Impact of Ocular Disease on Circadian Rhythms and Brain Connectivity

The work for this thesis was carried out in the Oxford Eye Hospital and in the Nuffield Department of Ophthalmology, Oxford University.

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“Do but consider what an excellent thing sleep is...that golden chain that ties health and our bodies together”

Thomas Dekker (c. 1572 – 1632)
Declaration

The work in this thesis was conceived and planned by the author in conjunction with her supervisors Professor Susan Downes and Professor Russell Foster. The author completed the amendment for the study protocol, ethics committee approval, the major parts of patient recruitment, data collection and data input, and preliminary statistical analysis of the work. Professor Downes assisted with the ethical approval and the Eye Research group facilitated with the study coordination and patient recruitment. Assistance with further statistical analysis was obtained from Dr Iona Alexander, Dept. of Circadian Neuroscience, Dr Gaelle Coullan and Professor Holly Bridge, Dept. of FMRIB and Dr Lunn, Dept. of Statistics. I confirm that no part of the material offered has previously been submitted by me for a degree in this or any other University. Material generated through joint work has been acknowledged and the appropriate publications cited. In all other cases, material from others has been acknowledged, and quotations and paraphrases suitably indicated.

I, Rupal Morjaria confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Miss Rupal Morjaria, MBCHB, FRCOphth. Date 18/8/2018
Investigation into the impact of ocular disease on sleep and mood has shown that in humans eyes have an important role, and that absence of eyes or interference with light reaching the retina can have deleterious effects. Light is the main zeitgeber ‘time-giver’ used by most species for the regulation of circadian rhythms and is detected by rods, cones and photosensitive retinal ganglion cells (pRGCs) in mammals. The aims of this research project were to investigate this from three different perspectives.

Three prospective studies were undertaken. The first, studied the impact of ocular disease on the sleep/wake cycle in diabetic retinopathy (DR) and in bilateral anophthalmia. There was no significant difference found between the severity of DR and global sleep scores, however the acquired anophthalmic groups have significantly raised global sleep scores compared to controls and the congenital anophthalmic group. Both anophthalmic groups had varying sleep/wake cycles on their actograms depending on the lifestyle (independent of the urinary melatonin). All the anophthalmic participants showed a non 24 hour sleep-wake rhythm disorder after melatonin profiling.

The second study investigates the evidence for the presence of extraocular circadian photoreceptors (EOCP) in participants with anophthalmia and sighted controls. Changes in brain activity using a functional MRI scan was assessed, when a bright white light is shining in different locations. This study did not reveal any evidence of EOCP.
Finally, structural brain MRI differences in anophthalmic groups were investigated. While similar changes in structural reorganisation occur in all anophthalmic groups in the occipital cortex, the acquired anophthalmic groups show an inverse relation with the time since becoming anophthalmic and the volume of optic radiation and optic nerve volume. The acquired anophthalmic group did not show increase in hippocampal volume (memory areas) or in the precuneus (spatial navigation) contrast to the congenital anophthalmic groups.

**Impact Statement**

The evidence of the impact of circadian de-synchrony on health is increasing especially in people subject to shift work and jet lag. The International Agency for Research on Cancer (IARC) classified shift-work with circadian disruption as a probable carcinogen (Straif *et al.*, 2007; Fritschi, 2009). It is known to cause prolonged fatigue, insomnia, appetite changes, mood changes, impaired alertness and performance and increased risk of hypertension and cardiovascular disease. Using the 2016 Organisation for Economic Cooperation and Development purchasing power parity of United States 0.702 pounds sterling per US dollar and the 2016 UK population of 65.64 million the financial impact of sleep disorders in UK is estimated to be £17 billion (£17,154,402,599.11). This emphasises the huge impact sleep disturbance has on productivity, economics, and gross domestic productivity. Sleep/Wake problems represent a substantial emotional and social burden on
totally blind subjects and studies such as this have a key role in exploring the
contribution of ocular disease to sleep/wake disturbance.

The aim of these studies are to help improve quality of life by developing
strategies to improve the sleep/wake disturbance. Where participants have
blind eyes with no perception of light (NPL) vision, previously eyes were
enucleated if they were unsightly and phthisical. However, there may be a role
to leave these eyes in place (if they are not painful), so that the residual pRGCs
in the retina and ocular structures can help entrain the non-image forming (NIF)
functions (Zaidi et al., 2007). Once the eyes are removed and participants are
anophthalmic, there are no remaining ocular or extraocular structures to input
light signals to the suprachiasmatic nucleus (SCN). Ocular prosthesis also
blocks out all light therefore SCN affecting light input to the via the visual
pathway.
Acknowledgements

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Statement of Copyright

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<td>AAV</td>
<td>Adeno-associated virus</td>
</tr>
<tr>
<td>AGEs</td>
<td>Advanced glycation end products</td>
</tr>
<tr>
<td>AMD</td>
<td>Age related macular degeneration</td>
</tr>
<tr>
<td>AMP</td>
<td>Amplitude</td>
</tr>
<tr>
<td>aMT6</td>
<td>6-sulfatoxymelatonin</td>
</tr>
<tr>
<td>An</td>
<td>Anxiety</td>
</tr>
<tr>
<td>BDZ</td>
<td>Benzodiazepine</td>
</tr>
<tr>
<td>BERK</td>
<td>Royal Berkshire hospital</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>Bipolar cell</td>
</tr>
<tr>
<td>BRVO</td>
<td>Branch retinal vein occlusion</td>
</tr>
<tr>
<td>BUCK</td>
<td>Buckinghamshire Healthcare NHS Trust</td>
</tr>
<tr>
<td>C</td>
<td>Rhythm adjusted daily mean</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic guanosine monophosphate</td>
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<td>COPD</td>
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<td>Control</td>
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<td>DCCT</td>
<td>Diabetes control &amp; complications trial</td>
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<td>Depression</td>
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<td>DLMO</td>
<td>Dim light melatonin onset</td>
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<td>DLMOff</td>
<td>Dim light melatonin offset</td>
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<td>Diabetic retinopathy</td>
</tr>
<tr>
<td>DRS</td>
<td>Diabetic retinopathy study</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
</tr>
<tr>
<td>ELM</td>
<td>External limiting membrane</td>
</tr>
<tr>
<td>EOCP</td>
<td>Extraocular circadian photoreception</td>
</tr>
<tr>
<td>EOP</td>
<td>Extraocular photoreception</td>
</tr>
<tr>
<td>ERM</td>
<td>Epiretinal membrane</td>
</tr>
<tr>
<td>IS/OS</td>
<td>Ellipsoid zone</td>
</tr>
<tr>
<td>FA</td>
<td>Fractional anisotropy</td>
</tr>
<tr>
<td>FDT</td>
<td>FMRI's diffusion toolbox</td>
</tr>
<tr>
<td>FEAT</td>
<td>FMRI Expert Analysis Tool</td>
</tr>
<tr>
<td>FMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>GC</td>
<td>Ganglion cell</td>
</tr>
<tr>
<td>GCL</td>
<td>Ganglion Cell Layer</td>
</tr>
<tr>
<td>GHQ</td>
<td>General health questionnaire</td>
</tr>
<tr>
<td>GLM</td>
<td>Generalised linear model</td>
</tr>
<tr>
<td>GMP</td>
<td>Guanosine monophosphate</td>
</tr>
<tr>
<td>HADS</td>
<td>Hospital anxiety and depression scale</td>
</tr>
<tr>
<td>HADS-A</td>
<td>HADS-anxiety</td>
</tr>
<tr>
<td>HADS-D</td>
<td>HADS-Depression</td>
</tr>
<tr>
<td>HM</td>
<td>Hand movements</td>
</tr>
<tr>
<td>ICA</td>
<td>Independent component analysis</td>
</tr>
<tr>
<td>INL</td>
<td>Inner nuclear layer</td>
</tr>
<tr>
<td>IPL</td>
<td>Inner Plexiform Layer</td>
</tr>
<tr>
<td>LGN</td>
<td>Lateral geniculate nucleus</td>
</tr>
<tr>
<td>LM</td>
<td>Light micrograph</td>
</tr>
<tr>
<td>MH</td>
<td>Mental health</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>ms</td>
<td>Milliseconds</td>
</tr>
<tr>
<td>MT</td>
<td>aMT6 secretion rate ng/h</td>
</tr>
<tr>
<td>NIF</td>
<td>Non-image forming</td>
</tr>
<tr>
<td>NIHR</td>
<td>National institute for health research</td>
</tr>
<tr>
<td>NFLDR</td>
<td>Non-proliferative diabetic retinopathy</td>
</tr>
<tr>
<td>NIF</td>
<td>Non-Image Forming</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NPL</td>
<td>No perception of life</td>
</tr>
<tr>
<td>OCT</td>
<td>Optical coherence tomography</td>
</tr>
<tr>
<td>ONL</td>
<td>Outer nuclear layer</td>
</tr>
<tr>
<td>OPL</td>
<td>Outer plexiform layer</td>
</tr>
<tr>
<td>OPN3</td>
<td>Encephalopsin</td>
</tr>
<tr>
<td>OS</td>
<td>Outer segments</td>
</tr>
<tr>
<td>OSA</td>
<td>Obstructive sleep apnoea</td>
</tr>
<tr>
<td>PDR</td>
<td>Proliferative diabetic retinopathy</td>
</tr>
<tr>
<td>PEDF</td>
<td>Pigment epithelium-derived factor</td>
</tr>
<tr>
<td>PIGF</td>
<td>Placental growth factor</td>
</tr>
<tr>
<td>PG12</td>
<td>Prostacyclin</td>
</tr>
<tr>
<td>PLR</td>
<td>Pupillary light response</td>
</tr>
<tr>
<td>R&amp;D</td>
<td>Research and development</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>PL</td>
<td>Perception of light</td>
</tr>
<tr>
<td>pRGC</td>
<td>Photosensitive retinal ganglion cell</td>
</tr>
<tr>
<td>PRP</td>
<td>Panretinal photocoagulation</td>
</tr>
<tr>
<td>pSS</td>
<td>Primary Sjogrens syndrome</td>
</tr>
<tr>
<td>PSQI</td>
<td>Pittsburgh sleep quality index</td>
</tr>
<tr>
<td>Qs</td>
<td>Questionnaires</td>
</tr>
<tr>
<td>RGC</td>
<td>Retina ganglion cell</td>
</tr>
<tr>
<td>RHT</td>
<td>Retinohypothalamic tract</td>
</tr>
<tr>
<td>RP</td>
<td>Retinitis pigmentosa</td>
</tr>
<tr>
<td>RPC</td>
<td>Retinal progenitor cell</td>
</tr>
<tr>
<td>RPE</td>
<td>Retinal pigment epithelium</td>
</tr>
<tr>
<td>RHT</td>
<td>Retinohypothalamic tract</td>
</tr>
<tr>
<td>SCN</td>
<td>Suprachiasmatic nucleus</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error mean</td>
</tr>
<tr>
<td>SOL</td>
<td>Sleep onset latency</td>
</tr>
<tr>
<td>SS</td>
<td>Sjogren’s syndrome</td>
</tr>
<tr>
<td>sSS</td>
<td>Secondary Sjogren’s syndrome</td>
</tr>
<tr>
<td>Synoff</td>
<td>Termination of melatonin synthesis</td>
</tr>
<tr>
<td>T</td>
<td>Period</td>
</tr>
<tr>
<td>TBSS</td>
<td>Tract based spatial statistics</td>
</tr>
</tbody>
</table>
1 General Introduction
1.1 Sleep

Sleep is an important biological process that occurs in all living organisms. Humans spend up to a third of their lives asleep and during this time the primary function is cerebral restoration and memory consolidation (Killgore, 2010). Sleep restriction has been shown to cause impaired glucose metabolism, reduced insulin response (Spiegel, Leproult and Van Cauter, 1999), changes in satiety hormones leptin and ghrelin (Spiegel et al., 2004), increased triacylglycerol concentrations (Costa, 1996; Morgan et al., 1999) and reduction in 24-hour thyrotrophin secretion (Spiegel, Leproult and Van Cauter, 1999). The metabolic effects of sleep deprivation and shift work have shown to increase cardiovascular risk such as ischaemic heart disease and hypertension, gastrointestinal diseases like colitis, gastroduodenitis and peptic ulceration, psychiatric disturbance including anxiety, depression, social phobia and chronic fatigue, increased risk of cancer and mortality (Ford and Kamerow, 1989; Stein, Kroft and Walker, 1993; Billiard et al., 1994; Costa, 1996; Gregory et al., 2006a; Alfano, Ginsburg and Kingery, 2007; Cosgrave et al., 2018). Sleep curtailment has become epidemic with 21% of the people in US to have reported less than 6 h of sleep on workdays (National Sleep Foundation, 2013).

Night-shift workers are known to have poorer daytime sleep, reduced nighttime alertness and performance and an increased accident rate compared to day-workers (Smith, Folkard and Poole, 1994; Åkerstedt, 1995; Akerstedt, 2000; Monk, 2000; Lo et al., 2012). Reduced cognitive function has been
shown during sleep deprivation, and performance measurements after 24 hours of wakefulness have been found to be as poor as during alcohol intoxication (Belenky et al., 2003; Van Dongen et al., 2003).

Two processes, namely Process C (the circadian process) and Process S (the homeostatic drive) interact to determine the timing, duration and structure of sleep, ‘Process S’ is a predictor of sleep pressure and it is reduced during sleep and the amount of slow-wave sleep increases with a higher sleep pressure. ‘Process C’ is guided by the internal circadian clock and determines the 24-hour sleep and wake patterns.

1.2 Circadian process

The term circadian is derived from Latin word *circa*, which means about, and *dies*, which means day. Virtually all living organisms possess internal daily rhythms and in humans the internal circadian clock varies around 24 hours (Aschoff and Aschoff, 1981; Hut and Beersma, 2011). Light is the most reliable zeitgeber ‘time-giver’ however other entraining signals are temperature, food availability or social contact which in turn can influence the production of hormones such as melatonin, cortisol (Lockley, 2009) prolactin and growth hormone (Roenneberg and Foster, 1997; Foster and Helfrich-Forster, 2001; Rajaratnam and Arendt, 2001; Peirson et al., 2005). Entrainment co-ordinates biological events such as sleep-wake cycles, food intake, alertness, performance patterns, core body temperature rhythms and heart rate.
Circadian rhythms also occur at a cellular level and are the product of an intracellular clock mechanism, which comprises of a set of interlocking transcriptional-translational feedback loops (TTFLs) involving core clock genes.

### 1.3 The Mammalian Molecular clock (TTFL)

The mammalian molecular clock consists of the main genes; CLOCK, BMAL1, *Per1, Per2, Cry1 and Cry2* which aid the intracellular core clock mechanism comprised of the transcriptional-translational feedback loop (TTFL). The clock genes are rhythmically transcribed and translated in single cells and negatively regulate their own expression in a period close to 24 hours (Reppert and Weaver, 2001). The SCN, which is the main central clock helps to synchronise the peripheral tissue clocks.

The TTFL is initiated with transcription of CLOCK and BMAL1 in the nucleus and translation in the cytoplasm. They both dimerise, translocate in the nucleus and bind E-box enhancer elements, activating transcription of *Per1, Per2, Cry1* and *Cry2* (Udoh et al., 2015). *Pers* and *Crys* are then translated in the nucleus forming *Per-Cry* complexes which in turn inhibit CLOCK-BMAL1 complex binding to E-box and resulting in inhibition of their own transcription and translation. As the levels of *Per* and *Cry* decrease, CLOCK and BMAL1 transcription starts over. Other feedback loops also regulate this process such as REV-ERBα, phosphorylation of *Per* and *Cry* casein kinase 1 isoforms, ROR/REV-ERB binding element (RORE) regulation of *Bmal1* and *F-box* proteins.
1.4 Discovery of Circadian Rhythms

Early human experiments investigated subjects in forced desynchrony. Biological rhythms of human subjects living in isolation in underground caves for several years found that the circadian pacemaker does not oscillate exactly on a 24-hour day but has a circadian period ($\tau$) slightly longer than 24 hours (average 24.2 h) (Czeisler et al., 1999; Wyatt et al., 1999; Lockley, Arendt and Skene, 2007). Under non-entrained conditions rhythms ‘free-run’ at the endogenous period of the biological clock for example, an individual with an endogenous period of 24.5 h will sleep half an hour later each day (Lockley, Arendt and Skene, 2007).

Real-world environments when altered light-dark exposure can result in circadian de-synchrony in sighted individuals include submarines (Kelly et al., 1999), astronauts during spaceflights (Monk et al., 1998; Dijk et al., 2001) and in polar winters where there is an absence of the environmental light dark cycle (Broadway and Arendt, 1988).

Lockley et al measured the circadian resetting response in humans by exposing individuals to 6.5 hours of monochromatic light at 460nm and 6.5 hours of monochromatic light at 555nm (Lockley et al. 2003). The former light stimulus induced double the circadian phase delay, indicating the peak
sensitivity of the human circadian pacemaker is blue-shifted (Lockley et al. 2003).

1.5 Discovery of the Photosensitive Retinal Ganglion Cells (pRGCs)

The notion of a third type of photoreceptor in the eye that contributed to an individuals’ circadian rhythm was originally inconceivable to many scientists. The discovery of the pRGC began in the Foster lab with studies on retinal degenerate mice. Thapan et al later used action spectroscopy which showed a spectral peak at short wavelength light which did not fit the absorption spectra of the human rod, S-, M- and L cone photopigments (Thapan, Arendt and Skene, 2001). This contributed to the increasing theories suggesting the presence of a novel opsin with a peak sensitivity around 459nm responsible for light induced melatonin suppression and for other non-image forming light.

1.6 Absorbance Spectra of Photopigments

Not many photopigments have emerged during evolution (Wolken and Mogus, 1979) as they must be able to absorb a photon with high probability and then pass on this information with high quantum efficiency to a transduction mechanism. Photopigments have a characteristic absorbance spectrum and can be identified using action spectroscopy. There has been a significant
amount of work using action spectroscopy in different species summarised
below (Table 1), leading to the discovery of the pRGC.
Table 1 Action Spectroscopy of non-image forming (NIF) responses to light

<table>
<thead>
<tr>
<th>Biological response</th>
<th>$\lambda_{\text{max}}$</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal organ response</td>
<td>500-520</td>
<td>Clawed toad</td>
<td>Korf et al (1981)</td>
</tr>
<tr>
<td>Pineal NAT suppression</td>
<td>495</td>
<td>Rat</td>
<td>Bronstein et al (1987)</td>
</tr>
<tr>
<td>Pineal melatonin suppression</td>
<td>500</td>
<td>Atlantic Salmon</td>
<td>Max and Menaker (1992)</td>
</tr>
<tr>
<td>Pineal NAT suppression</td>
<td>500</td>
<td>Chicken</td>
<td>Deguchi (1981)</td>
</tr>
<tr>
<td>Melatonin aggregation</td>
<td>461</td>
<td>Clawed toad</td>
<td>Lythgoe and Thompson (1984)</td>
</tr>
<tr>
<td>Contraction of isolated iris</td>
<td>500</td>
<td>Frog</td>
<td>Barr and Alpern (1963)</td>
</tr>
<tr>
<td>Phase-shifting rd/rd</td>
<td>511 Mouse 480 Mouse</td>
<td>Mouse</td>
<td>Provencio and Foster (1995)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Yoshimura et al (1994)</td>
</tr>
<tr>
<td>Pupillary light response</td>
<td>479</td>
<td>Mouse</td>
<td>Lucas et al (2001)</td>
</tr>
<tr>
<td>Melatonin suppression</td>
<td>446-77</td>
<td>Human</td>
<td>Brainard et al (2001)</td>
</tr>
<tr>
<td></td>
<td>459</td>
<td>Human</td>
<td>Thapan et al (2001)</td>
</tr>
<tr>
<td>Cone ERG</td>
<td>483</td>
<td>Human</td>
<td>Hankins and Lucas (2002)</td>
</tr>
<tr>
<td>Melanopsin retinal ganglion cells</td>
<td>484</td>
<td>Rat</td>
<td>Berson et al (2002)</td>
</tr>
</tbody>
</table>
1.6.1 *The Suprachiasmatic Nucleus (SCN)*

The master circadian pacemaker in mammals is located in the SCN, which is in small, paired nuclei in the anterior hypothalamus superior to the optic chiasm (Klein *et al.*, 1994). It receives light input from the eye via the retinohypothalamic tract (RHT) (Hankins, Peirson and Foster, 2008; Lockley, 2009). The circadian system needs relatively bright and long exposure to light to achieve photoentrainment as it is a thousand times less responsive to light than the visual system (Foster and Hankins, 2002; Peirson *et al.*, 2005).

Once entrained the electrical activity of the SCN is high during the day and low during the night, and this information is transmitted to many parts of the central nervous system and indirectly to peripheral organs. A lesion of the SCN results in behavioural arrhythmicity (Moore and Eichler, 1972) and transplantation of SCN tissue restores behavioural rhythms in SCN-lesioned animals, to a circadian period matching the donor (Ralph *et al.*, 1990).

