to control fixation and a foam facemask to exclude ambient light. The light stimulus consisted of a uniform white square extending ±7.5° from fixation along the vertical and horizontal meridians presented for 0.4 seconds; the intensity could be adjusted to one of three settings (0.04, 0.4 and 4.0 Lux). Recordings lasted 28 seconds during which a train of 14 light stimuli were presented, alternating between the right and left eyes at 1.6 second intervals. The relative difference in responses following stimulation of right and left eyes (i.e. the 'pupillometric' RAPD or pRAPD) was defined in log units using a proprietary algorithm (Procyon Ltd, UK).

All experiments were conducted in the same quiet room. Subjects were dark-adapted for 30 seconds before each recording, then two acquisitions were carried out at three stimulus intensities (0.04, 0.4 and 4 Lux). The mean results of the two were used to calculate pRAPD. These tests were repeated at five different times during the clinic hours, namely 9 AM, 11 AM, 1 PM, 3 PM and 5 PM.

Statistics

Normal distribution was ascertained prior to parametric testing. Unpaired Student t-test was used to assess the significance of any difference between mean pRAPD in the two cohorts (glaucoma vs. healthy controls). The dispersion (within-subject measurement variability) across the working day for each subject was defined as the coefficient of variability (CV = standard deviation (SD)/mean) of the estimates over the five repeated measurements between 9 AM and 5 PM. The percentage of frequency of zenith (highest) and nadir (lowest) pRAPD measurements were calculated for each test hour, and for the morning (9 AM and 11 AM testing) and the afternoon (1 PM, 3 PM and 5 PM) testing. A simple linear regression and Pearson's correlation was used to regress the relation of age or gender to the pRAPD estimates. A multi-variant analysis model was constructed to estimate effect of age or mean pRAPD on the dispersion using STATA 10 data analysis software (Stata Corp LP).[20]

RESULTS

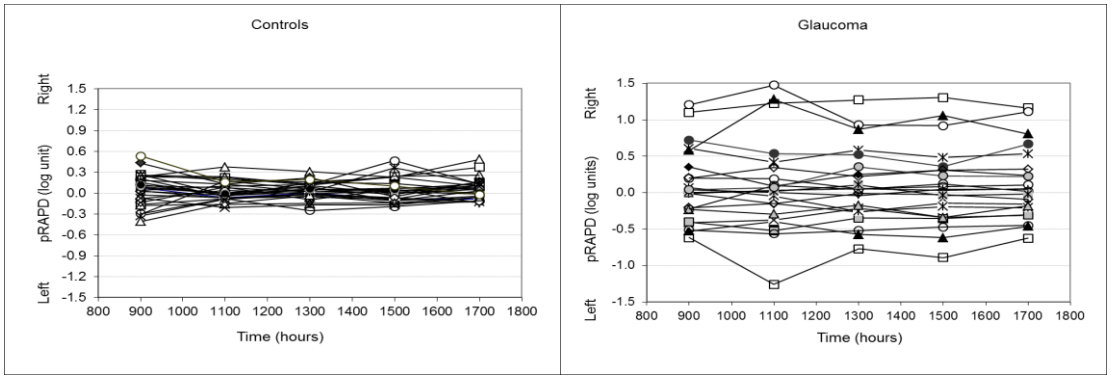
In total, 28 healthy subjects were recruited (20 female) with mean age 46 years (range 20 to 73); estimates of their pRAPD ranged from 0 to 0.53 log units (mean 0.11, SD 0.11). In the glaucoma cohort 22 patients were recruited (9 female) with mean age 72 years (range 53 to 88); estimates of their pRAPD ranged from 0 to 1.47 log units (mean 0.38, SD 0.36). The difference in pRAPD measurements between the two groups was statistically significant (p = 0.001).

Influence of disease status on dispersion of pRAPD measurements

The mean within-subject measurement dispersion, CV, for normal subjects was 2.49 (SD 6.51, range 0.13 to 35.26) and for glaucoma patients was 1.10 (SD 2.39, range 0.07 to 11.23), a difference that did not reach statistical significance (p = 0.36).

Influence of time of day on pRAPD measurements

The temporal curves of pRAPD estimates for each subject across the five time points in the day are shown in figure 1. For both healthy controls and glaucoma patients some variation is seen in the pRAPD estimates; on average, pRAPD measurements varied by ± 0.09 log units (95% CI = 0.34) in normal subjects and ± 0.11 log units (95% CI = 0.22) in glaucoma subjects across the working day. In general, no consistent pattern emerges to suggest a diurnal influence on this measurement (figures 1A and 1B). This impression was tested by calculating the frequency with which the highest (zenith) and lowest (nadir) pRAPD estimates were observed in either the morning clinic (9 AM& 11 AM time points) or the afternoon clinic (1 PM, 3 PM & 5 PM time points), table 1. The data confirm that the chance of measuring a high or low estimate of pRAPD is similar in morning and afternoon clinics.



Figures 1A and 1B. Dispersion of pupillometric relative afferent pupillary defect measurements across the working day for normal subjects (1A) and glaucoma patients (1B).