<table>
<thead>
<tr>
<th><strong>Table 2: Differences in Processing of the two visual pathways</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Circadian</strong></td>
</tr>
<tr>
<td>Sum light information over the entire light environment - irradiance</td>
</tr>
<tr>
<td>Insensitive to dim light</td>
</tr>
<tr>
<td>Insensitive to short duration light stimuli</td>
</tr>
<tr>
<td>Integrate light information over many minutes</td>
</tr>
</tbody>
</table>


1.7 Anatomy of the eye

The eyeball is set in a protective cavity in the skull called the orbit or socket. The eye is made up of three layers which enclose the transparent structures. The outer layer is made of two parts which are continuous: the anterior cornea and the posterior sclera (Figure 1B). The cornea is transparent and allows entry of light into the eye; the sclera creates the “white” of the eye and is composed of fibrous tissue. The middle layer is known as the uveal tract and is divided into three structures: the iris, the ciliary body and the choroid. The iris is the coloured part of the eye, which forms the pupillary aperture that regulates the levels of light admitted to the eye. The ciliary body produces aqueous humour, the clear watery fluid which fills the anterior chamber of the eye. The most posterior segment of the uveal tract is the choroid, a richly vascular structure essential for the high metabolic demands of the posterior segment of the eye. The innermost layer of the eye is the retina, which is an extension of the central nervous system and is connected to the brain through the optic nerve. The inside of the eye is divided into three transparent segments: the aqueous humour anteriorly, the lens and vitreous cavity posteriorly (which contains the gel-like vitreous humour).
1.7.1 The Functional Anatomy of the Retina

The retina is between 100-500μm thick and it is one of the most biologically active tissues in the body. It is a complex layered structure with approximately 60 types of neurons belonging to 5 classes, interconnected by synapses (Strettoi and Parisi, 2014). The outer nuclear layer contains cell bodies of the rods and cones, the inner nuclear layer contains cell bodies of the bipolar, horizontal and amacrine cells and the ganglion cell layer contains cell bodies of ganglion cells and displaced amacrine cells (Figure 2).

The retina has refined its mechanisms for light and dark adaptation and its own circadian clock to allow for the variation in luminance imposed on the retina between day and night. In summary, the retina, a single sensory organ informs the brain of light changes functional to vision, as well as to variations of light.
occurring in time, providing the core information for the existence of circadian rhythms (Strettoi and Parisi, 2014).

Figure 2 Light Micrograph (LM) of a human Retina showing the retinal layers
Courtesy of Ngai Victor Chong

Figure 3 Optical Coherence Tomography (OCT) of a healthy retina showing a cross-section of retinal layers
Acquired by RM with permission from subject
1.7.2 Retinal Pigment Epithelium (RPE)

The RPE is a monolayer of cuboidal supporting cells, important for photopigment regeneration, carrying out phagocytosis of the rod and cone outer segments, synthesis of inter-photoreceptor matrix, absorption of light and reduction of light scatter within the eye.

1.7.3 Photoreceptors: Rods and cones

There are approximately 120 million rods and 6 million cones, making them the most numerous photoreceptors in the eye. However, there are also other photoreceptors present including the photosensitive retinal ganglion cells (pRGCs) that capture a portion of this light. The rods mediate scotopic vision (dim light conditions), sense contrast, brightness and motion. The cones, short (S), medium (M) and long (L) wavelength, mediate fine resolution, spatial resolution and colour vision. Rods have extremely high sensitivity and can detect single quanta of light, however they respond slower than cones. The peripheral retina is mainly rod dominated (30 000 per mm²) and the macula region has a higher cone density (150 000 per mm²).

In addition to photoreceptors, the retina has five major classes of retinal neurons whose cell bodies are in the inner nuclear layer: bipolar cells, horizontal cells, amacrine cells, Muller cells, ganglion cells.
1.8 Photosensitive retinal ganglion cells (pRGCs)

pRGCs are a small subset of the retinal ganglion cells are referred to as photosensitive retinal ganglion cells, melanopsin retinal ganglion cells, intrinsically photosensitive retinal ganglion cells first identified in 2002. For the purposes of this thesis they will be referred to as pRGCs. pRGCs express melanopsin photoreceptor protein on the cell surface, which after activation by light initiates neuronal signals to the brain concerning environmental light intensities (Do and Yau, 2010). Melanopsin appears to function as a bistable pigment, able to regenerate its chromophore utilising all-trans-retinal and long-wavelength light (Hankins, Peirson and Foster, 2008). The peak sensitivity of the pRGC is in the blue part of the spectrum close to the peak sensitivity of melanopsin (483nm) (Dacey, H.-W. Liao, et al., 2005; Hughes et al., 2013).

In mice and in rats, pRGCs are represented by five sub-types (M1-M5) (Schmidt, Chen and Hattar, 2011; Cui et al., 2015; Reifler et al., 2015). Dacey et al. described found pRGCs to have the highest density in the central retina representing approximately 0.2% of the total ganglion cells (Dacey, H.-W. Liao, et al., 2005). More recently, Hannibal et al. studied two human retinas and found total cell counts from these contained 7283+/−237 pRGCs (0.63-0.75% of the total RGCs)”. They found the highest density in the central retina close to the fovea, followed by inferior quadrant, temporal quadrant, nasal quadrant, superior quadrant and the least in the peripheral retina (Hannibal et al., 2017a).
1.9 pRGC Sub-types

Hannibal et al used antibodies against N and C terminal parts of the human melanopsin, confocal microscopy and 3D reconstruction of pRGCs (Hannibal et al., 2017a). M1, displaced M1, M2, M4, gigantic M1 (GM1) and gigantic displaced M1 (GDM1) cells were identified. Few M3 cells and no M5 subtypes were labelled. These sub-types were present in different quantities throughout the retina (Hannibal et al., 2017a). M1 and displaced M1 cells were the major cell types particularly concentrated in the temporal retina. GM1 were abundant in the nasal retina, whilst GDM1 were found to be sparse and the inner stratifying M2/M4 cells were most numerous in the nasal part. The perifoveal
area contained the highest density of pRGCs with processes from M1, DM1 and M2 cells encircling the foveal pit however, there was a complete absence in the foveal region (Hannibal et al., 2017a).

1.10 pRGC sub-types connectivity within the retinal layers

pRGC processes form regular dendritic mosaics in rodents, monkeys and in humans and are considered as spatially independent (Berson, Castrucci and Provencio, 2010; Liao et al., 2016). Hannibal et al found clusters of M1 and DM1 formed complementary mosaics while GM1 is superimposed on M1 and DM1 mosaics (Hannibal et al., 2017b). M1, DM1 and/or GM1 dendritic processes were found to have direct contacts suggesting functional connectivity. M2/M4 cells had much fewer contacts (Hannibal et al., 2017a).

pRGCs were found to have large diameter axons splitting into two or double axons, one projecting to the brain via the suprachiasmatic nuclei, and the other axon projecting intra-retinally (Hannibal et al., 2017a). Other groups have reported this finding; pRGC axon collaterals have been described in mice, monkeys and human gigantic melanopsin cells (Joo et al., 2013).

Whilst Hannibal et al characterised M1 cells as GM1, M1 and GDM1, in mice studies Schmidt et al described two distinct subpopulations of M1 cells. Brn3b positive M1 cells and Brn3b negative M1 cells (Schmidt and Kofuji, 2009). Brn3b positive M1 cells do not project to the SCN and ablation of these
impaired the pupillary light reflex but did not affect circadian entrainment. It is therefore assumed that Brn3b positive M1 cells play a key role in the cone mediated pupillary light responses (Schmidt and Kofuji, 2009) (Figure 5).

M1 pRGCs have been the most extensively characterised compared to the other pRGCs. They are described as being predominantly responsible for circadian entrainment and pupillary light responses (Güler et al., 2008; Hatori et al., 2008). The non-M1 cells are likely to be responsible for image-forming behaviours and are thought to rely on rod and cone signalling. They are suspected of contributing to pain, fear and anxiety via their projections to the dorsal lateral geniculate nucleus, periaqueductal gray and amygdala (Maren and Fanselow, 1996; Hattar et al., 2006; Ecker et al., 2010).
Table 3: Summary of Findings for Photosensitive Retinal Ganglion Cells (pRGCs) in Humans, Mice and the Macaque Retina, Reporting Distribution and Connectivity of pRGCs

<table>
<thead>
<tr>
<th>Cell</th>
<th>Soma Diameter</th>
<th>Soma Shape</th>
<th>Distribution</th>
<th>Dendritic Field</th>
<th>Light Response</th>
<th>A11 Cells</th>
<th>Summary of Findings from Hannibal et al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer Stratifying</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM1 “Gigantic” M1 cell</td>
<td>32.7± 1.1μm</td>
<td>Round, oval cell bodies</td>
<td>Nasal retina</td>
<td>4/5 dendrites, Largest dendritic field, Overlap M1+DM1 Area 0.64±0.05mm²</td>
<td>Large response which rapidly decays. Higher input resistance and more depolarised resting membrane potential, spike to lower frequency (mice)</td>
<td>GM1 cells in ganglion cell layer and displaced in INL</td>
<td></td>
</tr>
<tr>
<td>M1 cell</td>
<td>19.6± 0.6μm</td>
<td>Circular, fusiform soma</td>
<td>Temporal retina and perifovea</td>
<td>2/3 sparsely branching dendrites. Limited overlap. Area 0.39±0.03mm²</td>
<td>More highly branched</td>
<td>Double or splitting axons; one to brain and one intraretinally</td>
<td></td>
</tr>
<tr>
<td>GDM1 “Gigantic displaced” M1</td>
<td>28.7± 1.0μm</td>
<td>Larger soma size than M1 (mice)</td>
<td>Nasal retina</td>
<td>4/6 branching dendrites. More highly branched</td>
<td>In opposition with calretinin only</td>
<td>Increased cell counts found (c.f Dacey 2005, Liao 2016). 7520 and 7046 in 2 retinas</td>
<td></td>
</tr>
<tr>
<td>Inner Stratifying</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>M2-24.5± 2.03μm</td>
<td>Smaller soma</td>
<td>Nasal retina</td>
<td>3/5 branching dendrites. Larger dendritic arbors. Higher melanopsin expression than M4</td>
<td>Respond to smaller steady light current Less intrinsically photosensitive than M1</td>
<td>In opposition with calretinin only</td>
<td>Significance of highest concentration of M1 and DM1 in temporal retina?</td>
</tr>
<tr>
<td>M4 cells</td>
<td>30.3±1.0μm</td>
<td>Largest somas (mice)</td>
<td>Nasal retina</td>
<td>4/6 branching dendrites. Both smaller dendritic fields-0.27±0.02mm² (similar finding as Ecker 2010)</td>
<td>In opposition with calretinin only</td>
<td>M1, DM1 ±GDM1 dendritic processes have direct contacts indicating functional connectivity. Fewer contacts M2/M4 cells</td>
<td></td>
</tr>
<tr>
<td>M3 cells (Inner and outer sublamina)</td>
<td>23.0± 0.5μm</td>
<td>Few cells found</td>
<td>Inferior and Nasal</td>
<td></td>
<td></td>
<td>M1 &amp; DM1 form complementary mosaics with GM1 superimposed ontogeny</td>
<td></td>
</tr>
</tbody>
</table>

Text in black from (Hannibal et al., 2017b), Red from (Ecker et al., 2010) and Blue from (Schmidt and Kofuji, 2009)
M1 cells project to the OPN controlling pupil constriction, and the SCN, controlling circadian photoentrainment. M2-M5 cells, send projections to the LGN and are involved in low-acuity visual function. Modified from (Schmidt et al., 2011). Image of brain courtesy of www.bigthink.com

1.11 Phototransduction and Image-Forming Vision

Rods contain a vitamin A-based chromophore called 11-cis retinaldehyde, which is covalently bound to an opsin protein, rhodopsin. All mammalian photopigments contain this chromophore. The opsin protein in cones is the cone opsin. This is present in the outer segment of the photoreceptors in a lipid bilayer membrane, arranged as flattened ‘discs’. Light activation causes photoisomerisation of 11-cis-retinaldehyde to change from an inactive state to an active state in the phototransduction cascade. 11-cis retinal is converted to all-trans retinal and then to all-trans retinol which is taken up by the RPE. It is
then converted back to 11-cis retinol in the dark. The conversion of the energy stored in a single photon of light to an electrical impulse occurs because of the extensive amplification of the molecular cascade. In the dark, resting state Na\(^+\) channels in the rod outer segment are held open by cGMP. This provides a relative depolarised state of the photoreceptor cell, relative to other cells. Light activation causes sequential activation of membrane bound proteins, transducin and phosphodiesterase to lower the cytosolic concentration of cGMP, thus closing the Na\(^+\) channels and causing a relative hyperpolarisation. The greatly amplified cascade involving hydrolysis of cGMP by phosphodiesterase generates an electrical response. Although photoreception is best understood in retinal rods and cones, photoreception is not confined to these structures.
1.12 Non-Image forming (NIF) vision

Environmental light is detected by three classes of ocular photoreceptor, the rods and cones of the outer retina and pRGCs. pRGCs are depolarised after light activation of their photopigment melanopsin and via input from the rod and cones via bipolar and amacrine cells (Berson, Dunn and Takao, 2002; Dacey, H. W. Liao, *et al.*, 2005; Jusuf *et al.*, 2007; Wong *et al.*, 2007; Schmidt and Kofuji, 2009; Estevez *et al.*, 2012; Weng, Estevez and Berson, 2013; Zhao *et al.*, 2014; Reifler *et al.*, 2015; Liao *et al.*, 2016). The light information is used by the brain to regulate NIF tasks such as entrainment of the circadian timing system (Panda *et al.*, 2002) and sleep induction (Lupi *et al.*, 2008), the pupillary light response (PLR) (Hattar *et al.*, 2003; Lucas *et al.*, 2003; Schmidt *et al.*, 2011) and the acute suppression of activity in response to light (Mrosovsky and Hattar, 2003) (Figure 5). pRGCs also contribute to pattern vision, photosensitivity, activity masking, sleep/arousal and neuroendocrine systems, anxiety and even make significant contribution to thalamocortical visual function (Güler *et al.*, 2008; Hatori *et al.*, 2008; Tsai *et al.*, 2009; Brown *et al.*, 2010; Ecker *et al.*, 2010; Johnson *et al.*, 2010; Thompson *et al.*, 2010; Estevez *et al.*, 2012; Procyk *et al.*, 2015).

In summary, the retina has two key roles; image forming and non-image forming. The outer retinal rod and cone photoreceptors gather light information to construct an image whilst pRGCs detect irradiance and arise from the inner
retina. The two systems interact and rods and cones relay information into pRGCs.

1.13 Melatonin secretion

Photic information affects melatonin synthesis, where the peak secretion of the hormone by the pineal gland is high during the night-time i.e. during the sleep period and low during the day-time (Cagnacci, Elliott and Yen, 1992; Lockley et al., 1997; Bjorvatn and Pallesen, 2009; Lockley, 2009). Melatonin synthesis and secretion is mediated via the light/dark cycle. A light signal to the retina results in inhibition of melatonin synthesis via an afferent signal from the SCN which projects to the pineal gland via the paraventricular nucleus and superficial cervical ganglion (Lockley, 2009).

Melatonin is one of the most reliable markers of the periodicity of the endogenous clock unlike other circadian rhythms such as core body temperature or cortisol, which are readily influenced by sleep, activity or stress (Vaughan et al., 1978; Åkerstedt et al., 1979). The major metabolite of melatonin is 6-sulfatoxymelatonin (aMT6s), measured in saliva, plasma or urine.

1.13.1 Melatonin Sampling

The most widely used method for melatonin sampling in clinical studies is measuring 48hour urine collections for aMT6. However, saliva and serum
sampling methods are also used. Measuring aMT6s from urine collected every 2 to 8 hours over a 24-48 h is the most practical method for estimating global timing and amount of melatonin production (Benloucif et al., 2008). It is feasible in both the elderly or in children with the help of a carer or investigator. Using this method overnight aMT6s excretion can be calculated using the first morning void. The phase of the rhythm is estimated from the timing of the acrophase (time of fitted peak) of a cosine fitted curve.

Saliva sampling is a second method. This is a fairly practical and reliable method however requires samples to be taken every 30-60 minutes at dim light (<30 lux) for at least 1 hour prior to and throughout the expected rise in melatonin (Benloucif et al., 2008). Saliva sampling also results in disrupted sleep and its overnight use is therefore limited.

Blood sampling is the most accurate and produces the largest melatonin volumes with a greater sensitivity of the results, however the technique is invasive requiring venous cannulation and the presence of a trained doctor/nurse (Benloucif et al., 2008).

### 1.14 Circadian Rhythm Sleep-wake Disorders

Circadian rhythm sleep-wake disorders (CRSWD) are caused by a misalignment between the endogenous rhythm and the environmental 24-hour day. The following criteria need to be met for a diagnosis of CRSWD:

“1) A chronic or recurrent pattern of sleep-wake rhythm disruption primarily caused by an alteration in the endogenous circadian timing system or
misalignment between the endogenous circadian rhythm and the sleep-wake schedule desired or required by an individual’s physical or social/work schedules, 2) The circadian rhythm disturbance leads to insomnia, excessive sleepiness or both, and 3) The sleep and wake disturbance is associated with significant distress or impairment in functioning”, lasting a minimum of 3 months (except jet lag) (American Academy of Sleep Medicine, 2014). The International Classification of Sleep Disorders (ICSD-3) recognises 7 distinct CRSD. The 7 disorders listed in the ISCD-3 are “1) delayed sleep-wake phase disorder, 2) advanced sleep-wake phase disorder, 3) irregular sleep-wake phase disorder 4) non-24-h sleep-wake rhythm disorder 5) shift work disorder 6) jet-lag type disorder and 7) circadian sleep-wake disorder not otherwise specified (NOS) (American Academy of Sleep Medicine, 2014),” Patients with CRSWD have a variety of complaints ranging from insomnia, excessive daytime sleepiness, early awakening impacting on their health, quality of life and mood.

1.1.1 Circadian Rhythm Sleep-wake Disorder, Non-24h sleep-wake rhythm disorder

As described in the section above (1.14) there are several types of CRSWD. For the purposes of this work, the non-24h sleep-wake rhythm disorder of CRSWD will be reviewed.

Non-24h sleep-wake rhythm disorder of CRSWDs commonly occur in the blind who have no light perception (Barion, 2011). Patients with a non-24h sleep-
wake rhythm disorder have periods of poor sleep and the symptoms mentioned above (section 1.14) are followed by brief episodes of complete disappearance when their internal clock is briefly aligned with the environment (Wright, Drake, Lockley, 2008). Each episode lasts several weeks to months where the timing progressively gets later if their circadian period is > 24 hours or earlier, if their circadian period is <24 hours (Wright, Drake, Lockley, 2008).

### 1.14.1 Actigraphy

Actigraphy was introduced in 1926 by Maynard Johnson (Johnson, 1926). A chronogram (a time plot) is created by ‘cutting’ out a segment of each day and ‘stretching’ it to the full length of a page, and then pasting it below the data of the day before. This method allows sleep and wake activity for each day to be viewed on a separate line, creating an actigraph. The vertical alignment of the data provides information about the duration of the circadian cycle (period) i.e. drifts to the left indicate a cycle is shorter than 24 h and if the cycle drifts to the right, it is longer than 24 h (Refinetti, Lissen and Halberg, 2007).

### 1.15 CRSWD and Ocular disease

The effect of the types of visual impairment and the photoreceptors affected on the circadian system has been used as an indirect method to assess the impact of light on circadian rhythms (Czeisler et al., 1981; Tabandeh et al., 1998; Leger et al., 1999; Lockley et al., 1999). Historically studies looking at sleep wake disruption have been described in a clinically heterogeneous population with conditions ranging from optic nerve degeneration, retinal
degeneration and anophthalmia. Abnormal hormonal patterns in blind individuals have been reported in over 5 decades however there have been confounding results. Circadian rhythms have been looked at in a varying population of blind people ranging from hand movements (HM) vision to no perception of light (NPL) (Table 4).

1.15.1 **CRSWD in Blind People**

The prevalence of sleep disturbances and abnormal melatonin phases in visually impaired individuals particularly those with no light perception has been shown to be five times higher (40-76%) compared to healthy sighted individuals (Davitt *et al.*, 1997; Lockley *et al.*, 1997; Tabandeh *et al.*, 1998; Barion, 2011). Totally blind persons cannot make use of light as the strongest zeitgeber and therefore have circadian rhythms that are not entrained (Miles, Raynal and Wilson, 1977; Nakagawa, Sack and Lewy, 1992; Sack *et al.*, 1992; Barion, 2011).

Tzischinsky *et al* investigated the relationship between melatonin secretion and sleep quality and subjective complaints in eleven totally blind children (Tzischinsky *et al.*, 1991). Nine children were congenitally blind and two had become blind in the two years prior to the study. Nine children had retrolental fibrosis, one had an optic tract tumour, and one had congenital glaucoma. Sleep-wake cycles were recorded using actigraphy and urine was collected to measure the melatonin metabolite 10 times over a 48-hour period. Delayed secretory peaks in aMT6s were significantly associated with disturbed
nocturnal sleep and with complaints about morning fatigue (Tzischinsky et al., 1991).

In another study, 127 blind women with no perception of light (NPL) and with light perception (LP) were investigated over 8 weeks with daily sleep diaries, 4-8 hour urine collections over 48 hours on 2/3 occasions separated by at least 2 weeks (Flynn-Evans et al., 2014). A greater proportion of individuals who had NPL vision were abnormally phased (24%) and non-entrained (39%) than those in the LP group; 21% and 10% respectively (Flynn-Evans et al., 2014). Eye conditions most associated with abnormal phase and/or non-entrained circadian rhythms were bilateral enucleation (67%) and retinopathy of maturity (57%). 84% of participants with retinitis pigmentosa and 83% of those with age-related macular degeneration were normally entrained. The group suggested that the aetiology of blindness in addition to LP status was related to an individual’s ability to process the circadian light signal (Flynn-Evans et al., 2014).

There is extensive work to show that individuals with varying degrees of light perception, show sleep-wake disruption (Davitt, Morgan, & Cruz, 1997; Flynn-Evans, Tabandeh, Skene, & Lockley, 2014; Leger, Guilleminault, Defrance, Domont, & Paillard, 1996; Lockley et al., 1995; Lockley et al., 2008; Lockley, Skene, Arendt, et al., 1997; Lockley, Skene, Tabandeh, et al., 1997; Moseley, Fouladi, Jones, & Tobin, 1996; Nakagawa, Isaki, Sack, & Lewy, 1992; Nakagawa, Sack, & Lewy, 1992; Sack & Lewy, 2001; Sack, Lewy, Blood, Keith,
& Nakagawa, 1992; Skene & Arendt, 2007; Tabandeh et al., 1998) (Table 4).

However, non-visual functions of the eye may still be preserved in the absence of rods and cones (Zaidi et al 2007).

Table 4 Table Summarising the Additional Studies Investigating Sleep/Wake disturbance in the blind

<table>
<thead>
<tr>
<th>Authors (et al)</th>
<th>Measure</th>
<th>Subjects</th>
<th>Results summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migeon 1956</td>
<td>17-OHCS</td>
<td>'blind' &amp; controls</td>
<td>No difference</td>
</tr>
<tr>
<td>Remler 1940</td>
<td>Rectal temp, HR, BP, Urine</td>
<td>'blind'</td>
<td>Some had normal rhythms, some abnormal</td>
</tr>
<tr>
<td>Orth &amp; Island 1969</td>
<td>17-OHCS</td>
<td>3 NPL</td>
<td>1= normal (N), 1 = abnormal (Abn), 1 = free running</td>
</tr>
<tr>
<td>Kreiger &amp; Rizzo 1971</td>
<td>17-OHCS</td>
<td>7LP and 12 NPL</td>
<td>5 LP &amp; 9 NPL abn 17-OHCS</td>
</tr>
<tr>
<td>Bodenheimer 1973</td>
<td>17-OHCS</td>
<td>'blind' &amp; controls</td>
<td>7 NPL phase difference in cortisol</td>
</tr>
<tr>
<td>Weitzman 1973</td>
<td>17-OHCS, GH</td>
<td>'blind' &amp; controls</td>
<td>No difference</td>
</tr>
<tr>
<td>D’Alessandro 1974</td>
<td>17-OHCS</td>
<td>NPL</td>
<td>11 NPL 24h arrhythmic</td>
</tr>
<tr>
<td>Schievng 1987</td>
<td>Epinephrine &amp; Norepinephrine</td>
<td>14 ‘blind’ &amp; controls</td>
<td>Blind = increase in Mesor of norepinephrine levels</td>
</tr>
<tr>
<td>Smith 1981</td>
<td>Melatonin</td>
<td>‘blind’</td>
<td>4 blind; Abn melatonin rhythms</td>
</tr>
<tr>
<td>Lewy &amp; Newcombe 1983</td>
<td>Melatonin</td>
<td>10 NPL &amp; PL</td>
<td>6 abn timed melatonin; 3 phase delayed of which 1 abn entrained and 1 free running</td>
</tr>
<tr>
<td>Sack &amp; Lewy 1992</td>
<td>Melatonin</td>
<td>20 ‘blind’</td>
<td>2 N phased, 3 Abn, 11 free-running, 3 arrhythmic</td>
</tr>
<tr>
<td>Miles &amp; Wilson 1977</td>
<td>Qualitative assessment</td>
<td>50 ‘blind’</td>
<td>76% complained of sleep/wake disturbance</td>
</tr>
<tr>
<td>Miles 1977</td>
<td>sleep/wake cycle, alertness, performance, 17-OHCS</td>
<td>1 ‘blind’ man</td>
<td>Free-running</td>
</tr>
<tr>
<td>Ortho 1979</td>
<td>17-OHCS</td>
<td>NPl</td>
<td>Free-running</td>
</tr>
<tr>
<td>Sasaki 1992</td>
<td>Qualitative assessment</td>
<td>‘blind’</td>
<td>40% of 73 blind sleep/wake complaints</td>
</tr>
<tr>
<td>Moseley 1996</td>
<td>Qualitative assessment</td>
<td>‘blind’</td>
<td>18% subjective sleep disorder</td>
</tr>
<tr>
<td>Tabandeh 1998</td>
<td>PSQI</td>
<td>‘blind’</td>
<td>NPL higher sleep disturbance</td>
</tr>
<tr>
<td>Leger 1999</td>
<td>Qualitative assessment</td>
<td>‘blind’</td>
<td>83% blind at least 1 sleep disorder (sleep latency, night time &amp; early am wakenings, reduced sleep duration &amp; quality)</td>
</tr>
<tr>
<td>Skene 1999</td>
<td>Sleep log, alertness, mood, aMT6 urine</td>
<td>67 ‘blind’</td>
<td>30 LP (77%) Abn aMT6 4 LP (13%) Abn phased rhythms 2 no detectable rhythm 1 LP free-running</td>
</tr>
<tr>
<td>Lockley 2007</td>
<td>Qualitative assessment</td>
<td>‘blind’</td>
<td>388 blind registered. 189/388 disturbed sleep NPL 66% mean PSQI ± SD= 8.1 ±1.1</td>
</tr>
</tbody>
</table>

Abbreviations: temp; temperature, HR; heart rate, BP; blood pressure, 17-OHCS; Cortisol concentration, LP; light perception, NPL; no light perception, Abn; abnormal, N; normal, GH; growth hormone
As part of my project was to investigate sleep wake disorders in diabetic retinopathy (DR) and anophthalmia, I have singled out these two conditions for a more detailed overview in my thesis.

1.16 Impact of diabetic retinopathy (DR) on sleep and mood

DR is the fifth common cause of reversible-treatable blindness in the world (Bourne et al., 2013) and a major cause of blindness among working population in developed countries (Mohamed, Gillies and Wong, 2007). The prevalence of DR among type I and II diabetics has been reported as high as 82.3% and 40.3%, and 32.2% overall with vision threatening DR (Roy et al., 2004) and 8.2% respectively (Kempen et al., 2004). The global diabetes prevalence in 2017 was 8.8% of a 7.5 billion population which is expected to rise to 9.9% in an estimated population of 9.5 billion by 2045 (Karuranga Suvi, Fernandes Joao da Rocha, Huang Yadi, 2017). With an increasing prevalence of diabetes and DR, understanding the potential implications of DR on sleep is of utmost importance.

1.16.1 Diabetes and its impact on sleep and mood

Reduced sleep duration is a risk factor for insulin resistance in type 2 diabetes and has been linked to abnormal glucose metabolism, increase diabetes risk (Knutson and Van Cauter, 2008; Cappuccio et al., 2010; Donga and Romijn, 2014; Jee et al., 2017), obesity and cardiovascular risk (Nedeltcheva and Scheer, 2014) (Figure 6). In addition, many of the conditions strongly linked to
diabetes, such as cardiovascular disease, obesity, restless leg syndrome, nocturia and obstructive sleep apnoea syndrome (OSAS), are also associated with poor sleep (Miyaoka et al., 1997; West, Nicoll and Stradling, 2006; Algul et al., 2009; Bansil et al., 2011; Plantinga, Rao and Schillinger, 2012; Aggarwal et al., 2013). The mechanism proposed for insulin resistance after sleep deprivation is an altered autonomic nervous system, endocrine changes and an altered inflammatory state (Leproult et al., 1997; Boudjeltia et al., 2008; Reynolds et al., 2012).

![Diagram showing the relationship between decreased sleep, change in hormonal profiles resulting in increased risk of diabetes, obesity, and cardiovascular disease. Modified from (Nedeltcheva and Scheer, 2014).](image)

**1.16.2 Diabetic Retinopathy (DR) and its impact on sleep and mood**

DR has been linked to longer duration of diabetes, poor glycaemic control, hypertension, cardiovascular disease, lipid profiles and obesity. DR is characterised by non-perfusion and ischaemia within the retina leading to loss
Chapter 1

of visual function as a result of macular oedema and retinal neovascularisation (Figure 7). The DR study in 1976 reported panretinal photocoagulation (PRP) as an effective treatment for DR (DRS study) (‘Preliminary report on effects of photocoagulation therapy. The Diabetic Retinopathy Study Research Group’, 1976), however this doesn’t come without side effects. Laser can cause peripheral and central scotomas, loss of central vision from macula laser, laser creep and poor dark adaptation (Alasil and Waheed, 2014).

Until recently, the mainstay treatment for proliferative DR (PDR) has been laser photocoagulation with management of risk factors such as blood pressure, cholesterol, diet and exercise. Despite considerable improvements in laser technology even targeted laser at the retinal pigment epithelium results in collateral damage to the neural retina and the choroid as a result of scatter rays. In summary, the pathological processes of DR and the treatment result in secondary photoreceptor death, visual deterioration and poor dark adaptation. It is not known however, how the disease process affects photosensitive retinal ganglion cells (pRGC) within the ganglion cell layer.

Little work has been done on the impact of DR and sleep/wake disorders. Jee et al have performed the largest cross-sectional study to date investigating the association between sleep duration and DR (Jee et al., 2017). 1670 subjects with diabetes aged over 40 were investigated, who participated in the Korean National Health and Nutrition Examination Survey between 2008-2012 (Jee et al., 2017). No significant association between DR and sleep duration was found.
in women and there was no significant association between vision threatening DR and sleep duration (Jee et al., 2017). However short sleep (≤ 5 hours) and long sleep (≥ 9 hours) was associated with a higher prevalence of DR in men (Jee et al., 2017). Further sleep wake phenotyping in DR of different severity to determine its effect on sleep, wake and mood is required.

**Figure 7** Pathogenesis of DR showing the pathway from hyperglycaemia to retinal neovascularisation

Upregulation of nitric oxide (NO), prostacyclin (PGI2), growth factors; vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGFβ) and basement membrane thickening lead to vascular occlusion and cell death. Subsequently, there is a rise in advanced glycation end products (AGEs), placenta growth factor (PIGF), reduction in pigment epithelium-derived factor (PEDF) leading to neovascularisation. Modified from (Cai and Boulton, 2002).
Figure 8 Optos Wide-field multi-colour images of a participant with proliferative diabetic retinopathy
Fundus image of right eye with extensive peripheral panretinal (prp) laser, intra-retinal microvascular abnormalities (IRMA), blot haemorrhages, cotton wool spots and disc new vessels.
Acquired by RM with permission from subject

1.17 Anophthalmia

True congenital bilateral anophthalmia is classified as the absence of both eyes from birth and is characterised by a ‘visual’ system that has never experienced pre- or post-natal visual stimulation. Acquired bilateral anophthalmia is where both eyes are lost either through trauma or surgical removal of both eyes due to diseases such as retinoblastoma, or end stage glaucoma. This occurs after development of the visual system has taken place.

Congenital anophthalmia/microphthalmia have a wide phenotypical variability and clinical diagnosis can often be difficult. Investigations used to aid diagnosis
include imaging either CT and or MRI, ultrasound scans and in some cases electrophysiology to detect whether there is any retinal function. Differential diagnosis include cryptophthalmos (completely fused eyelid margins without lashes), cyclopia/synophthalmia (which represent a rare lethal form of holosencephaly characterised by a failure of the embryonic prosencephalon to divide into left and right hemispheres and the orbits of the eye do not divide into two cavities) (Verma and Fitzpatrick, 2007; Salama et al., 2015).

The International Clearinghouse for Birth Defects Monitoring Systems defines anophthalmia and microphthalmia as ‘apparently absent or small eyes, with some normal adnexal elements and eyelids are usually present’ (International Clearinghouse for Birth Defects Monitoring Systems: Annual Report, 2003). Anophthalmia/microphthalmia can occur in isolation or be syndromic, as in one third of cases (Verma and Fitzpatrick, 2007; Bardakjian and Schneider, 2011).

The combined prevalence of these conditions is up to 30 per 100,000 population and the prevalence of anophthalmia is estimated to be 3 per 100,000 (Campbell et al., 2002; Morrison et al., 2002). Anophthalmia and microphthalmia have a complex aetiology with chromosomal, monogenic and environmental causes identified (Verma and Fitzpatrick, 2007). Chromosomal duplications, deletions and translocations are implicated (Verma and Fitzpatrick, 2007). Causative genes linked with anophthalmia are SOX2, PAX6, OTX2, CHX10, SMOC1 and RAX. Environmental factors such as gestational-acquired infections, maternal vitamin A deficiency, exposure to X-rays, solvent misuse and thalidomide exposure are also linked. Epidemiological data
suggests risk factors for these conditions are maternal age of over 40, multiple births (Kallen, Robert and Harris, 1996; Shaw et al., 2005), infants of a low birth weight and low gestational age (Forrester and Merz, 2006). Both anophthalmia and microphthalmia are more commonly bilateral and there is no predilection with regards to race or sex (Kallen, Robert and Harris, 1996; Shaw et al., 2005; Verma and Fitzpatrick, 2007).

Subjects with congenital anophthalmia differ from those with acquired anophthalmia as those who lost their eyes later in life have had normal brain development and connectivity. In subjects with congenital anophthalmia the input from the optic nerve to the thalamus and the brain never existed or may have existed only temporarily, early in development before the embryonic eyes degenerated (Bridge et al., 2009).

1.18 Impact of Anophthalmia on Sleep, Mood and Sleep

Wake Rhythms

Individuals with no eyes (bilateral anophthalmia) where light input to the SCN is completely obliterated, provide a unique opportunity to study circadian biology. In the study assessing sleep and mood in participants with anophthalmia (Chapter 3.2), I investigate behavioural (actigraphy) and physiological (endogenous melatonin) rhythms as well as subjective sleep quality and mood in individuals with congenital and acquired anophthalmia with consideration to the integrity of any residual pathways that may facilitate entrainment and the
impact of non-photic zeitgebers on circadian sleep-wake and hormone cycles as well as mood.

To investigate this further, I carried out two experiments, investigating light that is applied to extraocular sites (skull and knee) to assess its influence on circadian rhythms. Previous work on these two main sites are discussed below.

1.18.1 Extraocular Phototransduction in Mammals; Skull Experiments

It has been known for over 100 years that vertebrates, specifically birds, fish and reptiles possess extra-retinal photoreceptor organs in addition to external eyes that are useful for detecting light. For example photoreceptors have been located in the pineal complex, deep brain, skin, cornea and iris (Foster and Soni, 1998). Several types of vertebrate photoreceptors have been described including photosensitive retinal ganglion cells (pRGCs) in lateral eyes, horizontal cells in teleost retina (Peirson, Halford and Foster, 2009), Pinopsin (Avian pineal), VA-opsin and rod-like opsins (exo-rhodopsin/extra-retinal rod-like opsin) in the teleost pineal (Shand and Foster, 1999) and uncharacterised opsin/vitamin A based photopigments in the deep brain of the Japanese quail (Peirson, Halford and Foster, 2009).

In humans, reports have suggested that there may be an extraocular light input to circadian system e.g. behind knee, or transcranial (Campbell, Murphy and Suhner, 2001; Persinger, Dotta and Saroka, 2013). Persinger et al reported
that very bright light (10 000 lux) applied tightly against the left temporal skull, could be measured on the opposite side of the skull (Persinger, Dotta and Saroka, 2013). Another study stimulated brain tissue with light through the ear canal and a change in the functional MRI BOLD signal was assessed (Starck, Nissilä, Aunio, Abou-elseoud, et al., 2012). Results found a gradual increase in functional connectivity in the lateral visual network during the course of the stimulus (Starck, Nissilä, Aunio, Abou-elseoud, et al., 2012) compared to the sham controls.

Transcranial bright light via the ear canals has been used to treat seasonal affective disorder (SAD), raise serotonin levels and measure attention-related brain responses (Timonen et al., 2012; Jurvelin et al., 2014; Sun et al., 2016).

1.18.2 Extraocular Phototransduction in Mammals; Knee Experiments

Campbell’s group studied the effect of bright blue light (400-450nm) via fibre optic pads (Biliblanket ® Plus system) behind the knees (popliteal region) on circadian rhythms, and reported 3 hour light pulses produced phase shifts of body temperature and melatonin (Campbell and Murphy, 1998). Several experiments have since been repeated to look at the effect of light applied to the knee, which have failed to induce circadian phase shifts (Lushington et al., 2002; Wright Jr., 2002; Rüger et al., 2003) Eastman et al investigated this using the same fibre optic pads in three groups 1) 13000 lux of bright extraocular light to the popliteal region, 2) a control with dim light of 0-20 lux
and 3) medium intensity light of 1000 lux. Circadian phase markers- salivary dim light melatonin onset (DLMO) was used. This study found no evidence that popliteal extraocular light had a phase-shifting effect in either experiment (Eastman, Martin and Hebert, 2000). Hébert et al used the same apparatus as Campbell and Murphy and Eastman et al, to investigate the effect of extraocular light exposure on the secretion of nocturnal salivary melatonin in humans. 3 groups were studied; ocular light group, an extraocular light group presenting light behind the knee (with Biliblanket® Plus, Ohmeda Inc) and a control group (Hébert, Martin and Eastman, 1999). Extraocular light exposure again did not suppress melatonin secretion (Hébert, Martin and Eastman, 1999). Lockley et al conducted two studies investigating ocular and extraocular light exposure, measuring urine samples for 6-sulphatoxymelatonin (aMT6s) and core body temperature. No evidence of suppression of plasma melatonin levels was found during 180 minutes of 14000 lux (90 Wm⁻²) and 67, 500 lux (430 W/m²) of popliteal light exposure (Lockley et al., 1998). Wright and Czeisler conducted twenty-two 10-day phase-resetting trials. One of three, 3 hour long interventions was used a) 0 lux ocular and behind the knee light b) 0 lux ocular and up to 13000 lux behind the knee light and lastly 9500 lux ocular light and 0 lux behind the knee (Wright Jr., 2002). The same Biliblanket® Plus, Ohmeda Inc system was used as Campbell and Murphy and phase shifts in melatonin were measured. Although the ocular light exposure produced significant phase shifts this was not found in the light behind the knee group. The Campbell and Murphy study did not measure melatonin phase in the control group and all the subjects eyes were exposed to continuous low
intensities of light during the knee illumination (Campbell and Murphy, 1998; Wright Jr., 2002).

Recent advances in functional MRI have permitted an increase in the resolution with which the human brain can be imaged and this method has therefore been chosen to study the effect of extraocular light on the change in BOLD signal during functional MRI scanning.

1.19 Structural MRI changes in Congenital and Acquired Anophthalmia

Approximately a fifth of the cerebral cortex in the human brain is dedicated to the processing of visual information and in the case of visual loss, this neural tissue may be recruited for other functions. In many cases, however, the visual loss is not complete, leaving some residual visual perception, even if only the ability to perceive light. The most extreme form of visual deprivation occurs when there is no input from the eyes due to their absence, as in anophthalmia.

Congenital anophthalmia without any other neurological impairment provides an ideal population to study the effects of complete visual sensory deprivation on healthy brain tissue. The same is true of acquired bilateral anophthalmia. Comparing subjects with congenital and acquired bilateral anophthalmia thus allows the study of the visual pathway and the whole-brain reorganisation
and/or degeneration in the context of complete vision loss taking place either before (congenital) or after (acquired) visual system development.

Structural brain changes due to both congenital and acquired blindness have been described in grey and white matter. Grey matter atrophy (up to 20-25%) has been noted in primary ‘visual’ areas (Noppeney et al., 2005; Ptito et al., 2008) and extra-striate regions (Ptito et al., 2008) in early blind individuals compared to sighted controls. Reduced white matter volume and FA were found in the optic chiasm, optic nerves and optic radiations, as well as regions of the occipital lobe and corpus callosum (Noppeney et al., 2005; Ptito et al., 2008) in congenital and early-onset blindness, although the global organisation of the splenium remains unchanged (Bock et al., 2013). While there is considerable variability in the patterns of brain structural changes in blindness, some features are consistently present, such as a reduction in white matter microstructure in the optic radiation (Bridge et al., 2009; Wang et al., 2013). However, given these differences are consistent across congenital and acquired blindness, they are likely to result from different plastic processes; altered development in the former case and degeneration in the latter.
2 Aims and Objectives

The broad aims for this project are to study the impact of profound visual loss on a number of ‘extra-visual’ parameters. First, impact on the sleep/wake cycle as may be demonstrated in anophthalmia and diabetic eye disease; second, the hypothesis that there may be EOCP and to investigate the evidence of EOCP in congenitally and acquired anophthalmic individuals, by investigating for any brain activity following bright light stimulation in the ear, into the eyes/head and under the popliteal fossa using a functional MRI scan; and third that in the complete absence of vision, ‘brain rewiring’ may allow adaptation to utilise visual areas for other functions.

The aim of this project is to test the hypothesis that all individuals with bilateral acquired or congenital anophthalmia will have disrupted sleep-wake patterns, free-running hormonal rhythms and disrupted mood irrespective of time since enucleation or integrity of visual pathways. Individuals with bilateral congenital anophthalmia, may have developed coping strategies in response to the impact of circadian disruption on daily life and may have better sleep quality and improved mood compared to those who have become blind later in life. Individuals with anophthalmia who were blinded/lost their eyes early in life within the first 5 years may show changes in their structural MRI features similar to those seen in congenital anophthalmia and that depending on the
timing of eye loss, the structural changes in the acquired group may be more similar to the corresponding controls.