Clinics	Zenith		Nadir	
	Glaucoma	Control	Glaucoma	Control
AM clinic (9 AM to 11 AM)	59%	50%	50%	43%
PM clinic (1 PM to 5 PM)	41%	50%	50%	57%

Table 1.The chances of observing a high (zenith) or a low (nadir) pRAPD measurement in the morning or the afternoon clinics.

Influence of Age & Gender on dispersion of pRAPD measurements

Age: A multi-variant analysis model was constructed investigating the effect of age and mean RAPD on measurement variability. Increase in age by 1 year was associated with 0.00017 log units increased variability in pRAPD. A change of 0.1 unit in the mean pRAPD was associated with 0.024 log units increase in variability of pRAPD ($p<0.001$).

Gender: although the sample sizes are small, no significant gender effect on pRAPD variability was apparent in either cohort: among normal subjects, $R^2 = 0.01$, $p = 0.64$; among glaucoma patients, $R^2 = 0.09$, $p = 0.17$; for all subjects, $R^2 = 0.01$, $p = 0.44$.

DISCUSSION

Patients who have or are suspected of having glaucoma may be booked for evaluation at any time within the working day, and so it is important to know whether time of day affects our clinical measurements. For instance, it is well known that measurements of intraocular pressure vary significantly across the day, with the highest levels usually being recorded in the morning.[21]

The pupil is subject to several central influences, including state of arousal, fatigue and autonomic equilibrium,[17] all of which may affect pupil size and reactivity to light at different times of day. As a result, it is well known that pupil size fluctuates constantly even in a dark quiet room,[16]and when the eye is repeatedly stimulated with the same light stimulus the dynamic responses of the pupil can be seen to vary from one stimulus to another.[22;23] Since pRAPD estimates are a comparative metric of the difference in PLR amplitudes between the right and left eyes, on theoretical grounds we might expect this metric to be less sensitive to central influences as long as these are distributed equally across both eyes. However, this is not necessarily the case; for example, a previous study showed that repeated anisocoria measurements in healthy subjects show significant variation day to day suggesting that these central influences on the pupil are not always symmetrically distributed to the two eyes.[24] Moreover, the gain of the PLR is also asymmetric even in healthy individuals, giving rise to a small degree of normal variation in pupillary response between eyes. The magnitude of this variation is typically small and not clinically apparent.[25] Various hypotheses have been put forward to account for it including small differences in the number of ganglion cells, neuronal sensitivity, retinal adaptation, afferent pathway anatomy, higher centre input and efferent innervation of the pupillary muscles.[23;25] Just as with

anisocoria, repeated measurements of the difference in pupillary response between eyes also show variation; Kawasaki and her colleagues estimated the immediate short-term fluctuation of pRAPD to be approximately 0.1 log units in normal subjects.[22]The same authors also studied the long-term variability of pRAPD (4 measurements over 3 years) and estimated median change in pRAPD between any 2 sessions to be < 0.08 log units.[23]To our knowledge there is no study which has formally assessed if the time of day influences RAPD, either in normal healthy subjects or in patients with glaucoma.

This study was designed as a hospital-based study in order for it to be generally applicable to clinical use. Measurements were recorded using a simple, commercially available pupillometer in a separate quiet room, similar to those used routinely for testing visual fields. No attempt was made to synchronise the pupil measurements to equivalent times within the circadian rhythm of different study participants because in practice patients attending eye clinics will not be synchronised with respect to their day/night cycling. Apart from ensuring no recent travel and asking for a good night's rest prior to the examination, no attempt was made to record or control the alertness/sleepiness of the study participants, their sleep habits, the number of hours that they had slept or the amount of caffeine that they had ingested prior to the study period. Only the coffee intake during the test hours was restricted. We deliberately chose to dark-adapt subjects for only 30 seconds before each data acquisition because otherwise the total time to perform the test would become impractical for routine patient work-up as well as allowing other confounders such as alertness to have an influence within each test.

For similar reasons, in this study patients were asked to continue taking their regular glaucoma medications. All glaucoma patients took one or various combinations of prostaglandin analogues (latanoprost, bimatoprost), beta-blocker (timolol maleate), and carbonic anhydrase inhibitors (brinzolamide, dorzolamide). Only 2/22 were unilaterally treated with latanoprost, the rest were bilaterally treated with the same set of medication(s). None were using alpha adrenergic agonists or parasympathomimetics. Although some animal studies have suggested an influence of beta blockers and prostaglandin analogues on pupil size,[26;27]there is no reported effect of these on the amplitude of the PLR in man documented in the literature. Johnson tested human subjects with timolol 0.5% ophthalmic solution and measured PLR parameters using a pupillometer; he found no effect on pupil size or constriction amplitude.[28]Although we cannot rule out the possibility that glaucoma medications may have had an influence on the pupil measurements in this study, it is likely to have been symmetric between the two eyes because in almost all cases the treatment was bilateral, and so this may not have had much influence on our RAPD estimates. Only 2 patients were unilaterally treated, but the medication used (latanoprost) has no measurable effect on the PLR amplitude in man.[29] There was no notable difference of their diurnal variability compared to those of other subjects.

There is a significant age difference between the normal and glaucoma cohorts which makes age a potential confounding factor. However, the regression analyses showed there was only 0.00017 log units of change in pRAPD for each year of life, and there was no significant relationship between age and the variability of these pRAPD measurements.

With regards to the pupillometric test paradigms, a number of factors can induce measurement variability – for example a higher variability of pRAPD estimates is associated with using fewer stimulus pairs (gives a smaller sample to average),[22] and a lower stimulus intensity[22;30;31](gives a poorer 'signal to noise' ratio).The instrument used in this study had already been calibrated and the stimulus parameters optimised for the most accurate and repeatable measurement of the PLR.[11] Prior to the study, the repeatability of pRAPD with the same test protocol was evaluated separately on a cohort of 11 normal subjects and 12 glaucoma patients; the immediate test-retest variability was low, with the second measurement varying from the first on average by only 0.09 log units in normal subjects and 0.08 log units in patients with glaucoma ($p = 0.9$). The time-of-day variability between any 2 sessions in this study for the normal and glaucoma groups were 0.09 and 0.11 log units respectively. The immediate repeatability is of a similar magnitude to the time-of-day variability implying negligible diurnal variation. Although the test paradigm of this study is different from that of Kawasaki (1996)[23] and the temporal factors are different (immediate vs 4 year), a similar amount of test-retest variability (0.08 log units) was noted in their report.

As noted by other groups, [1;4;5] the estimates of pRAPD in this study were much higher in the glaucoma patients than in the normal controls (mean pRAPD = 0.38 vs 0.11 log units respectively, p =0.001). This observation confirms the asymmetric nature of optic nerve damage commonly found in this disease.

Despite there being a clear difference in pRAPD estimates between glaucoma patients and normal subjects, there was only a small difference noted in the variability of these pRAPD estimates across the working day. In fact, if we take into account the heteroscedasticity in the data (i.e. the increase in measurement variance expected for larger pRAPD estimates: from our regression model we estimate this effect to be equivalent to an increase of 0.024 log units variance in pRAPD measurements for every 0.1 log unit increase in mean pRAPD), the variability estimates (CV) adjusted with respect to these different means were greater in the normal controls (2.49) than in the glaucoma patients (1.10), although this difference did not achieve statistical significance (p=0.36). In addition, among the glaucoma patients there was no correlation between the variability of the pRAPD measurements and the degree of asymmetry in disc appearances (DDLS; R^2 = 0.015, P = 0.58), visual field loss (mean deviation; R^2 = 0.027, P = 0.47), overall disease severity (as evidenced by the worse DDLS score: R^2 = 0.01, P = 0.65; or by the worse mean deviation value: R2 = 0.06, P = 0.30). This is contrast to other studies which have shown test-retest variability to increase with defect severity[33]. It may be that pRAPD measurements, based on an involuntary brain stem reflex, are more robust than psychophysical measurements of visual threshold. Another reason may be that the light stimulus employed in pupillometry covers a wide central area and stimulates a much larger number of retinal ganglion cells, thus increasing the signal-to-noise ratio.

In conclusion, the results from this study show that the within-subject variability of pRAPD measurements across the working day is small in both glaucoma and normal subjects. Furthermore the pattern of the time-of-day variability shows an equal chance of having a higher or lower pRAPD in the morning compared with the afternoon in both normal and glaucoma cohorts. This suggests there is no influence of time of day on pRAPD estimates between 9AM and 5PM. We cannot exclude the possibility that there are significant fluctuations of pRAPD occurring in the evening or at night time however diurnal variation outside working hours has less practical relevance in routine clinical practice. It is therefore valid to compare serial pRAPD measurements made at different times in the working day.

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Appendix (E)

Poster presented at the Association for Research in Vison and Ophthalmology (ARVO) 2009 annual conference at Fort Lauderdale, Florida.

Title: Binocular infrared pupillometry for detecting relative afferent pupillary defect in patients with primary open angle glaucoma.

Binocular infrared pupillometry for detecting relative afferent pupillary defect in patients with primary open angle glaucoma

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UCL

Introduction and Objective

Relative afferent pupillary defect (RAPD) is one of the most important clinical signs of optic nerve diseases such as glaucoma. The traditional swinging flash light method of testing RAPD requires considerable skill from the examiner to elicit reliably. Pupillometers are known to be able to measure RAPD more objectively. This study evaluates the diagnostic accuracy of a commercially available binocular digital infrared pupillometer (Procyon P3000) in diagnosing primary open angle glaucoma (POAG) based on measuring RAPD.