To test this hypothesis, I carried out structural MRI imaging of individuals with congenital and acquired bilateral anophthalmia and compared these to healthy controls, in order to characterise the patterns of degeneration and reorganisation in these two different groups (Chapter 3.4). Specifically, the anterior visual pathway (optic nerve) and cerebral cortex grey and white matter were compared to relative to healthy, sighted control participants.

The specific objectives are listed below:

1. To assess the impact of diabetes and diabetic retinopathy on sleep quality and mood.
2. To assess the impact of congenital and acquired anophthalmia on sleep/wake timing, mood and circadian rhythm.
3. To investigate the presence of extraocular photoreceptors using functional MRI in congenital and acquired anophthalmic participants compared to sighted controls.
4. To investigate structural MRI changes in participants with congenital anophthalmia due to trauma or disease compared to acquired anophthalmia and healthy control
3 Methods
3.1 Assessments of sleep and mood in diabetic retinopathy

3.1.1 Study design

Sleep and mood in diabetic retinopathy was studied as part of “Effect of ocular disease on sleep and body clocks (circadian rhythms)”, a multi-centre study conducted in the NHS (UK) on which I was the study sub-investigator. This was a multi-site study. Participants with diabetes and those with DR were recruited from ophthalmology and specialist diabetic clinics at the Oxford University Hospitals NHS Trust (Oxford), NHS Frimley Health Foundation Trust (Frimley), Buckinghamshire Healthcare NHS Trust (Buck), Royal Berkshire Hospital (Berk) and Centre of Manchester NHS Trust. The study was approved through the Oxfordshire Research Ethics Committee South Central Oxford B (Ref: 11/SC/0093). This study adhered to the tenets of the Declaration of Helsinki. Informed written consent was obtained from those patients willing and able to take part in the study.

I was involved in the study research and development (R&D) applications and applications for the ethics amendment to recruit control participants together with SD and RD, including development of research protocols and control participant leaflets and consent forms (See Appendix). I coordinated the study
between multiple sites with the help of a research assistant (RD) and assisted in the R&D applications for each site.

Data collection was carried out by all the external site principal investigators, myself the Eye Research Group Oxford (RP and RD). All the questionnaire data from external sites was collected personally by RD or myself (Manchester data) and then manually entered into the password protected excel file in the Oxford Clinical research facility by myself, the Eye Research Group Oxford. Preliminary data entry on the excel was checked by myself and where there was missing data, the data was cleaned by the Eye Research Group Oxford. Any participants with a missing DR grading were reviewed by myself. If a Medisoft™ ETDRS grading was available it was entered by the Eye Research Group Oxford otherwise a digital grading was given where fundal images were available by myself.

3.1.2 Participants

Three different groups of participants were recruited to this study:

Participants who had diabetes and evidence of DR but no other ocular pathology.

Participants who had diabetes but had no evidence of DR and no ocular pathology.

Normal healthy controls without any self-reported ocular disease.
296 diabetic participants were recruited between 2011 to 2015 and 175 healthy controls were recruited between 2013-2015, due to delays with obtaining additional ethical approval for healthy controls. 169 diabetic participants and 23 control participants had to be excluded as they did not meet all the inclusion or exclusion criteria (Table 5). A total of 471 participants took part in the study.

A standard proforma was used by all the recruiting centres, (Appendix 10.1). Basic information collected in the proforma included study site, study number, diabetic retinopathy grading, ocular co-pathology e.g. AMD, primary open angle glaucoma, inherited eye disease or optic nerve disease, logMAR visual acuity and additional comments including previous laser, cataract surgery or medication. For all diabetic participants, medical notes on the clinic list (Ophthalmology diabetic clinics and medical diabetic clinics) were screened for inclusion/exclusion criteria before approaching individuals at their clinic appointment. Inclusion criteria included: age greater than or equal to 18 years; proficient in English and a diagnosis of diabetes type I or II. Participants were excluded if they had any coexistent eye disease, previous eye surgery or an intervention that could affect retinal function. Other exclusion criteria were primary or secondary sleep disorders; past or present alcohol or substance abuse; organic physical or psychiatric conditions; prescription of benzodiazepines; head injury; travelling through two or more time zones in the previous fortnight and a history of continued shift work. All controls were required to have a screening Snellen visual acuity of 6/6 or better at recruitment. All eligible participants were invited to take part and signed a study
consent form. General physical and psychological health of all participants was confirmed using our General Health Questionnaire adapted from the Patient Health Questionnaire (Spitzer et al 1999).

Table 5 Showing diabetic and control participants excluded for entry into the study

<table>
<thead>
<tr>
<th></th>
<th>Frimley DR</th>
<th>Berk DR</th>
<th>Buck DR</th>
<th>Oxford DR</th>
<th>Ctrl</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ETDRS</td>
<td>2</td>
<td>4</td>
<td>9</td>
<td>40</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>MH issues</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Qs Incomplete</td>
<td>0</td>
<td>1</td>
<td>13</td>
<td>31</td>
<td>17</td>
<td>62</td>
</tr>
<tr>
<td>Refused</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>25</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Shiftworker</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Anti-depressant</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>10</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Sleep apnoea</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>AMD</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Cancer treatment</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>ERM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CRVO</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>BRVO</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Chronic pain</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>BDZ use</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Morphine use</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>No longer diabetic</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>7</td>
<td>27</td>
<td>133</td>
<td>23</td>
<td>192</td>
</tr>
</tbody>
</table>

Abbreviations: Ctrl, Control participants, MH, mental health, Qs, Questionnaires, AMD, Age related macular degeneration, ERM, Epiretinal membrane, CRVO, central retinal vein occlusion, BRVO, branch retinal vein occlusion, BDZ, benzodiazepine use.

### 3.1.3 Clinical assessment

All study participants with known diabetic retinopathy underwent an ophthalmological examination by the most senior ophthalmologist in clinic. Snellen visual acuity and fundus photos were taken prior to examination. Slit-lamp fundoscopy was carried out in an eye clinic and a diabetic grading given using the diabetic grading software tool on the standard EPR system (Medisoft™). Participants who were attending annual diabetic screening in the community and had a confirmed diabetic screening grade of no retinopathy and
no known ocular disease had a Snellen visual acuity test at 6 metres in the diabetic clinic. This grading had occurred within one year of the study questionnaires being administered.

Healthy volunteers with no underlying medical or ocular disease were recruited. These included healthy staff members, friends or relatives accompanying patients in eye clinics.

The primary end points for this study were investigating sleep quality and quantity in participants with varying grades of diabetic retinopathy compared to healthy volunteers. The secondary end points were measuring anxiety and depression scores in these groups.

Participants were classified in to five groups according to the DR grading of the better eye (Table 6). DR was graded according to the international clinical diabetic retinopathy disease severity scale (Table 7).
### Table 6: Groups of Participants recruited in the DR arm of the study

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls no diabetes</td>
<td>175</td>
</tr>
<tr>
<td>No to mild DR</td>
<td>65</td>
</tr>
<tr>
<td>Moderate non-proliferative DR (NPDR)</td>
<td>128</td>
</tr>
<tr>
<td>Severe NPDR</td>
<td>15</td>
</tr>
<tr>
<td>Proliferative DR (PDR)</td>
<td>88</td>
</tr>
</tbody>
</table>

### Table 7 A table to show the international clinical diabetic retinopathy disease severity scale used to grade retinopathy in individuals with diabetes

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Retinopathy</td>
<td>No retinopathy</td>
</tr>
<tr>
<td>Microaneurysms Only</td>
<td>Mild diabetic retinopathy (DR)</td>
</tr>
<tr>
<td>Microaneurysms plus retinal haemorrhages and/or exudates (lipid deposits and/or</td>
<td>Moderate diabetic retinopathy (DR)</td>
</tr>
<tr>
<td>cotton wool spots)</td>
<td></td>
</tr>
<tr>
<td>At least one of: extensive &gt;20 intraretinal haemorrhages in each of the 4</td>
<td>Severe non proliferative diabetic retinopathy (NPDR)</td>
</tr>
<tr>
<td>quadrants, venous beading in 2+ quadrants, IRMA in 1+ quadrant, Absence of PDR</td>
<td></td>
</tr>
<tr>
<td>Neovascularisation +/- Vitreous/pre-retinal haemorrhage</td>
<td>Proliferative diabetic retinopathy (PDR)</td>
</tr>
</tbody>
</table>
3.1.4 Questionnaires

At recruitment, individuals were asked to complete self-rated questionnaires. In order to account for the impact of Body Mass Index (BMI) which is associated with poor sleep and has a high prevalence in diabetes on sleep scores, BMI was recorded and accounted for in the statistics.

Subjective sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI), which measures sleep quality over the preceding month across seven subscale domains (sleep quality; sleep latency; sleep duration; habitual sleep efficiency; sleep disturbance; use of sleeping medication and daytime dysfunction) (Buysse et al., 1989). These subscales are rated on a scale of 0-3 and their sum represents the global sleep quality score with a range from 0-21, where higher scores indicating poorer sleep quality. Scores of 0-5 are considered as good sleep, while scores above 5 are considered poor sleep quality and greater than or equal to 10 as severely impaired sleep quality score. “Using the cut-off score of 5, the PSQI has a sensitivity of 89.6% and specificity of 86.5% for identifying individuals with a sleep disorder” (Buysse et al., 1989).

Mood scores were recorded using the Hospital Depression and Anxiety Scale (HADS). The HADS is used to estimate the level of anxiety and depression experienced by each individual over the preceding week (Zigmond and Snaith, 1983). “The HADS comprises 14 rated questions on a scale of 0-3, with seven relating to anxiety and seven related to depression with a maximum of 21 for
 each category. Scores up to seven indicate no distress, above that threshold may be indicative of significant anxiety or depression, with a range of 8-10 indicating mild, 11-15 moderate and 16-21 severe distress”. This questionnaire is not a diagnostic tool but it helps to look for confounders as individuals with diabetes tend to have a higher incidence of depression (Anderson et al., 2001).

3.1.5 **Statistical analysis**

PSQI and HADS component scores were evaluated for each DR group and controls, ETDRS VA scores were converted to LOGMAR (RM). All data were manually entered into the Excel and subsequently analysed using R-2.15 (R Development Core Team 2012, University of Auckland, Auckland, New Zealand; available at [http://www.R-project.org](http://www.R-project.org)). Data were fitted to a multilevel linear regression model. All PSQI and HADS scores (including anxiety and depression sub-scores) were log transformed in order to meet the assumption of linearity. The most parsimonious linked mixed-model (using the ordinal library in R) was used to analyse the component scores of the PSQI. Graph Pad Prism version 7.0 for Mac was used for figures and calculation of p values for the sleep component scores. ANOVA test, an unpaired t-test with Welch’s correction and Tukey’s multiple comparison test was used. I present P values with statistical significance assumed when P <0.05.

Final statistical analysis was performed by myself with guidance from IA and Dr Dan Lunn (Statistician at the University of Oxford).
3.2 Assessments of Participants with Anophthalmia on Sleep and Mood

3.2.1 Study design

This was a multi-site study. The study was conducted following ethical approval by South Central-Oxford Research Ethics Committee – Oxford B/11/SC/0093 according to the tenets of the Helsinki agreement. All documentation was provided in Braille, read out verbally or emailed as per the participant’s preference. All the Braille documents were sourced by myself. All participants gave informed consent prior to participation.

I drafted the initial ethics, protocols, patient information sheets and consent forms and R&D submissions and was involved in the subsequent amendments while I was working on the project. I coordinated the Manchester R&D/ethics application and patient involvement, with input from my supervisors (SD) and the administration team (Eye Research Group Oxford). All study paperwork was available to the blind participants in braille, as a paper form to be read out or scanned or an electronic form.

The primary end points for this study were investigating sleep quality and quantity in participants with congenital and acquired anophthalmia compared to healthy volunteers. The secondary end points were measuring anxiety and depression scores in these groups.
3.2.2 Participants

Participants with bilateral anophthalmia and healthy controls were recruited from Manchester Eye Infirmary (9 acquired anophthalmic), Oxford Eye hospital (1 acquired anophthalmic), self-referral (1 congenital anophthalmic) (by RM) and 7 bilaterally congenitally anophthalmic participants were recruited from a previous study (Bridge, Cowey, Ragge, & Watkins, 2009; Bridge, Ragge, Jenkinson, Cowey, & Watkins, 2012).

For individuals with acquired anophthalmia the reason for enucleation, years since enucleation and age of onset of blindness were recorded for individuals. The integrity of the visual system according to previous work (Bridge, Cowey, Ragge, & Watkins, 2009; Bridge, Ragge, Jenkinson, Cowey, & Watkins, 2012) were recorded for individuals with congenital anophthalmia.

Twenty controls (11 male, age 25-61, mean age 36.85 years) were recruited from Oxford University, Oxford NHS trust and Manchester Eye Infirmary (by RM). Exclusion criteria for the control group were sleep apnoea, chronic pain, alcohol abuse, urinary incontinence, renal problems, existing psychiatric disease, foreign travel over two or more time zones in the last 2 weeks, pregnancy or breast feeding.

Anophthalmic and sighted control participants were recruited between 2011 to 2015.
For all the acquired anophthalmic participants, medical notes at the Manchester eye Infirmary and Oxford Eye Hospital were reviewed and participants were screened for inclusion/exclusion criteria before approaching individuals.

Inclusion criteria included: age greater than or equal to 18 years; proficient in English and willing to take part in the study. Participants were excluded if they had organic physical or psychiatric conditions; prescription of benzodiazepines; head injury; and a history of continued shift work. All of the anophthalmic participants except 2 acquired anophthalmic participants had a high alcohol intake and this was accounted for during the statistical analysis. All eligible participants were invited to take part and general physical and psychological health of all participants was confirmed using our General Health Questionnaire adapted from the Patient Health Questionnaire (Spitzer et al 1999).

3.2.3 Questionnaires

Please refer to the Questionnaire methods referred to in section 3.1.4.

3.2.4 Data Analysis

Refer to section 3.1.5

3.2.5 Actigraphy

All the anophthalmic participants were invited to take part in a non-invasive quantitative assessment of sleep/wake timing using actigraphy. Those who
consented were given a wrist actigraph (Camnitech MotionWatch 8 Actiwatch®) to wear 24 hours a day on their non-dominant wrist for minimum period of 2 consecutive weeks and a maximum of 6 weeks. The actiwatch is worn continuously day and night except when the participant is in the bath or shower. The actiwatch cannot be covered with sleeves indoors or outdoors unless there is risk of it getting very wet.

### 3.2.6 Actiwatch

An Actiwatch© is a wristwatch like device which detects wrist movements by means of a piezoelectric acceleration sensor and light levels through an integrated light sensor. This gives information about the correlation between patient’s light exposure and rest or sleep periods. Data obtained from actigraphy show a subject’s circadian rest activity pattern and can indicate disruption in the daily rest activity rhythm. Intrinsic variability among the watches was addressed by ensuring the same watches were used equally by both groups. The MotionWatch has been validated against polysomnography on 70 healthy and suspected sleep disordered subjects.

### 3.2.7 Light recording

The MotionWatch has a built in ambient light sensor. Light recording can be enabled or disabled by checking or unchecking the ‘Record light’ checkbox during set up. For the data collection, this is kept enabled, so that the MotionWatch can store light values. These are stored in lux at the same epoch.
(60 seconds) as the activity samples. The light sensor reads white light with a response optimised to match the human eye.

### 3.2.8 Data collection

During the course of the study the participants’ continued their normal daily routines as much as possible. The watch is used in conjunction with a standardised diary of sleep timings and daily activities that are used to annotate the actigraphy data. The diary entries included a record of bed-time, get-up time, naps, daytime activities and medication for the duration of the actigraphy recording period. Participants were asked to provide very detailed contextual information for 2 consecutive days during the first week. This was used as a real-time ‘bio-calibration’ record for the interpretation of individual actigraphy activity over the succeeding weeks. The consistency and accuracy of diaries were then monitored weekly by email or telephone conversations with the researcher (RM and IA). Diaries were kept by the blind participants via a dictaphone, email, word document (using specialist computer software such as JAWS) or taken daily over the phone by myself or written down by a family member.

Actigraphy data is sampled at one-minute epochs and MotionWare software (version 1.1.22 CamNtech Ltd.) was used to calculate the fragmentation index (an index derived from the frequency and intensity of physical movement during the sleep period, sleep onset latency (SOL; the amount of time between bedtime and sleep onset), wake after sleep onset (WASO; the amount of time...
spent above a predefined activity threshold), total sleep time (TST; time between sleep onset and final wake time, excluding WASO), and variability in sleep onset and sleep duration (measured by standard deviations).

### 3.2.9 Interpreting Actigraphy data

Once data collection is completed the actiwatch is connected to the computer via a USB port and the data is downloaded to a Windows computer (RM and IA). Actigraphs showing the rest-activity and light patterns were generated and analysed with “Actiwatch Activity and Sleep Analysis” software (Actiwatch software version 1.1.3, Cambridge Neurotechnology, UK. When a recording is opened the MotionWise window is displayed as shown below.

The actigraphs were printed and the first stage of analysis involved adding the diary information manually to them. This was to verify concordance with the actigraphy data, to determine ‘bedtime’, ‘get-up time’ and to edit gaps when the watch was removed, for example to avoid water when swimming, showering or bathing. It is important that the investigators had a good knowledge of the participants lifestyle and routine to be able to annotate the actograms as thoroughly as possible. To reduce discrepancies RM annotated all the acquired anophthalmic actigraphs while investigator IA annotated the congenital anophthalmic actograms. These were then cross checked by each other and where required a third opinion was sought from senior circadian scientist KW.

The MotionWise window provides a multiple-day, single or double plotted motion graph (actigraph) within a flexible, scalable and scrollable window. The
MotionWise window is the key tool for the selection of data for more detailed analysis. The top region of the window provides summary patient information. The entire window may be stretched and dragged by manipulating the corners of the window. The days with data are then viewed by using the slider control at the right-hand side of the screen or by using the mouse scroll wheel. The actigraph view can be adjusted for different purposes using the window below (Figure 10).

![Figure 9 MotionWise window display as shown above.](image)

The sleep diary is used to mark sleep and wake times as seen by the red highlighted sections above. Activity is shown by linear peaks. Area of average activity calculated has been shown in the image, where the watch was taken off.
The settings shown above have been used for the analysis of the actigraphs. By selecting 48 hour graphs, a double plotted graph can be viewed. The slider is used to adjust the heights of each row so the data can be easily viewed.

3.2.10 Selecting actigraphy data for Analysis

There are three main selection options which may be chosen by use of the control buttons in the MotionWise window:

By using ‘Select Any’ analysis is more flexible and an area of the actigraph can be selected to analyse the sleep. For purposes of the analysis, select all was only used in participants who did not take any breaks without the watch off, for greater than 3 hours. If there were days when the watch was not worn for greater than 3 hours, the days are excluded from analysis and select day(s) option is selected to analyse the days before the excluded day and the days after the excluded day separately.
When the watch is taken off for less than 3 hours, a period of no activity is seen on the actigraph. This is confirmed with the diary to check the participant took the watch off for example during showers or swimming. In these cases, the area is highlighted and “average for day’ selected to replace the blank area with average activity for the day (see Figure 11).

Figure 11 Image showing method of averaging activity when actiwatch is taken off for less than 3 hours.

Shown above, time period between 7.30 -9.00am has been selected to be ‘averaged for data in day’.
3.2.11 **Output from MotionWare software**

Once the actigraphs are annotated on paper and transcribed to the MotionWare software the results can be pasted onto an excel spreadsheet by selecting Select Tools, Report, Sleep summary table and paste to spreadsheet (RM) (See Figure 12 and Table 8).

![Figure 12 Image showing selection of MotionWare data for conversion to an excel format.](image-url)
3.2.12 **Actigraphy analysis**

Periodogram analysis and cosinor analysis was carried out across the 2 or 3 weeks of rest-activity data with the software El Temps (A. Diez-Noguera, University of Barcelona, Spain; www.el-temps.com/contact.htm) to determine the period length and peak of the rest-activity cycles respectively (by IA).

Automated algorithms within ‘MotionWare’ software (www.camntech.com) were used to obtain sleep-wake parameters, ‘sleep onset’; ‘sleep offset’; ‘sleep period’ (or ‘assumed sleep’, time between sleep onset and sleep offset); ‘total sleep time’ (‘sleep period’ minus periods of ‘wake time’ detected on the actogram); ‘sleep latency’ (the time between ‘bedtime’ and ‘sleep onset’); ‘sleep efficiency’ (percentage of time spent asleep between ‘bedtime’ and ‘sleep onset’); ‘actual sleep (%)’ (Percentage of time spent asleep between ‘sleep onset’ and ‘sleep offset’, thereby separating it from ‘sleep latency’); and ‘fragmentation index’ (this is used as an indicator of restlessness).