Methods

The pupillometer, figure 1(a), elicits the swinging flash light test. Each eye is illuminated for 0.4 seconds followed by 1.6 seconds of darkness. This sequence is repeated seven times. The different amplitudes of each pupillary response for direct and consensual light responses are used to calculate RAPDs: $RAPD_{DIR}$ and $RAPD_{CONS}$, figure 1(b). Final result, $RAPD_{FINAL}$, is the linear combination of the two RAPDs:

$$RAPD_{FINAL} = \alpha\, RAPD_{DIR} + (1-\alpha)\, RAPD_{CONS}$$

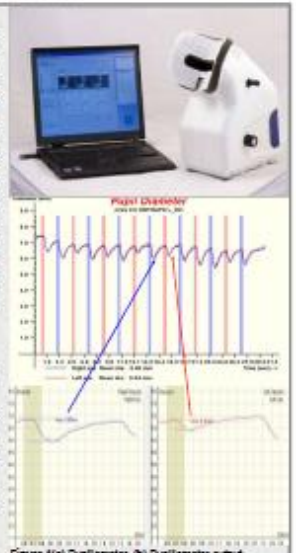

Figure 1(a) Pupillometer, (b) Pupillometer output

The study was approved by the National Research Ethics Service, UK. Patients with POAG were recruited from the glaucoma clinic, and the healthy volunteers from the staff and their friends.

Exclusion criteria

- Secondary glaucoma, visually significant media opacity, amblyopia (VA worse than Snellen 6/9), retinal disease, other optic nerve disease, diabetes, conditions or treatments that affect pupil mobility.

Inclusion criteria

- Unilateral or bilateral POAG, regardless of disease asymmetry
- Healthy volunteers

Results

Demographics

Normal group: n=58 (♀=39) mean age ± SD (range) = 48 ± 16 (16-80)
POAG group: n=58 (♀=38) mean age ± SD (range) = 74 ± 9 (56-91)

RAPD measurements

The optimisation of α and the cutoff are determined from a series of Receiver Operative Characteristic (ROC) curves each with α ranging from 0 to 1 in steps of 0.1. The ROC curve with the maximum Area Under the Curve (AUC), figure 2, is obtained when α is 0.6. The AUC is 0.936 (asymptotic 95% CI = 0.896 to 0.976). Sensitivity and specificity using the optimum cutoff of 0.064 dB are:

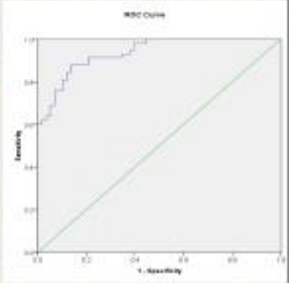

Figure 2. Receiver Operator Characteristic Curve

Sensitivity = 87.9% (Binomial 95% CI = 76.7 - 95.0)
Specificity = 86.2% (Binomial 95%CI = 74.6 - 93.9)

The generalisability of the test is further evaluated by running separate training (reference) models and test models using 50% of the sample for training and 50% for the test model, which is repeated 10 times with different combinations of patients and controls.

The optimum values of α and the cutoff learned from the training and test models are studied for their dispersion (maximum, minimum, median). Similarly, the dispersion of the sensitivity and specificity results of the 10 test models are calculated, table 1.

Dispersion	α	Cutoff	AUC	Sens (%)	Spec (%)
Median	0.55	0.054	0.94	89.7	89.7
Maximum	0.80	0.085	0.96	93.1	93.1
Minimum	0.50	0.033	0.91	82.8	65.5

Table 1: Dispersion and central values (Sens = sensitivity, spec = specificity)

Discussion

The sensitivity and the specificity are better than other commercially available methods of optic nerve function assessment when used in isolation: standard automatic perimetry, 52-68% and 80%; frequency doubling technology perimetry 71-74 % and 80%; short-wavelength automatic perimetry, 42-48% and 80%; high-pass resolution perimetry, 39-65% and 80%.

We reviewed the false positive and false negative cases to assess if any characteristics were associated with incorrect test results. No major differences were found in the mean age ± SD and range of false positives (45 ± 16, 22 to 66) from that of true negatives (47 ± 16, 16 to 79); and false negatives (76 ± 9, 63 to 87) from that of true positives (74 ± 9, 56 to 91).

The RAPD is a test of relative difference in the afferent pathway performance. Asymmetry of disease between eyes was estimated using the Disc Damage Likelihood Scale (DDLS) and visual field Mean Deviation (MD). In both instances a lack of asymmetry was associated with false negative test results. All of the false negative results occurred when the difference in DDLS between the eyes was ≤ 1. Similarly, all false negative results occurred when the MD differences between the eyes was ≤ 3.8dB, table 2.

Δ DDLS	Total	TP	FN	FN%
0	14	10	4	29%
1	29	26	3	10%
2	10	10	0	0%
Δ MD (dB)	**Total**	**TP**	**FN**	**FN%**
Δ MD<1.5	17	14	3	18%
1.5 ≤Δ MD<4	17	13	4	24%
4 ≤ Δ MD <8	16	16	0	0%

Table 2. Δ DDLS, Δ MD, and their false negative rates

Conclusions

- **The RAPD measured by the pupillometer differentiated subjects with POAG from the normal subjects with high sensitivity and specificity.**
- **The method is objective, and quickly and easily administered.**
- **False negative may occur if the DDLS ≤ 1 or ΔMD ≤ 3.8 dB.**

Financial disclosure: Dr Shwe - Tin receives part of her research funding from Procyon Instruments Ltd, UK. Dr Dan Taylor is a research and development director of Procyon Instruments Ltd, UK.

Appendix (F)

Binocular infrared pupillometry in detecting relative afferent pupillary defect in glaucoma patients: sensitivity and specificity.

Audrey Shwe-Tin, MRCOphth, Guy T Smith, BSc (Hons) FRCOphth, Daniel Taylor, BSc (Hons) PhD, Ian E Murdoch, MSc MD FRCOphth

ABSTRACT

Purpose: To determine the diagnostic accuracy of a commercially available pupillometer (Procyon P3000) in differentiating patients with primary open-angle glaucoma from healthy subjects based on pupillommetric relative afferent pupillary defect (pRAPD).

Materials and Methods: A method comparison study was conducted to compare the diagnostic accuracy of the pupillometry against clinical diagnosis. Fifty eight controls and 58 primary open angle glaucoma patients were enrolled. The recorded pupillary responses were used to calculate the pRAPD. The clinical diagnosis and the pRAPD results were combined to generate a Receiver Operating Characteristic curve.

Results: The sensitivity, the specificity and the Area Under the ROC Curve were 87.9% (Binomial 95% confidence interval (CI): 76.7% – 95.0%), 86.2% (Binomial 95% CI: 74.6% – 93.9%), and 0.92 (Asymptotic 95% CI = 0.87 - 0.97). All false negative patients had difference in Disc Damage Likelihood Scale of ≤ 1 and visual field mean deviation of ≤ 3.8 dB between the two eyes.

Conclusions: The pupillometer can distinguish patients with POAG from normal subjects with high sensitivity and specificity based on pRAPD. The method is objective, and quickly and easily administered.

INTRODUCTION

Glaucoma is a leading cause of preventable blindness in the United Kingdom[1] and elsewhere.[2] It is estimated that there will be 79.6 million people with glaucoma in 2020; and of these, 74% will have open angle glaucoma.[2] Glaucoma damage to the retinal nerve fibre layer gives rise to a permanent visual field defect. Perceptible loss of field suggests that extensive and irreversible field loss has already occurred in both eyes. Early detection of the disease, therefore, may prevent significant morbidity. Nonetheless, definitive diagnosis of early glaucoma at a single visit is often unattainable.[3]

There has been much interest in the detection of a relative afferent pupillary defect (RAPD) in the diagnosis of glaucoma[4-8] because it is an important parameter in quantifying the loss of neuronal function, and glaucoma almost always involve asymmetrical damage of optic nerve. Unlike perimetry it offers an objective, rather than subjective, measure of optic nerve function. This may prove advantageous to some patient groups such as those with very poor vision or the very elderly patients who are less likely to produce a reliable perimetric result.

RAPD is present when the pupil reaction to light is asymmetrical between the eyes. In the absence of other causes, the eye with a smaller reaction to light has reduced afferent neural signals compared with the fellow eye due to a lesion somewhere along the neural pathway from photoreceptors to the pretectal region of the midbrain. Whereas visual field defects using Statpac1 and 2 Humphrey Analysis may require 25-30% loss in axonal activity[9], and Goldmann perimetry may remain normal with 40% axon loss[10], only 13% difference in axonal input to the pretectal nucleus has been found to be sufficient to produce a RAPD[6]. There have been cases reported where RAPD is elicited despite normal 30-1 Humphrey automated perimetry[11], and a normal Goldmann kinetic visual field test.[12,13]

The association of RAPD with retinal nerve fibre layer (RNFL) thickness loss[8,14] and its potential presence before a measurable field loss renders it a powerful tool in the early diagnosis of glaucoma. However, The traditional method of measuring RAPD with the swinging flash light test[4] is highly dependent on the skill of the examiner and may give rise to misinterpretation[7,15,16]. Using the swinging flash light test, Tatsumi and colleagues[8] found that a 0.6 log unit neutral density filter (NDF) eliminated the RAPD caused by a 27% RNFL loss. This is stated to be the minimum resolution of the

swinging flash light test for measuring the RAPD in their study. Because the clinical swinging flash light test only detects up to the smallest RAPD of 0.3 or 0.6 log units, there have been a great interest in detecting the RAPD far more accurately using automated devices such as pupillometers. Digital infrared pupillometry uses pupillographic parameters (*e.g.* amplitudes of pupillary constriction, latency, or velocity of constriction) to estimate the RAPD. Pupillometric RAPD (pRAPD) measurement is objective, accurate and more sensitive than the swinging flash light test[17-19]. Different types of infrared pupillometers have now been used to detect RAPD in glaucoma and afferent pathway lesions.[15,16,18,20,21] However, these machines are mainly the preserve of research-oriented hospitals and universities.