Non-parametric circadian rhythm analysis (Van Someren *et al.*, 1999) within MotionWare 1.1.22 was used to generate ‘average 24hr day’ profiles on continuous days (RM for acquired, IA for congenital). When days with incomplete data were excluded, weighted average were calculated. This protocol determined the least active 5 hours (L5), the most active 10 hours (M10). The ratio of M10 and L5 across 24 hours determined the amplitude (from 0-1) with higher values indicating low activity during sleep (L5) and high activity during the day (M10).
Table 8: Sample Output of Data from MotionWare Software for Selected Time Period

<table>
<thead>
<tr>
<th>Start date</th>
<th>03/10/2014</th>
<th>05/10/2014</th>
<th>06/10/2014</th>
<th>07/10/2014</th>
<th>09/10/2014</th>
<th>10/10/2014</th>
<th>12/10/2014</th>
<th>13/10/2014</th>
<th>15/10/2014</th>
<th>16/10/2014</th>
<th>17/10/2014 Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day of week</td>
<td>Friday</td>
<td>Sunday</td>
<td>Monday</td>
<td>Tuesday</td>
<td>Thursday</td>
<td>Friday</td>
<td>Sunday</td>
<td>Monday</td>
<td>Tuesday</td>
<td>Wednesday</td>
<td>Thursday</td>
</tr>
<tr>
<td>Brazil</td>
<td>04/10/2014</td>
<td>05/10/2014</td>
<td>06/10/2014</td>
<td>07/10/2014</td>
<td>09/10/2014</td>
<td>10/10/2014</td>
<td>12/10/2014</td>
<td>13/10/2014</td>
<td>15/10/2014</td>
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<td>Fall asleep</td>
<td>21:58</td>
<td>00:54</td>
<td>00:33</td>
<td>01:34</td>
<td>01:11</td>
<td>23:59</td>
<td>02:16</td>
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<td>00:47</td>
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<tr>
<td>Wake up</td>
<td>06:44</td>
<td>05:30</td>
<td>07:01</td>
<td>09:00</td>
<td>08:17</td>
<td>09:03</td>
<td>10:17</td>
<td>10:29</td>
<td>12:22</td>
<td>10:06</td>
<td>08:54</td>
</tr>
<tr>
<td>Get up</td>
<td>06:44</td>
<td>05:30</td>
<td>07:02</td>
<td>09:02</td>
<td>08:17</td>
<td>09:03</td>
<td>10:17</td>
<td>10:29</td>
<td>12:22</td>
<td>10:06</td>
<td>08:54</td>
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<td>07:28</td>
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<td>08:02</td>
<td>07:44</td>
<td>11:39</td>
<td>09:48</td>
<td>07:53</td>
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<td>04:36</td>
<td>06:28</td>
<td>07:26</td>
<td>07:06</td>
<td>09:04</td>
<td>08:01</td>
<td>07:42</td>
<td>11:35</td>
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<td>08:24</td>
<td>04:29</td>
<td>06:02</td>
<td>06:40</td>
<td>06:30</td>
<td>08:30</td>
<td>07:27</td>
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<td>00:24</td>
<td>01:00</td>
<td>02:27</td>
<td>01:22</td>
<td>00:52</td>
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<td>10.8</td>
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<td>95.8</td>
<td>97.5</td>
<td>93.1</td>
<td>89.3</td>
<td>91.4</td>
<td>93.4</td>
<td>92.7</td>
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<td>16</td>
<td>6</td>
<td>11</td>
<td>19</td>
<td>19</td>
<td>22</td>
<td>14</td>
<td>16</td>
<td>50</td>
<td>33</td>
<td>28.16</td>
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<tr>
<td>Wake bouts</td>
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<td>22</td>
<td>14</td>
<td>16</td>
<td>50</td>
<td>33</td>
<td>28.16</td>
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<td>Mean sleep bout</td>
<td>00:11:30</td>
<td>00:44:50</td>
<td>00:22:55</td>
<td>00:21:03</td>
<td>00:20:32</td>
<td>00:21:31</td>
<td>00:31:56</td>
<td>00:27:22</td>
<td>00:11:30</td>
<td>00:13:22</td>
<td>00:08:28</td>
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<td>Mean wake bout</td>
<td>00:00:22</td>
<td>00:01:24</td>
<td>00:02:36</td>
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<td>00:01:54</td>
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<td>00:01:30</td>
<td>00:02:24</td>
<td>00:04:27</td>
<td>00:02:50</td>
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<tr>
<td>Immobile mins</td>
<td>500</td>
<td>263</td>
<td>357</td>
<td>399</td>
<td>387</td>
<td>492</td>
<td>448</td>
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<td>245</td>
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<td>Immobile time (%)</td>
<td>95.1</td>
<td>95.3</td>
<td>92</td>
<td>89.3</td>
<td>90.8</td>
<td>90.4</td>
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<td>93.5</td>
<td>83.2</td>
<td>73.3</td>
<td>86.8</td>
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<td>Mobile mins</td>
<td>26</td>
<td>13</td>
<td>31</td>
<td>47</td>
<td>39</td>
<td>52</td>
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<td>74</td>
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<td>Mobile time (%)</td>
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<td>4.7</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>16</td>
<td>23</td>
<td>26</td>
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<td>00:12:52</td>
<td>00:13:49</td>
<td>00:13:43</td>
<td>00:17:14</td>
<td>00:20:31</td>
<td>00:08:54</td>
<td>00:08:08</td>
<td>00:14:53</td>
</tr>
<tr>
<td>Immobile bouts &lt;1mm</td>
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<td>4</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>14</td>
<td>16</td>
<td>9.5</td>
</tr>
<tr>
<td>Immobile mins &lt;1mm (%)</td>
<td>4.2</td>
<td>0</td>
<td>21.1</td>
<td>12.9</td>
<td>3.6</td>
<td>9.5</td>
<td>7.7</td>
<td>4.8</td>
<td>21.5</td>
<td>30.2</td>
<td>25.7</td>
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<tr>
<td>Total activity score</td>
<td>1240</td>
<td>382</td>
<td>2604</td>
<td>4389</td>
<td>2003</td>
<td>1873</td>
<td>2755</td>
<td>1443</td>
<td>8730</td>
<td>12278</td>
<td>4394</td>
</tr>
<tr>
<td>Total activity /epoch</td>
<td>2.36</td>
<td>1.38</td>
<td>6.71</td>
<td>5.84</td>
<td>4.7</td>
<td>3.44</td>
<td>5.73</td>
<td>3.56</td>
<td>12.56</td>
<td>20.88</td>
<td>17.77</td>
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<td>Mean nonzero activity /epoch</td>
<td>47.69</td>
<td>29.38</td>
<td>84</td>
<td>91.38</td>
<td>51.36</td>
<td>36.02</td>
<td>83.48</td>
<td>53</td>
<td>74.62</td>
<td>78.2</td>
<td>59.38</td>
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<td>Fragmentation Index</td>
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<td>4.7</td>
<td>29</td>
<td>23.4</td>
<td>12.7</td>
<td>19.1</td>
<td>14.6</td>
<td>11.5</td>
<td>38.4</td>
<td>56.9</td>
<td>48.9</td>
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<td>Threshold</td>
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<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Chapter 3

3.2.13 Melatonin Sampling

The estimated circadian phase of the participants circadian pacemaker was established by measuring weekly profiles of 6-sulphotocytomelanin (aMT6s).

To measure aMT6s, urine was collected by each participant over a 48-hour period for a minimum of 2 consecutive weeks. Each participant was asked to pass a full volume of urine into a pre-labelled bottle with braille every four hours (about eight hours when asleep). Each total voiding was collected and then the volume or weight measured. Two 5ml aliquots of each full urine voiding was kept in small containers and bags labelled in braille. The remainder of the voiding was then disposed. The time, date and total volume of each sample was logged against the braille numbered 5ml aliquots using the same media as used for the sleep diary (3.2.8). Urine volume and sample time was recorded.
by the participant using talking scales, or by a sighted helper of the participant, or an investigator (RM/IA). At the end of the sampling they were sent back to the laboratory at the University of Oxford. They were checked by the two main investigators to ensure adequate filling and any excess was removed and stored frozen at -20 degrees Celsius.

3.2.14 aMT6s analysis

Urinary aMT6s concentrations were determined by radioimmunoassay (Stockgrand Ltd., University of Surrey, UK) by BM (Aldhous and Arendt, 1988). The 24-hour rhythmicity of aMT6s was determined using a macro in excel Windows, Version 9.4 (Minors and Waterhouse, 1989). A non-linear regression model was applied to the data to account for unequally spaced collection times using the following equation: \( MT-c+(Amp*\cos)(2\pi*((t-tc)/T)) \). MT represents the aMT6s secretion rate (ng/h), \( t \) = time. C represents the rhythm adjusted daily mean; Amp=amplitude; \( tc \) = phase angle; and \( T \) period. Results with a cosine fit with a significance of \( p<0.05 \) were included in the analysis (Skene, Lockley and Arendt, 1999). The timing of the aMT6s peaks were estimated for each 48-hour session and these data points were subsequently fitted to a curve. Non-linear regression was performed using SAS software for windows, Version 9.4 (IA).

3.2.15 Statistical analysis

Data analysis comparing the two groups was based on t-tests for independent variables.
3.3 Non-visual phototransduction (extraocular) – Functional MRI Methods

3.3.1 Participants

Five subjects with congenital bilateral anophthalmia (mean age 30 years, range 21-38 years, sex male: female; 4:1) and eight subjects with acquired bilateral anophthalmia (mean age 51 years, range 25-70 years, sex male: female; 3:5) participated in this study (Table 20 for details). Genotyping was available for 2 participants with congenital anophthalmia: case 1 who had a mutation OTX2 and case 6 who had a mutation in NDP. Seventeen sighted controls with normal corrected vision also participated (controls had a Snellen best corrected acuity of 6/6 or better in each eye); nine were recruited as controls for the congenital anophthalmic group (mean age 33 years, range 25-46, male: female; 5:4) and eight as controls for the acquired anophthalmic group (mean age 41 years, range 33-61 years, male: female; 2:6) (by RM). The participants with congenital anophthalmia and their sighted controls were scanned at the University of Oxford (Oxford site), whilst the acquired anophthalmic participants and their controls were scanned at the Central Manchester University Hospitals (Manchester site) (by RM). This study was granted ethical approval by the South Central- Oxford Research Ethics Committee – Oxford B/11/SC/0093 and research and development approval from each site. The ethics amendment together with participant information sheets, study protocol and consent forms were written by RM with guidance from SD and HB. All participants gave...
informed consent prior to participation, and the study was carried out according to the tenets of the Helsinki agreement. Participant information sheets were provided in Braille for blind participants, and read out verbally or emailed as a word document as per the preferred method of communication requested by the participant.

**Table 9 Demographics and blindness information for all subjects with anophthalmia in the non-visual phototransduction study (extraocular).**

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Age blindness onset (years)</th>
<th># years since blind</th>
<th>% of life spent blind</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital 1</td>
<td>Male</td>
<td>34</td>
<td>0</td>
<td>34</td>
<td>100%</td>
</tr>
<tr>
<td>Congenital 2</td>
<td>Female</td>
<td>38</td>
<td>0</td>
<td>38</td>
<td>100%</td>
</tr>
<tr>
<td>Congenital 3</td>
<td>Male</td>
<td>29</td>
<td>0</td>
<td>29</td>
<td>100%</td>
</tr>
<tr>
<td>Congenital 4</td>
<td>Male</td>
<td>30</td>
<td>0</td>
<td>30</td>
<td>100%</td>
</tr>
<tr>
<td>Congenital 5</td>
<td>Male</td>
<td>21</td>
<td>0</td>
<td>21</td>
<td>100%</td>
</tr>
<tr>
<td>Acquired 1</td>
<td>Male</td>
<td>54</td>
<td>2</td>
<td>52</td>
<td>96%</td>
</tr>
<tr>
<td>Acquired 2</td>
<td>Male</td>
<td>47</td>
<td>1</td>
<td>46</td>
<td>98%</td>
</tr>
<tr>
<td>Acquired 3</td>
<td>Male</td>
<td>71</td>
<td>36</td>
<td>35</td>
<td>49%</td>
</tr>
<tr>
<td>Acquired 4</td>
<td>Female</td>
<td>60</td>
<td>40</td>
<td>20</td>
<td>33%</td>
</tr>
<tr>
<td>Acquired 5</td>
<td>Female</td>
<td>50</td>
<td>36</td>
<td>14</td>
<td>28%</td>
</tr>
<tr>
<td>Acquired 6</td>
<td>Female</td>
<td>25</td>
<td>20</td>
<td>5</td>
<td>20%</td>
</tr>
<tr>
<td>Acquired 7</td>
<td>Female</td>
<td>28</td>
<td>21</td>
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<td>25%</td>
</tr>
<tr>
<td>Acquired 8</td>
<td>Female</td>
<td>65</td>
<td>35</td>
<td>30</td>
<td>46%</td>
</tr>
</tbody>
</table>

### 3.3.2 MRI imaging acquisition

**Oxford**

Images were acquired using a Siemens Verio 3-Tesla whole body MRI scanner and a 32-channel coil at the Functional Magnetic Resonance Imaging of the
Brain Centre (University of Oxford). Structural images were acquired at 1 mm isotropic resolution using a T1-weighted MPRAGE sequence (TR=2040 ms, TE=4.7 ms, flip angle=8°, 192 transverse slices, 1 mm isotropic voxels). Four functional images were acquired axially using an echo-planar imaging sequence (TR=2500 ms, TE= 30 ms, 3 mm x 3 mm x 2.5 mm voxels, 43 axial slices, 192 volumes). Scanning protocols were devised by HB and the experiment was planned by RM, SP, SD and HB.

**Manchester**

Images were acquired using a Philips 3-Tesla whole body MRI scanner and a 32-channel coil at the NIHR/Wellcome Trust Clinical Research Facility (University of Manchester). Structural images were acquired at 1 mm isotropic resolution using a T1-weighted MPRAGE sequence (TR=2439 ms, TE= 8.7 ms, flip angle= 8°, 192 transverse slices, 1 mm isotropic voxels). Four functional images were acquired axially using an echo-planar imaging sequence (TR=2500 ms, TE= 30 ms, 3 mm x 3 mm x 2.5 mm voxels, 43 axial slices, 192 volumes).

### 3.3.3 Visual stimulus

All subjects underwent MRI scanning of their brain in a dark room and wore a blindfold. Participants were requested to lie still and try to relax during four fMRI scans. In three of these scans, a KL2500 cold light source (see Figure 7 for spectral power distribution 17.3 Log Quanta or 60.47mW/cm²) via 10 metre MRI safe fibre optic light guide was presented. This light is equivalent to
brighter than daylight on a sunny day. The light guide did not produce any sound or heat. The participants wore in-ear earplugs during the scan session to reduce sounds levels from the MRI machine, as required by safety procedures. All participants were accompanied in the scanner by RM and the light guide was set up for each sequence for all the scans by RM. The light was presented to participants at one of the following locations: behind the right ear (“ear”), just below the nasal bridge and in between the eyes (“head”), and at the right popliteal fossa (“knee”). The stimulus light was manually turned on for 30 seconds and then off for 30 seconds for a total of 8 minutes at each of these locations. In the fourth scan (“no light”), the light source was placed by the participant’s right ear but was never turned on. The light intensity was measured using a spectrophotometer and Ocean Optics Spectra Suite software (by RM and Dr Carina Pothecary). Light readings were taken from the following locations: the light source itself, immediately adjacent to the light source, through the ocular prosthesis, and through the blindfold. No significant light was detected through this blindfold (Figure 14) or through the ocular prosthesis.
Figure 13 Spectral power distribution of the bright light source by KL2500 cold light source

Figure 14 Image showing light measurements taken through the blindfold using the spectrophotometer
3.3.4 Image analysis

3.3.4.1 General Linear Model (GLM)

Functional images were analysed using tools from FSL (FMRIB’s software library, http://www.fmrib.ox.ac.uk/fsl). Pre-processing included motion correction using MCFLIRT (Jenkinson et al., 2002), brain extraction of motion corrected volumes using BET (Smith, 2002) (Figure 15 and Figure 16), smoothing using a smoothing kernel of 6-mm (full-width at half-maximum), registration to each participant’s T1-weighted structural image using BBR (Boundary Based Registration), and then non-linear registration to MNI-152 standard brain using FNIRT. A high-pass temporal filter of 70 seconds was used to remove low-frequency fluctuations (by RM with assistance from GC and HB).

Pre-processed functional images were analysed using FEAT (FMRI Expert Analysis Tool, version 6.00) (Figure 17 and Figure 18). FEAT is a software tool for high quality model-based FMRI data analysis, with an easy-to-use graphical user interface (GUI). FEAT is part of FSL (FMRIB’s Software Library). The FEAT produces a web page analysis report, including colour activation images and time-course plots of data vs. model to produce the first level analysis. The data modelling which FEAT uses is based on general linear modelling (GLM). It allows description of the experimental design; where the brain has activated in response to the stimulus light on vs. stimulus light off.
Motion correction parameters (translations and rotations in x, y and z) as well as the time-series of white matter and CSF BOLD signal were included as covariates of no interest in the general linear model, and thus removed potential motion and physiological noise artefacts from the data. Time series data were extracted from a 3 mm radius sphere within CSF in the anterior lateral ventricle (MNI coordinates: x=+2, y=+10, z=+8) and white matter in the dorsal posterior frontal lobe (MNI coordinates: x=-26, y=-22, z=+28) (Leech, Braga and Sharp, 2012). Group analyses were performed in FEAT using FLAME (FMRIB’s Local Analysis of Mixed Effects) (Figure 19, Figure 20, Figure 21) (by RM and GC).

3.3.4.2 Independent Components Analysis (ICA)

Functional images were pre-processed and registered using the same parameters described above, except the high-pass temporal filter was changed to 150 seconds in order to remove frequencies of less than 0.0067 Hz (Watkins et al., 2012). Independent component analysis (ICA) was performed for each site separately using multi-session temporal concatenation in FSL’s MELODIC software (Beckmann and Smith, 2004) (by GC). This meant that for each site, all pre-processed functional images from all subjects were temporally concatenated and 25 group-averaged components (or networks of correlated BOLD signal) could be identified from the entire dataset. The following seven classical functional networks were clearly identified for both sites: Default Mode Network, Dorsal Attention Network, Left fronto-parietal Network, Right fronto-parietal Network, Visual Network, Auditory Network and Sensorimotor Network.
Next, a dual-regression analysis (Filippini et al., 2009) was used to compare the spatial patterns of these networks across the four experimental conditions (GC performed this analysis with interpretation by HB). Firstly, each scan’s time course for each of the group ICA components was extracted. Secondly, these time courses were used to extract each scan’s spatial map for each of the resting state networks. Finally, these spatial maps were grouped by condition (ear, head, knee, no light) and voxel-wise testing for significant differences between conditions was performed with FSL’s randomised nonparametric permutation testing (5000 permutations) using a t-test, with correction for multiple comparisons carried out using threshold-free cluster enhanced (TFCE, $P < 0.05$) (Nichols and Holmes, 2002). If no group differences were found in the corrected statistical maps, uncorrected t-statistics were examined at to ensure that any subtle differences were not overlooked. ICA and GLM analysis and interpretation was carried out by Dr Gaelle Coullan and Professor Holly Bridge, using the pre-processing steps carried out above (by RM).
Chapter 3

Figure 15 Pre-processing steps using Brain Extraction Tool (BET)
Process to brain extract image on the FSL software, input image is loaded and output image used to rename new image.

Figure 16 Pre-processing output after Brain is extracted as seen on FSL view
Images need to be checked to ensure too much or too little skull is not extracted and image quality is adequate.
Figure 17 FSL window showing the analysis steps to compare functional MRI activity in congenital anophthalmic participants (A0)

FEAT MRI analysis for light behind the ear compared to the knee analysis is shown here. 10 inputs have been entered, i.e. 5 FMRI scans for light behind the ear and 5 FMRI scans for light behind the knee.

Figure 18 FEAT MRI analysis window
Showing functional MRI data selected for congenital anophthalmic participants with light behind the ear compared to light behind the knee under “Select FEAT directories” in Figure 17.
Figure 19 – Group analysis using FLAME showing the statistical model used

Figure 20 - Group analysis using FLAME showing the post stats settings
3.4 Methods for Structural MRI Imaging in Participants with Anophthalmia

3.4.1 Participants

This study was granted ethical approval by the South-Central Oxford Research Ethics Committee (B/11/SC/0093) and all participants gave written informed consent prior to participation. In the case of anophthalmic subjects, Braille consent forms and braille participant information sheets were provided when requested, or the information was emailed to participants in a readable form by computer software such as jaws.

Fourteen participants and 20 control participants were recruited in total. All the acquired anophthalmic participants were recruited from the Manchester Royal Eye Hospital, from the enucleation register and all the congenital anophthalmics were recruited from a previous study by HB and were resident in the Oxford/London region.

Entry criteria was the presence of bilateral clinical anophthalmia, congenital or acquired, age of 18 years or older and ability to consent for the study. Exclusions were; presence of a significant neurological or psychiatric disorder. Of the participants with anophthalmia six were originally classed as congenital bilateral anophthalmia (mean age 29 years, range 21-37 years, 4 males, 2 females). However, after scanning, five were truly bilateral anophthalmic and one had congenital bilateral microphthalmia. Eight had acquired bilateral
anophthalmia (mean age 51 years, range 25-70 years, 3 males; 5 females) (see Table 20 for details). Of the congenital anophthalmic group: case 1 had congenital anophthalmia with a pathogenic variant in the gene OTX2; case 6 had congenital microphthalmia due to Norrie’s disease with a pathogenic variant in the NDP gene. Case 6 with no light perception, was previously believed to be a bilateral congenitally anophthalmic some tiny ocular remnants in two phthisical globes were seen on the MRI. Neither these two cases, nor any of the others had any syndromal, in particular neurological, symptoms or signs and had all completed higher education. Twenty sighted controls with normal (Snellen best corrected visual acuity of 6/6 or better) were also recruited. Of these 12 were controls for the congenital anophthalmia group (mean age 31 years, range 24-46, 6 males, 6 females) and 8 were controls for the acquired anophthalmia group (mean age 41 years, range 33-61 years, 2 males, 6 females). Scanning was carried out at two sites: participants with congenital anophthalmia and their sighted controls at the University of Oxford (Oxford site); and acquired anophthalmia participants and their controls at the University of Manchester (Manchester site). Different scanning sites were used due to the geographic location of the congenital and anophthalmic participant groups.
Figure 21 Group analysis using FLAME
Figure showing the next stage of data analysis for FMRI images in acquired anophthalmics with light on the head compared to no light behind ear. 14 FMRI images have been selected (7 for light on the head and 7 for no light).

3.4.2 MR imaging acquisition

Oxford Images were acquired using a Siemens Verio 3-Tesla whole body MRI scanner and a 32-channel coil at the Functional Magnetic Resonance Imaging of the Brain Centre (University of Oxford). Structural images were acquired at 1 mm isotropic resolution using a T1-weighted MPRAGE sequence (TR=2040 ms, TE=4.7 ms, flip angle=8°, 192 transverse slices, 1 mm isotropic voxels). Diffusion-weighted images were acquired axially using echo-planar imaging with 2 mm$^3$ isotropic voxels. The diffusion weighting was isotropically
distributed through space along 60 directions using a b-value of 1500 s/mm². Four volumes with no diffusion weighting were acquired during the beginning of each sequence (See Appendix for Protocols).

Manchester Images were acquired using a Philips 3-Tesla whole body MRI scanner and a 32-channel coil at the NIHR/Wellcome Trust Clinical Research Facility (University of Manchester). Structural images were acquired at 1 mm isotropic resolution using a T1-weighted MPRAGE sequence (TR=2439 ms, TE=8.7 ms, flip angle=8°, 192 transverse slices, 1 mm isotropic voxels). Diffusion-weighted images were acquired axially using echo-planar imaging with 2 mm³ isotropic voxels. The diffusion weighting was isotropically distributed through space along 61 directions using a b-value of 1500 s/mm². One volume with no diffusion weighting was acquired during each sequence.

3.4.3 Data analysis

Structural MRI data were analysed using voxel-based morphometry in order to investigate voxel-wise differences in local grey matter volume between groups, specifically FSL-VBM (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM), an optimised VBM protocol (Good et al., 2001) carried out with FSL tools (Smith et al., 2004). Images from each site (Oxford and Manchester) were analysed separately. First, structural images were brain-extracted, tissue-type segmented and registered to MNI-152 standard space using non-linear registration (Andersson, Jenkinson and Smith, 2007) (by RM with instruction from HB). Each brain is
manually checked to ensure that only all non-brain tissue is removed and no brain tissue is removed.

The resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific grey matter template. Second, all native grey matter images were non-linearly re-registered to this study-specific template and "modulated" to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated grey matter images were then smoothed with an isotropic Gaussian kernel (3 mm sigma) (GC and HB).

Given the small number of subjects, we used the same procedure as a previous paper (Bridge et al., 2009) where uncorrected t-statistics (t = 3.0), corresponding to p< 0.001, were used as a threshold measure, with the additional constraint that in cortical regions outside of the occipital cortex differences should be bilateral as there are no prior hypotheses. As a further constraint only clusters containing 30 or more voxels are shown in the figures (GC and HB).

Diffusion data were analysed by calculating fractional anisotropy (FA) maps and comparing local FA between blind and sighted groups. FA is a measure of diffusion direction strength and can be used as an indication of fibre density. Voxel-wise statistical analysis of the FA data was carried out using TBSS (Tract-Based Spatial Statistics (Smith, Jenkinson and Johansen-Berg, 2006),
part of FSL (Smith et al., 2004). Images from each site (Oxford and Manchester) were analysed separately. First, FA images were created by fitting a tensor model to the raw diffusion data using FDT and then brain-extracting each image. All subjects' FA data were then aligned to MNI-152 standard space using the non-linear registration (Andersson, Jenkinson and Smith, 2007). Next, the mean FA image was created and thinned to create a mean FA skeleton that represents the centres of all tracts common to the group. Each subject's aligned FA data was then projected onto this skeleton (shown in green in figures) and the resulting data fed into voxel-wise cross-subject statistics. A similar procedure was undertaken to quantify differences in mean diffusivity (MD).

For both grey matter volume and FA, voxel-wise GLM was applied using permutation-based non-parametric testing (5000 permutations) to investigate significant differences between the 'anophthalmic' and control groups. However, as the acquired anophthalmic and Manchester controls were not well age matched, age was added as a covariate of no interest in both VBM and TBSS design matrices. An additional VBM and TBSS design was used to investigate the negative effect of blindness years in the Manchester data; demeaned percentage of total lifetime spent blind was calculated for each subject (0% for Manchester controls, see Table 14 for acquired anophthalmics). The Juelich Histological Atlas, Harvard-Oxford Cortical Structural Atlas and JUH White-Matter Tractography Atlas as implemented in
fslview (version 3.2.0) were used to identify structures and regions of interest for further analyses.

**Optic Nerve Analysis**

Structural T1-weighted MPRAGE images were used to assess the integrity of the optic nerve in congenital and acquired anophthalmia. In order to quantitatively compare optic nerve integrity across subject groups, a left and right optic nerve mask was made for each subject along one axial slice where the nerve appeared to be at its thickest. The volume (mm$^3$) of these masks was extracted and mean volume for all four subject groups was compared.

**Cortical thickness**

Structural T1-weighted MPRAGE images were also analysed using Freesurfer (Dale, Fischl and Sereno, 1999) ([http://surfer.nmr.mgh.harvard.edu](http://surfer.nmr.mgh.harvard.edu)). The images were segmented into tissue types (Fischl and Dale, 2000) and average cortical thickness values were calculated from those areas that can be reliably defined based on anatomical features (Brodmann area 17, extrastriate area MT, primary somatosensory cortex: Brodmann areas 3a and 3b) and primary motor cortex: Brodmann areas 4a and 4p).
4 Diabetic Retinopathy and Sleep

Results & Discussion
4.1 Introduction

One of the main aims of this thesis is to study the implications of DR on sleep/wake cycle. With an increasing prevalence of diabetes, morbidity from DR will continue to increase. Sleep problems are known to cause additional health problems in addition to reduced immunity and increase insulin resistance. This study will examine the effect of DR on subjective sleep quality scores and mood.

4.2 Participants

Data for 175 controls and 296 DR participants with type II diabetes was analysed. There were 4 DR groups, mild DR n=65, moderate DR n=128, severe non-proliferative DR (NPDR) n=15 and proliferative DR (PDR) >60 n=88. The BMI was significantly higher in individuals with DR (mean 29.21) compared to controls (mean 25.90), p<0.001, using an unpaired t-test with Welch’s correction (Figure 22). There was a significant difference between BMI in the controls and mild DR (p=0.0005), moderate DR (p=0.0007) and PDR (p=0.001). There was no significant difference in the BMI between individual DR groups (p>0.05). No significant difference was found between age of the controls and each DR group (p>0.05). An ANOVA test and Tukey’s multiple comparison test was also carried out.
There was no significant difference found in PSQI scores between the diabetic groups and control groups \( p > 0.05 \), however the mean PSQI was lower among the control group compared to the diabetic groups. This analysis was conducted using a multiple regression model and studying the eye with the lowest DR score. (Analysis was repeated using the worst eye and the average DR category for both eyes, and no significant difference between different levels of DR and PSQI score was found using each method.) Data presented in this thesis uses the DR score for the better eye, thus assuming less retinal damage/loss and thus less possible loss of pRGCs to help regulate the sleep/wake cycle.

The mean PSQI scores for each group were; Controls: 4.8 (±3.1SD), mild DR: 5.49 (±3.8SD), moderate DR: 5.04 (±3.6SD), severe non-proliferative DR (NPDR): 6.53 (±4.6SD) and proliferative DR (PDR): 5.10 (±3.9SD).
NPDR group had a slightly higher mean PSQI compared to the other DR groups, however this group only had 15 participants, 5 of whom had a mean PSQI >9 misrepresenting the final result. All the groups had the highest number of participants with a PSQI score within normal range (0-5) (Table 11).

Participants with a PSQI score of greater than or equal to 10 have a worse visual acuity score (p=0.046) however this isn’t significant for those with a PSQI score of greater than 5. There was no significant difference in visual acuity scores between the DR groups. A raised depression score (p<0.001), raised anxiety score (p<0.01) and a raised BMI (p<0.0001) was associated with a raised PSQI score. Having a history of heart disease, respiratory disease, a physical disability, psychiatric disease or hospitalisation in the last year and living situation were not associated with an increase incidence of poor sleep.
Table 10 Demographics of Diabetics and Controls

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Mild DR</th>
<th>Moderate DR NPDR</th>
<th>Severe DR</th>
<th>Proliferative DR</th>
<th>All DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (male/female)</td>
<td>175 (123/52)</td>
<td>65 (14/51)</td>
<td>128 (49/79)</td>
<td>15 (5/10)</td>
<td>88 (33/55)</td>
<td>296 (101/195)</td>
</tr>
<tr>
<td>Age (mean, SD)</td>
<td>57.61 (17.8)</td>
<td>61.57 (14.1)</td>
<td>58.85 (13.7)</td>
<td>57.8 (12.7)</td>
<td>54.31 (12.8)</td>
<td>58.07 (13.8)</td>
</tr>
<tr>
<td>Mean BMI (SD)</td>
<td>25.90 (5.2)</td>
<td>29.72 (6.8)</td>
<td>28.93 (7.4)</td>
<td>29.22 (9.2)</td>
<td>29.24 (6.8)</td>
<td>29.21 (7.2)</td>
</tr>
<tr>
<td>Normal BMI (20-25)</td>
<td>87 (49.7%)</td>
<td>13 (20%)</td>
<td>32 (25.6%)</td>
<td>5 (33.3%)</td>
<td>14 (15.9%)</td>
<td>64 (21.7%)</td>
</tr>
<tr>
<td>Underweight (&lt;20)</td>
<td>11 (6.3%)</td>
<td>1 (1.5%)</td>
<td>5 (3.9%)</td>
<td>1 (6.7%)</td>
<td>4 (4.6%)</td>
<td>11 (3.7%)</td>
</tr>
<tr>
<td>Overweight (26-30)</td>
<td>48 (27.4%)</td>
<td>33 (50.8%)</td>
<td>44 (34.4%)</td>
<td>3 (20%)</td>
<td>34 (38.6%)</td>
<td>114 (38.6%)</td>
</tr>
<tr>
<td>Obese (&gt;30)</td>
<td>29 (16.6%)</td>
<td>18 (27.7%)</td>
<td>47 (36.7%)</td>
<td>6 (40%)</td>
<td>36 (40.9%)</td>
<td>107 (36.3%)</td>
</tr>
</tbody>
</table>

Top row shows the total number of participants in each DR group (N) with the male to female ratio in brackets. Second and third rows show the mean age and mean BMI in each DR group with standard deviations in brackets. Fourth, fifth, sixth and seventh rows have the number of participants in each BMI group with the percentage of the whole group in brackets.
Table 11 Percentage of participants within normal PSQI scores, >5 and >9 in Groups 0, 1, 2, 3 and 4.

<table>
<thead>
<tr>
<th>Group</th>
<th>PSQI score 1-5</th>
<th>PSQI &gt;5</th>
<th>PSQI&gt;9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>114 (65.1%)</td>
<td>43 (24.6%)</td>
<td>18 (10.3%)</td>
</tr>
<tr>
<td>Mild DR</td>
<td>38 (58.5%)</td>
<td>1 (1.54%)</td>
<td>26 (40%)</td>
</tr>
<tr>
<td>Moderate DR</td>
<td>83 (64.8%)</td>
<td>10 (0.78%)</td>
<td>35 (27.3%)</td>
</tr>
<tr>
<td>Severe NPDR</td>
<td>6 (40%)</td>
<td>5 (33.3%)</td>
<td>4 (26.7%)</td>
</tr>
<tr>
<td>PDR</td>
<td>60 (68.2%)</td>
<td>17 (19.3%)</td>
<td>11 (12.5%)</td>
</tr>
</tbody>
</table>

Table showing the number of participants in each group with the percentage of total shown in brackets

Table 12 PSQI component scores, HADS-Anxiety (HADS-A), HADS-Depression (HADS-D) and HADS score for Controls and all DR groups.

<table>
<thead>
<tr>
<th>PSQI and subscales</th>
<th>Controls</th>
<th>Mild DR</th>
<th>Moderate DR</th>
<th>Severe NPDR</th>
<th>PDR</th>
<th>All DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSQI</td>
<td>4.80 (3.1)</td>
<td>5.49 (3.8)</td>
<td>5.04 (3.6)</td>
<td>6.53 (4.6)</td>
<td>5.10 (3.9)</td>
<td>5.23 (3.8)</td>
</tr>
<tr>
<td>Quality</td>
<td>0.92 (0.8)</td>
<td>0.96 (0.8)</td>
<td>0.91 (0.8)</td>
<td>1.33 (1.0)</td>
<td>0.93 (0.8)</td>
<td>0.95 (0.8)</td>
</tr>
<tr>
<td>Latency</td>
<td>0.72 (0.6)</td>
<td>0.94 (0.9)</td>
<td>0.72 (0.8)</td>
<td>1.00 (0.9)</td>
<td>0.84 (0.9)</td>
<td>0.82 (0.8)</td>
</tr>
<tr>
<td>Duration</td>
<td>0.51 (0.7)</td>
<td>0.70 (1.0)</td>
<td>0.74 (1.1)</td>
<td>0.73 (0.9)</td>
<td>0.73 (1.1)</td>
<td>0.73 (1.1)</td>
</tr>
<tr>
<td>Efficiency</td>
<td>0.65 (0.9)</td>
<td>1.0 (1.3)</td>
<td>0.88 (1.3)</td>
<td>0.87 (1.3)</td>
<td>0.78 (1.1)</td>
<td>0.88 (1.2)</td>
</tr>
<tr>
<td>Medication</td>
<td>0.15 (0.6)</td>
<td>0.07 (0.4)</td>
<td>0.07 (0.4)</td>
<td>0.13 (0.5)</td>
<td>0.08 (0.5)</td>
<td>0.08 (0.4)</td>
</tr>
<tr>
<td>Daytime Dysfunction</td>
<td>0.65 (0.7)</td>
<td>0.57 (0.9)</td>
<td>0.53 (0.8)</td>
<td>0.93 (1.2)</td>
<td>0.47 (0.8)</td>
<td>0.54 (0.9)</td>
</tr>
<tr>
<td>HADS-A</td>
<td>5.38 (3.2)</td>
<td>3.27 (2.7)</td>
<td>3.50 (3.0)</td>
<td>3.93 (3.1)</td>
<td>3.77 (3.3)</td>
<td>3.55 (3.0)</td>
</tr>
<tr>
<td>HADS-D</td>
<td>2.49 (2.3)</td>
<td>2.78 (2.1)</td>
<td>2.39 (2.8)</td>
<td>2.67 (3.4)</td>
<td>3.07 (3.1)</td>
<td>2.69 (2.9)</td>
</tr>
<tr>
<td>HADS</td>
<td>7.83 (4.8)</td>
<td>6.04 (3.9)</td>
<td>5.88 (5.1)</td>
<td>6.60 (5.9)</td>
<td>6.84 (5.6)</td>
<td>6.24 (5.1)</td>
</tr>
</tbody>
</table>

Mean values shown in each box with standard deviations in brackets

There was no significant difference in the sleep parameters sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance or daytime dysfunction (Figure 23) between groups controls and the DR groups. The
mean sleep quality score for controls was 0.92 and 0.95 for the all DR group (p=0.40) (mild DR, 0.96 (±0.79sd), moderate DR, 0.91 (±0.70sd), severe NPDR 1.33 (±0.98sd), PDR, 0.93 (±0.83sd), (Table 12). The sleep duration (0.51 ± 0.74sd) and sleep efficiency (0.65 ±0.93sd) scores were better among the control groups than among all of the DR groups (0.73 ± 1.04sd) and (0.88 ± 1.16sd), but not significantly different (p = 0.23 and p = 0.18 respectively).

Mean sleep disturbance in control group was 1.20 (±0.47sd) and 1.25 (±0.55sd) in all DR groups (mild DR, 1.25 (±0.56sd), moderate DR, 1.19 (±0.53sd), severe NPDR 1.53 (±0.52sd), PDR, 1.27 (±0.58sd). There was no significant difference in values for sleep medication between controls and DR groups (p=0.61) or within individual groups.

4.4 HADS and DR severity

A raised HADS score of >7 was significantly associated with a raised PSQI score (p<0.001), however mean HADS score, anxiety and depression scores remained within normal range for all DR groups (Figure 25, Table 12). The control group had a marginally higher mean HADS Score of 7.83 (±4.83sd) and a higher anxiety score of 5.38 (±3.19sd), p>0.05. Irrespective of the presence of DR, those participants with a higher BMI tend to have higher anxiety and depression scores (p<0.01).
Figure 23 Frequency distribution for PSQI category and sleep components.

(A) PSQI category. Subjects with scores >5 were classified as having a global poor sleep quality and >9 as very poor sleep quality. (B-H) show PSQI components.

B; Sleep quality C; Sleep latency D; Sleep duration E; Sleep efficiency F; Sleep disturbance G; Sleep Medication H; Daytime dysfunction. F-H not; not in the past month, less; less than once a week, once; once or twice a week, three; three or more times a week. There is no significant difference between control groups and DR groups in A-H (p>0.05).
Chapter 4

**A**

Bar chart showing the distribution of hours spent in bed with a peak around 7-8 hours.

**B**

Bar chart showing the distribution of hours spent in bed with a peak around 8-9 hours.
Figure 24 Frequency distribution histograms for self-reported sleep patterns derived from the PSQI in control and all DR groups.
A and B show the number of hours in bed for the control group and all DR group binned by 1-hour intervals from 2-10 hours. C and D show bedtime frequency distribution binned by 1-hour intervals from 20h to after 3h in the control and all DR group. E and F show wake up time frequency distribution binned by 1-hour intervals from 03h to 11h. There is no significant difference in the two groups for bedtime, wake up time or self-reported sleep duration.
Figure 25 Mean PSQI, Mean HADS, Mean HADS-Anxiety (an) and Mean HADS-Depression (dep) in controls and 4 DR groups

Blue bar shows mean PSQI scores in each group with the lowest score in the controls (p>0.05). Orange bars showing mean HADS scores in each group with the highest peak in the control group (p>0.05). Grey bars showing HADS-anxiety scores in all groups (highest mean anxiety score in control group p>0.05) and yellow bars showing HADS-depression scores in all groups with no significant difference within all the groups. Error bars showing standard errors.
4.5 Discussion Diabetic Retinopathy and Sleep

The aim was to investigate the impact of DR on subjective sleep and wake and mood. This is the second study to investigate the relationship between diabetic retinopathy and sleep in a large cohort. This data shows no relationship between sleep-wake disturbance and mood and DR grade. Jee et al. investigated sleep and DR in 1670 subjects over 40 years old (Jee et al., 2017). They found that DR increased in male participants with ≤ 5 hours or ≥ 9 hours of sleep compared to those with 6-8 hours of sleep (Jee et al., 2017). This difference was not found among diabetic women in the group. By contrast, this data does not show a significant difference between sleep duration and DR grading regardless of the sex. (Statistical analysis using a general linear model in the software programme R using the most parsimonious linked mixed-model).

There was no significant difference in HADS score, anxiety or depression scores among the DR groups and control groups. A raised HADS score was however significantly associated with having a raised PSQI score (p<0.001). This is expected as sleep is known to be linked to anxiety and depression (Reynolds and Kupfer, 1987; Ford and Kamerow, 1989; Stein, Kroft and Walker, 1993; Billiard et al., 1994; Gregory et al., 2006b; Alfano, Ginsburg and Kingery, 2007).
We can therefore conclude that even though some studies have shown a significant relationship between diabetes and poor sleep (Luyster and Dunbar-Jacob, 2011), this may be as a result of factors not dependent of the grade of retinopathy or potential damage to the pRGCs.

Studies in animal models of glaucoma, mitochondrial optic neuropathies such as Leber’s hereditary optic neuropathy and dominant optic atrophy have shown that pRGCs are more resistant to injury (Cui et al., 2015). Jakobs et al found that despite the fact that pRGCs are among the largest ganglion cells in the mouse retina, in glaucoma models the decrease in numbers of these pRGCs was proportionately less than for the total number of ganglion cells (Jakobs, 2005). It has also been reported in animal studies where the optic nerve is transected, that the pRGCs have a three times greater survival compared to normal ganglion cells (Robinson and Madison, 2004). From this, we can deduce that despite extensive DR damage, only a small number of RGCs may be affected thus not affecting sleep/wake cycles.

The second hypothesis is that in DR, laser treatment aims to preserve the central retina. pRGCs represent 0.63-0.75% of the total 1-1.2 million ganglion cells and are distributed throughout the human retina at approximately 7 cells/mm² (Hannibal and Fahrenkrug, 2004). Hannibal et al found pRGCs were highest in the central retina close to the fovea (34.4 cells/mm²), which is the area treated cautiously in diabetic retinopathy. First line treatment for proliferative diabetic retinopathy is extensive peripheral laser with relative
preservation of the macula region. This is to allow sparing of visual function and the central retina. As a result, there may still be sufficient pRGCs remaining to maintain a healthy sleep/wake cycle as our data suggest.