To pursue our interest in looking at pRAPD in glaucoma subjects, we used a commercially available pupillometer, Procyon P3000D standard dynamic pupillometer (Procyon P3000 D^{TM}, Procyon Instruments Ltd, UK). We asked Procyon if they would develop specialised software to measure pRAPD from the pupillographs the instrument produces. They developed a new method which they claimed would essentially give better sensitivity than standard methods, and we set out to examine whether this was correct. This paper describes the sensitivity and specificity with which the Procyon pupillometer and their algorithm of measuring pRAPD is able to differentiate a group of healthy subjects from patients with primary open angle glaucoma (POAG).

MATERIALS AND METHODS

The study was approved by the National Research Ethics Service, UK. Subjects were recruited from the Great Western Hospital, Swindon. Healthy volunteers were not relatives of the glaucoma patients. All subjects gave informed consent to the investigations performed. Glaucoma patients with unilateral or bilateral POAG were included.

Subjects were excluded if they had secondary glaucoma including pigment dispersion syndrome or pseudoexfoliation syndrome; visually significant media opacity (cloudy cornea, dense cataract); amblyopia (VA worse than 6/9); manifest squint; retinal or optic nerve disease (including anomalous optic discs) other than glaucoma which might contribute to producing a RAPD; diabetes; previous ocular inflammation or trauma. Patients were also excluded if they were taking ocular or systemic medications known to influence pupil movement, or suffer conditions that affect pupil motility (posterior synechiae, iris atrophy, Adies tonic pupils, and peripheral iridotomy). Patients who had uneventful phacoemulsification and intraocular lens implant were included if the surgery was carried out more than 3 months prior to the study with no history or evidence of iris trauma. Healthy volunteers were defined as having no eye disease, trauma or surgery; best spectacle corrected Snellen visual acuity of 6/9 or better; and normal optic nerve appearance.

A full medical history was taken. The Snellen's visual acuity and the Goldmann applanation tonometry recorded. A full dilated fundus examination was performed by a single ophthalmologist (GTS). The Disc Damage Likelihood Scale (DDLS)[22] was recorded using rim to disc ratio measured by a 90D lens with a projected graticule. The disc scores range from 1 to 10, 1 being the indicator of the least change (rim:disc > 0.4) and 10 represents more than 270º rim loss (rim:disc = 0). The glaucoma patients underwent field testing with Humphrey Sita fast field analyser (program 24-2), standard automated perimetry (SAP). The diagnosis of glaucoma was a clinical diagnosis based on all available information. For all early cases the clinical records were re-reviewed independently by another glaucoma expert (IEM). Any patients for whom the diagnosis was in doubt were excluded from the study.

Computerised pupillometric assessment was performed by an independent operator (AST) who was masked to the disease status of the subject. The details of the commercially available Procycon P3000D digital infrared pupillometer are described elsewhere.[23] Briefly, before any pupillometric data is acquired, the subject is asked to look at a small, dimly-lit target (size <0.5^{o}, illuminance <1 mlux) at an optical infinity during which the eyes, which are separated by the light channels, are dark adapted for a period of 30 seconds. Following this is an acquisition period of 28 seconds, in which 800 frames are recorded at a rate of 25 frames per second. Each acquisition includes stimulation of each eye alternately with a 15^{o} square stimulus, set at the virtual distance of more than 10 metres, for 0.4 seconds with 1.6 seconds of darkness in between. This sequence is repeated seven times. A total of two acquisitions at three intensity levels: scotopic (0.04 lux), low mesopic (0.4 lux) and high mesopic (4 lux) are performed. The pupillary responses are recorded and any blink artefacts eliminated.

The proprietary algorithm was used to estimate pupillommetric RAPD (pRAPD) from the direct light and consensual light responses and is displayed in log units. The sensitivity and the specificity in differentiating glaucoma patients form normal subjects were assessed using the Receiver Operative Characteristic (ROC) curves and the optimum percentage values were defined with binomial 95% confidence interval (CI). Disease asymmetry between the fellow eyes was assessed using visual field mean deviation (functional assessment) and the DDLS (structural assessment). The association of the amount of asymmetry and the false negative rates were examined.

RESULTS

A total of 116 subjects, 58 controls (39 female, 19 male) and 58 glaucoma patients (38 female, 20 male) were recruited. The mean age (SD, range) of the controls and glaucoma patients were 48 (8, 16 – 80) years and 74 (5, 56 - 91) years.

The majority of our patients have early glaucoma. In 76% (45/58) of patients the visual field mean deviation (MD) was <10 dB, and in 74% (43/58) the DDLS score was ≤ 5 (rim/disc ratio ≥ 0.01) in their worse eye. The mean MD ± standard deviation (SD) was −7.8 ± 6.7 dB in the worse eyes and −3.1 ± 4.6 dB in the better eyes. The mean DDLS ± SD was 5.0 ± 1.5 in the worse eyes and 3.7 ± 1.7 in the better eyes. Details of DDLS and MD of the fellow eyes are described in figures, 1 and 2.