The visual acuity of the DR groups in the best eye ranged from logMAR -0.1 to 0.6, with only one participant having 0.6 (Snellen acuity 6/24) and -0.1 to hand movements (HM) in the worse eye with one participant having HM vision and 4 having counting fingers vision. There were no truly severe DR cases with PL or NPL vision. Using visual acuity as an indication of the retinal damage, most of the DR cases in the study cohort had some preservation of the retina. There is also strong evidence from the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) (Keen, 1994; King, Peacock and Donnelly, 1999) that intensive glycaemic control can prevent or delay the development or progress of diabetic retinopathy (Keen, 1994; Cull et al., 2007). Tighter diabetic control, the national retinopathy screening programme and regular General Practitioner and hospital follow up in the UK have resulted in a smaller population having severe complications of diabetes, including DR.

In conclusion, this data did not show a significant difference in the global sleep scores or anxiety and depression scores between the 5 groups. Individuals with severe DR still retain a subjective sleep quality similar to those with moderate or mild or no DR and a non-diabetic population. This is encouraging news for an ageing population with an increasing prevalence of diabetes.
4.6 Limitations of the diabetic retinopathy study

This study is a large study and further data collection and analysis is still ongoing. The data used for the purposes of the thesis has some limitations. Firstly, the controls are not appropriately matched. The control group has 70% males compared to females while the whole DR group only has 34% males compared to females. 50% of the controls had a normal BMI (range 20-25) whilst the DR group has over 70% of the population within the overweight (BMI 26-30) and obese groups (BMI>30).

The data presented in this thesis has an unequal distribution of participants in each group in particular group 3 with moderate DR. Reasons for using the DR grading in the best eye is because I hypothesised that participants with severe retinopathy, would have damage to the pRGCs and in turn a disturbance to the sleep wake cycle. In view of this, the grading from the better eye was used to indirectly measure the impact of remaining healthy retina (and in turn pRGC function) on the sleep wake cycle.

The controls used in the study were predominantly relatives sitting in the waiting room accompanying patients attending for an ophthalmic/diabetic examination. It could be argued that these controls are subject to bias. It is well known that carers of patients with mental health issues are at higher risks of anxiety and stress (Wooff et al., 2003; Cooper et al., 2008). Diabetes has a
significant impact on life and can be associated with several co-morbidities and frequent hospital visits. Another chronic condition, chronic obstructive pulmonary disease (COPD) was studied where anxiety and depression among 203 family carers of people with COPD was examined using a HADS score (Jácome et al., 2014). 63.5% carers had a clinically significant raised HADS-anxiety score of >7, 34% had a significantly raised HADS-depression score >7 and 27.1% had both (Jácome et al., 2014). Another concern is that if the controls are partners of DR participants, they will be subject to similar environments. They may both sleep and wake up at the same time and in turn disturb each other’s sleep, affecting the sleep parameters.

The grading completed by ophthalmologists at each unit was used as the final grading. DR grading can vary from one clinician to another however consistency was maintained by ensuring the most senior ophthalmologist examined each study participant. Any participants without a grading were graded by RM using fundus photos.

Retinal function can be assessed using a wide array of assessments including visual acuity scores, visual fields, fluorescein angiograms to look at ischaemic areas, OCT and OCT angiography and microperimetry. Baseline investigations in diabetic clinics are visual acuity assessments and clinical assessment of DR grading, which are commonly used indicators for disease. Recent studies indicate better indicators to measure residual retinal function and perhaps
further assessments could have been done on our participants (Okada et al., 2006; Pearce, Sivaprasad and Chong, 2014; Edington et al., 2016).
5 Sleep/Wake Disruption in Subjects with Anophthalmia – Results & Discussion
5.1 Introduction

In the previous chapter, I discussed the impact of DR on sleep using subjective PSQI and HADS scores. This group of subjects have intact eyes and LogMAR visual acuity in the worst eye ranged from -0.1 to HM. It is important to establish the effect of retinal disease such as DR on sleep quality prior to assessing the impact if there is no retinal function and the complete absence of eyes.

The current chapter concentrates on sleep/wake disorders in a group of participants with congenital and acquired anophthalmia. Anophthalmia is a traumatic and debilitating condition that can affect an individual from birth (congenitally) or later in life (acquired). It can be unilateral or bilateral. The impact of bilateral visual loss from the condition is considerable socially and also has potential health implications from its effect on the sleep/wake cycle.

The subsequent sections of the chapter concentrate on the investigation of sleep/wake problems in participants with anophthalmia. Firstly, the subjective sleep quality (PSQI) and hospital anxiety and depression scores are assessed compared to healthy sighted controls. Next, the sleep/wake patterns will be measured using actigraphy and supported by 48-hour urinary melatonin (aMT6) sampling.
5.2 Participants

40 participants were recruited in total in the anophthalmia and control group. 2 participants with acquired anophthalmia were excluded. One did not complete the questionnaires and the second had severe learning difficulties and epilepsy and was on anti-epileptic treatment, which affected his sleep. 8 congenital anophthalamic participants, 10 acquired anophthalmic participants (eyes removed between 5-46 years ago) and 20 healthy sighted controls successfully completed the PSQI and HADS questionnaires. Data for co-existing health conditions was obtained using a general health questionnaire (GHQ).

Table 13 shows there was no significant difference in the BMI between the control groups and the acquired or congenital groups, however there was a significant difference in the mean age between the acquired anophthalmic participants and controls (p=0.02).

| Table 13 Demographics for Controls, Congenital and Acquired anophthalmic participants |
|-----------------------------------|-----|---------|------------------|------------------|
| Gender (M: F) | Mean Age | P value (compare to controls) | Mean BMI | P value (compare to controls) |
| Control | 9: 11 | 36.9 | - | 26.0 | - |
| Congenital | 5: 3 | 31.6 | 0.52 | 30.0 | 0.24 |
| Acquired | 3: 7 | 49.3 | 0.02* | 27.3 | 0.82 |

Ordinary one-way ANOVA test with Tukey’s multiple comparison used * marks statistically significant p<0.05
Figure 26 Frequency distribution for BMI category in controls, acquired anophthalmics (A1) and congenital anophthalmics (A1).

There is no significant difference between the 3 groups, p>0.05.
Table 14 Demographics and blindness information for all anophthalmic cases (Age in 2015).

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Age anophthalmia onset (years)</th>
<th>No of years since anophthalmic</th>
<th>% of life spent anophthalmic</th>
</tr>
</thead>
<tbody>
<tr>
<td>A0_01</td>
<td>Male</td>
<td>30</td>
<td>0</td>
<td>30</td>
<td>100%</td>
</tr>
<tr>
<td>A0_02</td>
<td>Female</td>
<td>26</td>
<td>0</td>
<td>26</td>
<td>100%</td>
</tr>
<tr>
<td>A0_03</td>
<td>Female</td>
<td>38</td>
<td>0</td>
<td>38</td>
<td>100%</td>
</tr>
<tr>
<td>A0_04</td>
<td>Male</td>
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<td>0</td>
<td>31</td>
<td>100%</td>
</tr>
<tr>
<td>A0_05</td>
<td>Male</td>
<td>35</td>
<td>0</td>
<td>35</td>
<td>100%</td>
</tr>
<tr>
<td>A0_06</td>
<td>Male</td>
<td>23</td>
<td>0</td>
<td>23</td>
<td>100%</td>
</tr>
<tr>
<td>A0_07</td>
<td>Female</td>
<td>44</td>
<td>0</td>
<td>44</td>
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</tr>
<tr>
<td>A0_08</td>
<td>Male</td>
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<td>0</td>
<td>26</td>
<td>100%</td>
</tr>
<tr>
<td>A1_01</td>
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<td>91.4 %</td>
</tr>
<tr>
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<td>54</td>
<td>2</td>
<td>52</td>
<td>96 %</td>
</tr>
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<td>1</td>
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<td>9</td>
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<td>65%</td>
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</tr>
<tr>
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<td>36</td>
<td>14</td>
<td>28 %</td>
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<tr>
<td>A1_08</td>
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<td>25</td>
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<td>5</td>
<td>20 %</td>
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<tr>
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<td>A1_10</td>
<td>Female</td>
<td>65</td>
<td>35</td>
<td>30</td>
<td>46 %</td>
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</table>
5.3 Subjective Sleep Scores (PSQI) Results

Poor sleep (global sleep score ≥5) was observed in all ten (100%) participants with acquired anophthalmia, 50% of individuals with congenital anophthalmia and 35% of control participants. The data show that individuals with acquired anophthalmia have higher global subjective PSQI scores (p<0.001 and p<0.0001), then individuals with congenital anophthalmia or sighted controls respectively. There was no significant difference between sleep scores of individuals with congenital anophthalmia and control participants (p>0.05). The amount of time that has elapsed since the enucleation does not have an impact on rest/activity patterns or on the PSQI and HADS scores (linear regression model using R). Although the acquired anophthalmic participants have disrupted sleep; the congenital anophthalmic patients and normal controls have similar PSQI scores (p=0.27). All individuals only conducted the PSQI questionnaires once during the study. This does not take into account any changes that may occur in the perception of sleep quality as a result of the participants being in phase or out of phase with their internal circadian rhythm. If participants carried out the questionnaires when their internal clock was aligned with the external clock they may perceive better sleep quality in the last month leading to a lower PSQI score compared to later in the sleep cycle when the internal clock and external clock may be the most misaligned.

Individuals with acquired anophthalmia had worse global sleep quality than controls (p<0.01) but there was no significant difference in those with congenital anophthalmia compared to controls. Participants with acquired
anophthalmia had a shorter sleep duration than controls (p<0.0001) than individuals with congenital anophthalmia (p<0.0005), worse sleep efficiency than controls (p<0.0005) and individuals with congenital anophthalmia (p<0.0005). Individuals with acquired anophthalmia had worse daytime dysfunction than controls (p=0.05) but there was no significant difference in daytime dysfunction between controls and congenital participants (p>0.05). There was no significant difference in sleep latency or the use of sleep medication in the 3 groups. Ordinary one-way ANOVA with Tukey’s multiple comparisons test was carried out. The acquired anophthalmic group also have a 3.45 times chance of having a higher PSQI score than the congenital or control group, p<0.0001.

5.4 Mood – Hospital Anxiety and Depression Score

Half of the acquired anophthalmic participants had a raised HADS-A score (HADS-Anxiety) however none of them had a high depression score. The mean HADS scores in each group was 11.4, 6 and 9.1 for the acquired, congenital and control groups with no significant difference between the three groups (p>0.05). There was no significant difference between the anxiety and depression scores of individuals with congenital and acquired anophthalmia and controls (Table 16)(RM).
Table 15 Global PSQI and mean sleep component scores with p values for differences in participants with acquired anophthalmia compared to congenital and control participants, and congenital anophthalmia participants with controls.

<table>
<thead>
<tr>
<th>Component Scores</th>
<th>Significant Differences (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global PSQI (H)</td>
<td>=0.0014**</td>
</tr>
<tr>
<td>Subjective Sleep Quality (PSQI)</td>
<td></td>
</tr>
<tr>
<td>Sleep Quality (A)</td>
<td>Acquired 1.7</td>
</tr>
<tr>
<td>Sleep Latency (B)</td>
<td>1.2</td>
</tr>
<tr>
<td>Sleep Duration (C)</td>
<td>2.2</td>
</tr>
<tr>
<td>Sleep Efficiency (D)</td>
<td>2.3</td>
</tr>
<tr>
<td>Sleep Disturbance (E)</td>
<td>1.4</td>
</tr>
<tr>
<td>Sleep Medication (F)</td>
<td>0.9</td>
</tr>
<tr>
<td>Daytime Dysfunction (G)</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* Indicates significant differences.
Figure 27 Frequency distribution for PSQI category and sleep components.

(A) PSQI category. Subjects with scores >5 were classified as having a global poor sleep quality and >9 as very poor sleep quality. (B-H) show PSQI components.

B; Sleep quality C; Sleep latency D; Sleep duration E; Sleep efficiency F; Sleep disturbance G; Sleep Medication H; Daytime dysfunction. F-H not; not in the past month, less; less than once a week, once; once or twice a week, three; three or more times a week.
Figure 28 Frequency distribution histograms for self-reported sleep duration derived from the PSQI.

Top: showing sleep duration in subjects in the congenital anophthalmic group (A0), Middle: showing sleep duration in subjects in the acquired anophthalmic group (A1), Bottom: showing sleep duration in subjects in the control group.
Table 16 Hospital anxiety and depression score (HADS), and its components cores, HADS-Anxiety (HADS-A) and HADS-Depression (HADS-D) for groups with anophthalmia and controls

<table>
<thead>
<tr>
<th>Component Scores</th>
<th>Significant Differences (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acquired</td>
</tr>
<tr>
<td>HADS (A)</td>
<td>11.4</td>
</tr>
<tr>
<td>HADS-A (B)</td>
<td>7.78</td>
</tr>
<tr>
<td>HADS-D (C)</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Figure 29 Bar graphs showing mean HADS-anxiety, HADS-depression and total HADS score for controls, congenital and acquired anophthalmic participants (p>0.05).
Error bars represent standard error (RM). Ordinary one-way ANOVA with Tukey’s multiple comparisons test was carried out.
5.5 Sleep-Wake Monitoring (Actigraphy) in Anophthalmic Participants

Actigraphy was completed in 8 individuals (mean age 55 years, 3 male) with acquired anophthalmia and 6 individuals (mean age 29.66 years, 4 male) with congenital anophthalmia. There was no significant difference in actigraphy sleep parameters between those with acquired and those with congenital anophthalmia. There was no significant difference in sleep onset, sleep offset, assumed sleep time, total sleep time, sleep efficiency, fragmentation index, L5 (least active 5 h period) and M10 (most active 10 h period) in the two groups with anophthalmia Table 17.

Below are some representative examples of phenotypic variability in rest-activity patterns derived from wrist activity monitoring in the home environment.

| Table 17 - Comparisons of Sleep Parameters (time in decimal) between the Groups with Congenital and Acquired Anophthalmia (Mann Whitney U test). |
|---------------------------------|---------------------------------|-----------------|
| Actigraphy Sleep Parameters     | Mean Sleep Parameter Scores (SD)/ Significance |
| Acquired (n=8)                  | Congenital (n=6)                 | P Value         |
| Sleep onset                     | 23.83 (1.52)                     | 1.31 (1.16)     | 0.1419           |
| Sleep offset                    | 7.85 (1.92)                      | 7.48 (2.16)     | 0.8518           |
| Assumed Sleep                   | 6.55 (1.15)                      | 7.07 (1.24)     | 0.1812           |
| Total sleep time                | 5.572 (1.16)                     | 6.47 (1.69)     | 0.1812           |
| Actual Sleep (%)                | 86.29 (5.97)                     | 81.86 (5.25)    | 0.2824           |
Standard deviation shown in brackets. (Results analysis by IA)

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep efficiency</td>
<td>85.85</td>
<td>79.08</td>
<td>0.1812</td>
</tr>
<tr>
<td>Fragmentation index</td>
<td>27.7</td>
<td>26.74</td>
<td>0.8518</td>
</tr>
<tr>
<td>L5</td>
<td>1297.94</td>
<td>1672</td>
<td>0.2824</td>
</tr>
<tr>
<td>M10</td>
<td>12151.33</td>
<td>11561.5</td>
<td>0.8518</td>
</tr>
</tbody>
</table>

Figure 30 Showing actigraphy of a congenital anophthalmic A0_01 with no ocular structures.

The PSQI score is normal (4). A; structural MRI scans of visual apparatus shows no ocular structures (Bridge et al., 2009), B; Actigraphs on the left are double plotted according to the time of day and study day. Actigraph for participant with some resemblance of a regular sleep/wake cycle marked by red lines, * marks disruption to the pattern from an early wake up-time from setting the alarm early for a planned journey B; Actigraph of the same participant with an irregular/fragmented sleep/wake pattern (courtesy of IA)
Figure 31 Actigraphy of congenital anophthalmic A0_01 with a graph showing the weekly 48-hour melatonin profiles.

Subjects collected sequential 4 to 8 hourly urine samples for 48 hours per week over 3 consequent weeks. The melatonin period is 24.9 resembling a non-24h sleep-wake rhythm disorder.
Figure 32 Showing actigraphy of a congenital ‘anophthalmic’ participant A0_03 with some residual ocular structures.

A; Actigraph showing participant with a non-entrained sleep/wake pattern which breaks down marked by * and shown by arrow. Sleep/wake patterns then become more regular as marked by red lines (IA) B; Graph showing weekly 48-hour melatonin profiles over 3 weeks (prepared by RM) C; Structural MRI showing some residual ocular structures and optic nerve (RM Chapter 4)
Figure 33 Showing a congenital anophthalmic participant A0_05 with a normal PSQI score (2) and an indeterminable circadian type due to low amplitude melatonin and significant melatonin data for 1 week only.

A; Actigraph with red lines showing approximate sleep and wake up times, actigraphy period is 24 hours (IA)

B; Graph showing weekly 48-hour melatonin profiles over 3 weeks with a low amplitude and a variable pattern. A melatonin period of 23.84 for 1 week (p value >0.05 for 2 weeks) (graph prepared by RM, data IA) C; MRI scan with no visual apparatus visible (Bridge et al., 2009)
Figure 34 Actigraphs of two acquired anophthalmic participants A; A1.03 and B; A1.10

A; There is no regular sleep/wake pattern on the actigraph (no melatonin data available) B; A1.10 Participant’s actigraph initially resembles a non-24h sleep-wake rhythm disorder then seems to become entrained with more consistent sleep and wake up times. Actigraphy period is 23.66 however sufficient urine data was not available to calculate melatonin period (Data acquired by RM)
Figure 35 Actigraphs of two acquired participants A1_02 and A1_05 with a high PSQI score, anophthalmic for 52 years (96% life) and 35 years (49% of life).

A and B both show regular sleep/wake patterns as seen by the areas marked in red and the participants appear entrained however the actigraphy period is 23.91 and 24. Melatonin period is 26.02 and 25.8 respectively, i.e. there is a non-24h melatonin rhythm. These two subjects A1_02 and A1_05 have a strict wake up and bed time because of work and family life respectively explaining the actigraphy patterns. (Data acquired by RM)
Figure 36 Actigraphs (above A1_04, below A1_07) with 48-hour melatonin peaks over 3 weeks

Both participants take regular naps as shown by the shorter red periods before the night-time sleep period. Grey areas represent the normal range of peak melatonin production for aMT6s (1.00-7.00 hours). The melatonin periods are 24.54 and 24.7 respectively, consistent with a non-24h sleep-wake rhythm disorder. Both participants have a flexible daily routine (Data acquired by RM)
5.6 Results from 48-hour urine melatonin

Of the 8 individuals with acquired anophthalmia who completed the actigraphy, 7 completed the melatonin collection. Of the 6 individuals with congenital anophthalmia who completed actigraphy, 4 successfully completed the melatonin collection. 1 of these was excluded due to taking exogenous melatonin during the collection period. Analysis was completed on 3 congenital anophthalmic participants.

Five individuals with acquired anophthalmia had an aMT6s period outside the normal range (23.88–24.12h) (Wright et al., 2001). Two participants with anophthalmia A1_09 and A1_10 could not have the circadian period calculated as the p value was >0.05 and A1_10 only had adequate urine aMT6 data for 1 week (Table 18).

A0_05 only had significant data for 1 week (p <0.05), and the melatonin period (24.9) and acrophase (15.67 ±1.096) for this week is outside the normal range and characteristic of a free-running period. However, where a single value is obtained, it is not possible to determine if it is normal, abnormal or part of a non-24h sleep-wake rhythm disorder or entrained rhythm (Lockley et al., 1997). Observation of the raw data points therefore, showed a low amplitude and also are suggestive of free-running pattern (Figure 33).
Chapter 5

There was no significant difference (p=0.24) between the melatonin period for those with acquired anophthalmia (24.35 ± 0.55) compared to those with congenital anophthalmia (25.17 ± 0.32), unpaired t-test. However, the number of participants in both groups are small, especially in the congenitally anophthalmic group. The melatonin analysis was completed with assistance from IA and BM.

Figure 37 Graph showing the mean melatonin period for the group with congenital anophthalmia (A0) compared to participants with acquired anophthalmia (A1) and standard error (SEM).

Mean Period in A0 is 24.35 ± 0.55 SEM and A1 is 25.17 ± 0.32 SEM, p = 0.24 with an unpaired t-test (RM).

Table 18 Results of the Mesor, Amplitude (Amp), lower and upper standard deviation (S.D), Acrotime (TC) and Period of aMT6s over three collection points.