The Area Under the receiver operative characteristic Curve (AUC) was 0.92 (asymptotic 95% CI = 0.87 - 0.97). Using the optimum cut-off of 0.06 log units, the sensitivity of the test was 87.9% (binomial 95% CI: 76.7% – 95.0%) and the specificity 86.2% (binomial 95% CI: 74.6% – 93.9%), table 1. 51/58 glaucoma patients had a pRAPD above the threshold and 50/58 controls had no significant pRAPD. The range of pRAPD found in the controls is from 0 to 0.21 log units. The mean pRAPD ± SD (range) of false positive subjects (8/58) was 0.13 ± 0.05 (0.08 to 0.21) log units.

Glaucoma diagnostic tests	AUC (95% CI)	Sensitivity (%)	Specificity (%)
Pupillometric RAPD by Procyon P3000	0.92 (0.87 - 0.97)	87.9	86.2
Pupillometric RAPD by a custom-built pupillometer[†]	0.92 (0.84-0.99)	86.7	90.0
Pupillometric RAPD by Procyon P3000	0.92 (0.87 - 0.97)	91.4	80
Pupillometric RAPD by a custom-built pupillometer[‡]	0.92 (0.84-0.99)	86.7	80
Standard Automated Perimetry[∫]	0.60 – 0.71	39 - 68	80
Short- Wavelength Automated Perimetry[∫]	0.60 – 0.73	42 - 48	80
Frequency Doubling Technology Perimetry[∫]	0.74 – 0.80	71 - 84	80
High Pass Resolution Perimetry[∫]	0.58 – 0.67	23 - 65	80

Table 1. Estimates of area under the curve of each of the main methods of assessing optic nerve function for patients with glaucoma are shown. The sensitivity and specificity of pRAPD for distinguishing glaucoma patients from normal subjects are shown in the upper half of the table. In the lower half of the table the specificity is fixed at 80% and the sensitivity estimated for each method. ([†] see reference 22, [‡] personal correspondence from L Kalaboukhova, [∫] see reference 24)

False negative patients (mean pRAPD ± SD, range = 0.01 ± 0.02, 0 to 0.04 log units) had small asymmetry of disc damage (ΔDDLS ≤ 1) and visual field mean deviation (ΔMD ≤ 3.8 dB), figures1 and 2. Four false negative patients had no DDLS difference between the eyes and the rest, n=3, had DDLS difference of only 1.

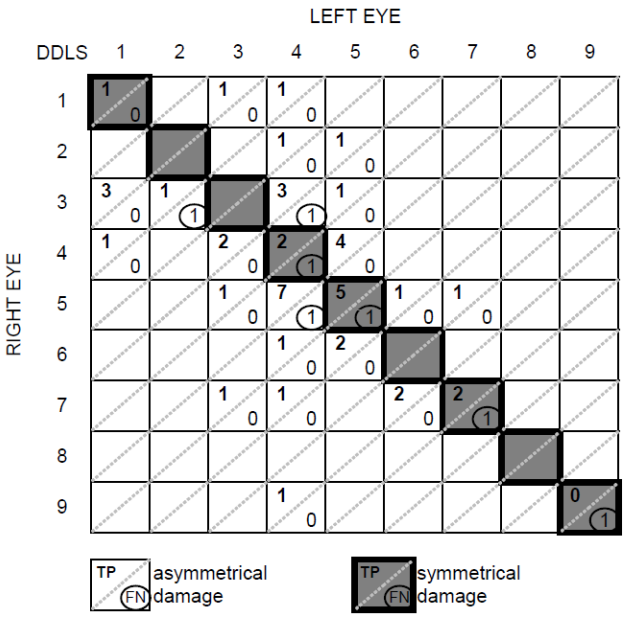

Figure 1. The true positive and false negative rates based on pRAPD are shown for each glaucoma patient tested (TP FN). The results are grouped by disc damage likelihood scale (DDLS) in each eye. Shaded boxes indicate patients with symmetric damage based on DDLS [22]

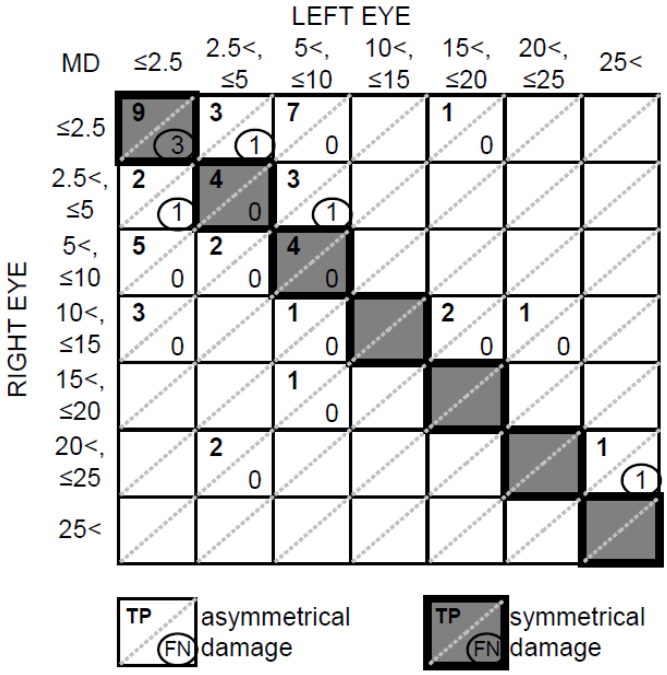

Figure 2. The true positive and false negative rates based on pRAPD are shown for each glaucoma patient tested (TP FN). The results are grouped by mean deviation (MD) in each eye. Shaded boxes indicate patients with symmetric damage based on MD.

DISCUSSION

The purpose of this study is to determine whether a RAPD measured by a highly accurate commercially available binocular infrared pupillometer (Procyon P3000) can be used to distinguish patients with glaucoma from normal subjects.

The results of this study are comparable with those of Kalaboukhova and colleagues [20] who tested 30 glaucoma patients with POAG, pseudoexfoliative glaucoma, pigment glaucoma and normal tension glaucoma, and 30 healthy participants using a custom-built pupillometer. They found that the pRAPD could distinguish between their glaucoma patients and the healthy participants with 86.7% sensitivity and 90% specificity, the area under their ROC curve was 0.92 (95% CI = 0.84 - 0.99). Although our patients tend to be older (74 vs 65 years old) and have less disease asymmetry (ΔMD = 4.7 vs 6.3 dB), both studies show that pRAPD has high sensitivity and specificity in distinguishing glaucoma patients from normal subjects, table 1.

In 2006, Sample and colleagues published a comparison of various methods of assessing optic nerve head function [24]. The authors fixed the specificity at 80% to allow direct comparison of the methods and then calculated the sensitivity of each method. For subjects with evidence of glaucoma progression the sensitivity of standard automatic perimetry was 39 to 68%. The sensitivity of short-wavelength automatic perimetry was 42 to 48% and frequency doubling technology perimetry 71 to 84%, table 1. The authors stated that sensitivities were slightly lower in another patient group who had evidence of glaucomatous optic discs at the time of study but had no progression status confirmed. Our study included patients with a range of severity of glaucoma with or without evidence of progression. If we fix the specificity at 80%, as per the protocol of Sample and colleagues, the Procyon P3000 pupillometer has 91% sensitivity in picking up glaucoma cases. Therefore it appears that the sensitivity of pRAPD compares very favourably with other methods of assessing optic nerve function.

Using a diagnosis of POAG based on all available evidence, we found that the pRAPD test generated 7 false negatives and 8 false positives. Although the mean age of our control group is much younger than that of the glaucoma patient group, there is no significant difference found in the mean age and range of false positives (ages 45 ± 16years, 22 to 66 years) from that of true negatives (47 ± 16 years, 16 to 79 years); and false negatives (ages 76 ± 9 years, 63 to 87 years) from that of true positives (ages 74 ± 9 years, 56 to 91 years). This suggests that false positives and false negatives are more likely to be linked to other factors.

The false negatives occurred when the asymmetry of disc and visual field changes are small between the eyes. The false negatives have MD difference of ≤ 3.81 dB, and difference in DDLS scoring of ≤1. However, in our patient group, 10/14 subjects with no difference in DDLS between the eyes, and 14/17 patients who have ΔMD of ≤1.5 dB are still detected (true positive) with our algorithm. This would suggest that patients whose disease appears symmetrical on clinical examination or SAP still have a degree of asymmetry detectable by pupillometry. Therefore, apparent disease symmetry should not be considered as a disqualifier for pupillometry. The range of pRAPD of the false positives was from 0.08 to 0.21 log units. This represents the range of physiological pRAPD (up to 0.3log unit)[25] that overlaps with pRAPD due to primary open angle glaucoma in our study.

There is a tendency for the sensitivity and specificity of the available glaucoma tests to fall off at the extremes of disease severity. Our sample included more patients with early glaucoma than those with late glaucoma, and yet pRAPD test discriminates early disease from normal with high sensitivity and specificity.

Nonetheless, there are limitations to this method. In this study we included glaucomatous patients with normal pupils but excluded secondary glaucoma patients and patients with previous peripheral iridotomy and other conditions that we believe may interfere with pupil dynamics. We expect the sensitivity and specificity to be lower if the inclusion criteria were broadened to include patients with pupil abnormalities, or other retinal problems.

At present we have aimed to balance sensitivity and specificity. If, however, one wanted to use this as a community based screening tool the cut-off value can be adjusted to maximise the specificity, at a cost of sensitivity. For the hospital eye services the sensitivity of the test may be increased at the expense of its specificity.

Measurement of the pupillometric RAPD in glaucoma patients is different to the traditional methods of assessing primary open angle glaucoma. Rather than looking at individual eyes it makes a comparison between them. The high sensitivity and specificity of this test show its agreement with the clinical diagnosis made from other available methods. It is quick and easy to perform and largely independent of

patient input. This means it may well have a valuable place in serial or parallel tests for glaucoma detection.

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