<table>
<thead>
<tr>
<th>ID</th>
<th>p value</th>
<th>Mesor</th>
<th>TC</th>
<th>Am</th>
<th>lower</th>
<th>upper</th>
<th>Period</th>
</tr>
</thead>
<tbody>
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<td>Value (3)</td>
<td>Value (4)</td>
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<td>-----------</td>
<td>-----------</td>
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<td>20.33</td>
<td>32.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2285</td>
<td>607.50</td>
<td>28.46</td>
<td>473.9</td>
<td>23.31</td>
<td>33.62</td>
<td></td>
</tr>
<tr>
<td>A1_10</td>
<td>0.8076</td>
<td>72.62</td>
<td>20.01</td>
<td>17.1</td>
<td>6.83</td>
<td>33.19</td>
<td><strong>NS</strong></td>
</tr>
</tbody>
</table>

ID “A1” represent the acquired anophthalmic participants, “A0” represents the congenital anophthalmic participants. A0_05 only significant data available for 1 week (Prepared by IA)
Table 19 Mean Acrophase, Melatonin Period, Circadian Type, Mean Melatonin Amplitude, Actigraphy Period and Time of Data Collection

<table>
<thead>
<tr>
<th>Subject</th>
<th>Mean Acrophase (+/-SD)</th>
<th>Melatonin Period</th>
<th>Circadian Type</th>
<th>Mean amplitude</th>
<th>Actigraphy Period</th>
<th>Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A0_01</td>
<td>15.69 (5.054)</td>
<td>24.9*</td>
<td>free-running</td>
<td>1001</td>
<td>24.08</td>
<td>3</td>
</tr>
<tr>
<td>A0_03</td>
<td>6.977 (1.253)</td>
<td>23.84</td>
<td>free-running</td>
<td>793.133</td>
<td>23.91</td>
<td>3</td>
</tr>
<tr>
<td>A0_05</td>
<td>15.67 (1.096)</td>
<td>23.84</td>
<td>? Insufficient data</td>
<td>19.89</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>A1_02</td>
<td>16.33 (5.735)</td>
<td>26.02*</td>
<td>free-running</td>
<td>697.233</td>
<td>23.91</td>
<td>3</td>
</tr>
<tr>
<td>A1_04</td>
<td>23.51 (2.338)</td>
<td>24.54*</td>
<td>free-running</td>
<td>796.9667</td>
<td>24.08</td>
<td>3</td>
</tr>
<tr>
<td>A1_05</td>
<td>26.91 (3.571)</td>
<td>25.8</td>
<td>free-running</td>
<td>45.705</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>A1_06</td>
<td>16.19 (1.919)</td>
<td>24.78*</td>
<td>free-running</td>
<td>419.3</td>
<td>23.83*</td>
<td>3</td>
</tr>
<tr>
<td>A1_07</td>
<td>23.9 (2.1)</td>
<td>24.7*</td>
<td>free-running</td>
<td>949.3</td>
<td>24.08</td>
<td>2</td>
</tr>
<tr>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>23.66</td>
<td>0</td>
</tr>
</tbody>
</table>

* Marks significant melatonin periods. A0_05 had very low melatonin amplitude and data for only 1 week gave a significant result. Ideally 3 weeks of data is required (Data by IA)
A0_03 has a circadian period just outside the ‘normal’ range however it is very close to 24 hours (23.84h). The mean peak in melatonin for week 1 and 3 is within a normal range at 3.27 h (min 2.02 and max 4.51) and 2.32 h (min 1.19 and 3.47). However, week 2 was 4.81 h (min 2.07 and 7.56). Week 2 melatonin range is slightly outside the normal range (1.3-7.1 h clock time) (Lockley et al., 1997). The circadian period close to 24 h and the normal range of melatonin peak for week 1 and 2 could explain why the actigraphy appears abnormally phased and not non-entrained (Figure 32).

5.7 Discussion of Sleep/Wake disruption and Mood in Participants with Anophthalmia

The aim of this project was to test the hypothesis that all individuals with bilateral acquired or congenital anophthalmia would have disrupted sleep-wake patterns, free-running hormonal rhythms and disrupted mood irrespective of time since enucleation or integrity of visual pathways. The data confirms that the complete absence of eyes from anophthalmia results in non-entrained rhythms, as seen by all the subjects and if the loss of eyes occurred after birth, it is accompanied by a high PSQI score.

All the participants with acquired anophthalmia had a raised PSQI score but not the participants with congenital anophthalmia. This could be considered surprising but might be explained as the anophthalmic participants have been blind since birth and are unaware of any ‘change’ to their current subjective
sleep quality. The participants with acquired anophthalmia however have had a period with both eyes present and varying visual acuity to a definite period with no light perception and loss of their eyes from enucleation. This means that they are be able to assess the change in their subjective sleep quality since the enucleation more accurately.

Lockley et al investigated 49 blind subjects, from the 30 NPL, 11 were bilaterally anophthalmic (Lockley et al., 1997). In their data, there was no significant difference in the PSQI score between the PL group and NPL group and 86% of the whole blind group had a PSQI ≥5 (Lockley et al., 1997). Tabandeh et al evaluated sleep quality using PSQI in 388 blind subjects and 44 normal sighted subjects (Tabandeh et al., 1998). 189 (48.7%) of the blind subjects had a raised PSQI score, however the prevalence was higher in the NPL group and the sleep disturbance was higher than in those with LP or better acuity (Tabandeh et al., 1998). In their data 65% of the NPL group (38/54) had a PSQI score ≥6 compared to 45% (151/330) of the PL group and 9% (4/44) of the sighted controls (Tabandeh et al., 1998).

Another group evaluated sleep in blind using a 48-item questionnaire, Stanford Questionnaire and Evaluation of Wakefulness (SQAW) (Leger et al., 1999). In their group, blind subjects reported a reduced sleep duration, a worse sleep latency and at least 1 sleep problem was reported in 82.7% of the blind subjects (Leger et al., 1999). Almost a third of the blind population reported waking up more than three times a night (Leger et al., 1999). Even though a
different questionnaire method was used to those in this study, these results are similar to those found in the anophthalmic group. The acquired anophthalmic participants had a reduced sleep efficiency, reduced sleep duration and reduced sleep quality compared to the control group (and reduced sleep efficiency and duration compared to congenital anophthalmic participants). 65% of the blind group in Leger et al’s study felt their sleep was linked to depression and anxiety and 63.2 % of the controls (Leger et al., 1999). There was no significant difference in anxiety and depression among the blind and control groups in this study.

We report an estimated prevalence of sleep problem of 78% among the blind group in this data (14/18) (congenital and acquired anophthalmia). This is similar to that reported in the previous studies.

### 5.8 Sleep-Wake Monitoring (Actigraphy) in Anophthalmic Participants

Flynn-Evans et al did not use sleep timing or duration information to classify circadian rhythm disorders. This is because there is a high prevalence of sleep disturbance among the blind who do not have misaligned rhythms (Lockley et al., 1997; Tabandeh et al., 1998; Leger et al., 1999; Flynn-Evans et al., 2014). Also sleep/wake cycles often correlate well with the circadian period in entrained and non-entrained individuals. In blind individuals with free-running rhythms, the sleep/wake activity either greatly underestimates the endogenous
period or indicates a 24-h sleep rhythm (Lockley, Skene and Arendt, 1999). It is suggested that this discrepancy is likely as blind people still try to sleep in the night and wake in the day despite their internal circadian phase. This can be seen in case examples A0_01 (Figure 31), A1_02 and A1_05 (Figure 35). Participant A0_01’s sleep/wake cycle has no particular rhythms however the actigraphy period is very close to 24 hours (24.08 h) but the melatonin period is 24.9 h. He has a flexible work routine and can schedule meetings in the evenings, resulting in the irregular sleep/wake patterns.

Examples of the two participants with acquired anophthalmia, A1_02 and A1_05 both have a raised PSQI score of 11 and 9 respectively. The actigraphy shows a regular sleep/wake cycle and actigraphy periods close to 24 hours (23.91 h and 24h). They both have strict wake up and bed times as they live with family, but the melatonin period (26.02 and 25.8) and mean acrophase (16.33 ±5.73 and 26.91 ±3.57) corresponding to a non-24h sleep-wake rhythm disorder. Participants A1_04 and A1_07 (Figure 36) have flexible schedules and they both have regular naps. Again, both have circadian periods close to 24 h (24.08 h) but melatonin periods (24.54 h and 24.7 h) and mean acrophase (23.51± 2.34 and 23.9 ±2.1) outside normal range indicative of a non-24h sleep-wake rhythm disorder.

In view of the findings described, strategies to help improve sleep in this group need to be in place. These include good sleep hygiene. Regular get up and bed times, sleep in a quiet (and normally dark) environment, regular meals,
reduction in alcohol and caffeine and the use of an alarm clock can help to regulate the sleep/wake cycle despite the internal circadian period.

5.9 Circadian type and Melatonin rhythms

Melatonin rhythms are the strongest marker of the internal circadian period and not subject to external factors such as the sleep/wake cycle (Flynn-Evans et al., 2014).

The period for all the participants with anophthalmia was non entrained and ranged from 23.8 to 26.02. 6 participants had a value above 24.12 and 2 participants below 23.88. All the participants with anophthalmia had a free-running circadian type. Two cases were excluded and are discussed (A0_03 and A0_05).

A0_03 has a circadian period very close to 24 hours (23.84 hours) which is therefore very difficult to distinguish from a non-entrained rhythm. Looking at the second half of the actigraphy, the rhythm has definite sleep and wake up times (shown by the red lines) as would be expected of an abnormally entrained rhythm (Figure 32). Secondly, it is possible that a longer course of data collection over several weeks would show significant changes in the circadian phase. Davitt et al. also reported that many blind appear to be continually free running, so that sometimes their sleep-wake activity cycles will be in phase with the external environment but not at other times (Davitt et al.,
Thirdly, although light is the strongest zeitgeber in humans, it has been reported that other non-photic zeitgebers in humans can help to entrain some individuals to a 24 hour cycle (Klerman et al., 1998; Mistlberger and Skene, 2004). This individual lives independently and has a job and a busy social life, all the factors that may helping to entrain the sleep/wake cycle. Additionally, Lockley et al mentioned that individuals who aren’t truly anophthalmic and have vestigial tissues, may have enough retinal tissue to maintain the integrity of the retina-RHT-SCN pathway (Lockley et al., 1997). The next chapter of this thesis examines light transduction through a cosmetic shell through a spectrophotometer, and no light can pass through it, therefore would block any potential entrainment from the vestigial retinal tissue. Secondly, humans do not have the presence of extraocular circadian photoreceptors (EOCP) (6.4).

This data is in keeping with previous studies on participants with NPL vision (Lockley et al., 1997; Lockley, Arendt and Skene, 2007; Flynn-Evans et al., 2014). Flynn-Evans et al studied 12 bilateral acquired anophthalmic participants and found 4 subjects were normally phased, 3 abnormally phased and 5 were non-entrained. Lockley et al found 76% of the subjects with NPL (28/37) had either non-entrained or abnormally entrained rhythms and from the 11 bilaterally enucleated participants 91% were free-running (Lockley et al., 1997; Lockley, Arendt and Skene, 2007).
5.10 Limitations of Sleep/Wake disruption & Anophthalmia Study

The main limitation of this study is the small number of anophthalmic participants. These participants were recruited after contacting all the ophthalmologists in the UK and the small number reflects the rarity of the condition and difficulties with accessing records from non-NHS patients that attend the ocular prosthetic departments only. As a result, the age and the sex in the groups could not be matched equally.

The interpretation and annotations of the actograms is very subjective especially for ‘noisy’ actograms. Accuracy of the diary, participant compliance and investigator bias can affect the result. Steps were taken to reduce any inaccuracies (3.2.9) and to allow for a rigid analysis process for all the actograms. Participant compliance was also excellent in all subjects included in the analysis. One example is for participant A1_05. He lived a very sedentary life and often fell asleep while watching television. The main issue was that it was difficult to identify particular activity on the actograms such as wake up or sleep time. This is because the participant always lay in bed listening to radio prior to bed and on waking. Annotations of the actograms relied on a detailed diary and looking for subtle peaks in activity when he moved to turn the radio on/off before going back to bed.
Participants were recruited in this study titled “Impact of ocular diseases on sleep/wake cycle”. The title of the study may have attracted individuals (including controls) with sleep disorders to complete the study, compared to those who only completed the subjective sleep questionnaires.

Circadian rhythms vary and a subject’s rhythm may be aligned and then the rhythm shifts becoming free running. This has been shown in some of the examples. The ethics for this study covered a sampling period of 3 weeks however ideally actigraphy and aMT6 recording should be done over a longer period of 12 weeks or even a year. 3 weeks was chosen as the cut off as this is the standard time used in most circadian studies and because of the impact of a prolonged study on the participants lifestyle.

Urine sampling was used as this was the most convenient method to be used for the participants with anophthalmia. The method does not disturb sleep and is fairly non-invasive. Saliva sampling is a much easier method of collection. However, this was not feasible in totally blind participants as they are not aware of the external light conditions and collection is required in dim light conditions. Collection of plasma aMT6 is the most invasive, requiring regular blood samples with 48-hour medical access.
6 Evidence of Extraocular Circadian Photoreceptors in Anophthalmia - Functional MRI Results & Discussion
6.1 Introduction

The previous chapter discussed the sleep/wake patterns in subjects with anophthalmia. Sleep/wake patterns together with other circadian rhythms such as cortisol and temperature regulation are driven by the endogenous circadian clocks that require daily adjustment, to synchronise them to the external environment. As discussed in the previous chapter, the non-visual photoreceptors (pRGC) help to synchronise the internal clock to the environmental light signal or other entraining signals in its absence.

Extra-retinal photoreceptors in invertebrates and non-mammals can also entrain circadian rhythms (Foster and Soni, 1998). However, in mammals there is no evidence of the presence of EOCP. Due to the lack of such evidence, this study investigates for any changes in the fMRI BOLD signal following extraocular bright light stimulation in sighted blindfolded subjects and subjects who have the absence of eyes from congenital or acquired anophthalmia.
### 6.2 Participants

<table>
<thead>
<tr>
<th>Congenital Anophthalmia</th>
<th>Gender</th>
<th>Age</th>
<th>Clinical description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital 1</td>
<td>M</td>
<td>34</td>
<td>OTX2 mutation-bilateral anophthalmia</td>
</tr>
<tr>
<td>Congenital 2</td>
<td>F</td>
<td>38</td>
<td>Isolated bilateral anophthalmia, family history of microphthalmia</td>
</tr>
<tr>
<td>Congenital 3</td>
<td>M</td>
<td>29</td>
<td>Bilateral microphthalmia, NDP mutation</td>
</tr>
<tr>
<td>Congenital 4</td>
<td>M</td>
<td>30</td>
<td>Isolated bilateral anophthalmia</td>
</tr>
<tr>
<td>Congenital 5</td>
<td>M</td>
<td>21</td>
<td>Isolated bilateral anophthalmia, no family history</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acquired Anophthalmia</th>
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<th></th>
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</thead>
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<tr>
<td>Acquired 1</td>
<td>M</td>
<td>54</td>
<td>Retinoblastoma. Bilateral enucleation by age 2</td>
</tr>
<tr>
<td>Acquired 2</td>
<td>M</td>
<td>47</td>
<td>Retinoblastoma. Bilateral enucleation by age 1</td>
</tr>
<tr>
<td>Acquired 3</td>
<td>M</td>
<td>71</td>
<td>Right eye; trauma age 6. Left eye lost age 36 from sympathetic ophthalmia</td>
</tr>
<tr>
<td>Acquired 4</td>
<td>F</td>
<td>60</td>
<td>Bilateral evisceration. Lost eyes age 40</td>
</tr>
<tr>
<td>Acquired 5</td>
<td>F</td>
<td>50</td>
<td>Sighted till age 4. Bilateral enucleation age 40</td>
</tr>
<tr>
<td>Acquired 6</td>
<td>F</td>
<td>25</td>
<td>Peter’s anomaly. Enucleation age 18</td>
</tr>
<tr>
<td>Acquired 7</td>
<td>F</td>
<td>28</td>
<td>Retinopathy of prematurity and congenital glaucoma. Early blind age 4</td>
</tr>
<tr>
<td>Acquired 8</td>
<td>F</td>
<td>65</td>
<td>Retinopathy of prematurity and congenital glaucoma. Bilateral enucleation; right eye age 16 left eye age 38</td>
</tr>
</tbody>
</table>

Age is at the time of scan
6.3 Results of FMRI analysis is Anophthalmia

FMRI data were analysed using two separate approaches to assess whether extra-ocular light presentation elicits more brain activity (compared to no light presentation) in both sighted and anophthalmic subjects. Firstly, a traditional model-driven GLM was used to model the 30 sec ON/OFF blocks in each experimental condition. Secondly, data-driven ICA was used to remove noise artefacts and extract functional networks from the entire fMRI dataset, followed by dual regression to compare the networks’ spatial patterns across the four experimental conditions.

6.3.1 General Linear Model (GLM)

A general linear model (GLM) was used to model 30 second blocks in the Ear, Head, Knee and No Light experimental conditions. These blocks corresponded to light ON and light OFF in the Ear, Head and Knee light conditions, and were also applied to the No Light condition in order to control for artefacts or signal which may have arisen during these 30 second blocks but were unrelated to extra-ocular light presentation. Figure 38 shows whole-brain results of light ON vs. light OFF for all extra-ocular light conditions combined (Ear, Head, Knee) in each of the four subject groups (congenital controls, congenital anophthalmia, acquired controls, acquired anophthalmia). The largest cluster is in the congenital control group, located in the region of the primary somatosensory cortex [42,-16,56]. Some activity was found in the occipital cortex of both the
acquired anophthalmia [+8,-92,+6] and congenital anophthalmia [-8,-74,+8] groups. However, all of these reported clusters are uncorrected with a very low threshold of $Z > 2.3$. No clusters survive correction for multiple comparisons.

Furthermore, similar small clusters appear when applying the same block design to the no light condition. This condition was intended as a control since subjects were scanned entirely in darkness with no visual stimulation. Figure 39 shows whole-brain results for the no light condition for each of the four subject groups (congenital controls, congenital anophthalmia, acquired controls, acquired anophthalmia), using the same uncorrected low threshold used in Figure 38. Panel A shows results when applying the same 30 ON/OFF model used for the light conditions in Figure 38. Some clusters of “activity” are found in most subject groups, especially across the visual occipital cortex in the acquired control group. Interestingly, when a 15 sec ON/OFF model was applied to the same No Light dataset (panel B), these clusters of “activity” disappear or appear in different locations in all subject groups.

Finally, in order to assess whether group differences (sighted vs. anophthalmia) in the four experimental conditions could be found in a “visual” region of interest level, mean percentage BOLD signal change was extracted from the primary visual cortex (V1, defined by the Juelich Histological Atlas as implemented in FSLview and thresholded at 30%). Figure 40 shows group mean (panel A) and median (panel B) results across the four experimental conditions for each anophthalmic group and their respective sighted controls.
V1 percentage BOLD change in the acquired control group during the no Light condition matches the cluster of visual cortex activity found at a whole-brain level in Figure 39 (panel A). Otherwise, considerable individual variability in each group precludes any clear trends being drawn from these graphs. Individual variability is most notable in the knee location; the difference between the Knee mean (panel A) and median (panel B) group values for the congenital anophthalmia and acquired anophthalmia groups suggest that some subjects in these groups have high percentage BOLD signal change in V1 whilst others do not.

6.3.2 Independent Component Analysis (ICA)

Independent component analysis of all functional data (Ear, Head, Knee, No Light) for each site separately (Oxford and Manchester) revealed the following classical functional networks: Default Mode Network, Dorsal Attention Network, Left Frontoparietal Network, Right Frontoparietal Network, Visual Network, Auditory Network and Sensorimotor Network. These networks are shown for each site separately in Figure 41. The spatial pattern of each of these networks is similar across the two sites.

Next, a dual regression approach was used to investigate network differences between the four experimental conditions (Ear, Head, Knee, No Light), specifically in networks likely to include the visual cortex. These were identified as the Visual Network (Figure 41, panel E) and the Default Mode Network.
Figure 41, panel A). Figure 42 shows the brain regions that contribute to the Visual Network in all four experimental conditions (panel A) as well as differences between conditions using contrasts of the three light conditions (Ear, Head, Knee) > No Light condition (panel B). Figure 43 shows the brain regions that contribute to the Default Mode Network in all four conditions (panel A) as well as differences between conditions using contrasts of the three light conditions (Ear, Head, Knee) > No Light condition (panel B). All 14 subjects from the Oxford site (congenital controls and congenital anophthalmia) are shown in the left column and all 16 subjects from the Manchester site (acquired controls and acquired anophthalmia) are shown in the right column. In both the Visual (Figure 42, panel A) and Default Mode (Figure 43, panel A) networks, brain areas contributing to these networks look virtually identical across all four experimental conditions (Ear, Head, Knee and No Light). The light > no light contrasts reveal some small clusters in the Visual (Figure 42, panel B) and Default Mode (Figure 43, panel B) networks. However, none of these clusters survive correction for multiple comparisons and are instead reported as uncorrected t-statistics at a very low threshold of $T > 2.5$. 

![Diagram of brain regions](image)
Figure 38 Whole-brain functional activation during all light conditions (Ear, Head, Knee) for each subject group (congenital controls, acquired controls, congenital anophthalmia, acquired anophthalmia).

For visualisation purposes, statistical maps are thresholded at $Z > 2.3$ (uncorrected) and overlaid on MNI standard brain using FSLview. No results survive cluster correction.

Prepared by HB and GC.

Figure 39 Whole-brain functional activation during the no light condition for each subject group (congenital controls, acquired controls, congenital anophthalmia, acquired anophthalmia)

Panel A shows results when applying the same 30 second ON/OFF block design as the light conditions in Figure 38. Panel B shows results using a 15 second ON/OFF block design. For visualisation purposes, statistical maps are thresholded at $Z > 2.3$ (uncorrected) and overlaid on MNI standard brain using FSLview. No results survive cluster correction.

Prepared by HB and GC.
Figure 40 Mean percentage BOLD signal change during each experimental condition (Ear, Head, Knee, No Light) extracted from the primary visual cortex (V1, as defined by the Juelich Histological Atlas in FSLview)

Group mean values for each of the four experimental conditions are shown in panel A, group median values are shown in panel B. Error bars represent standard error.
Figure 41 The main ICA networks for all subjects at the Oxford (left) and Manchester (right) sites

For visualisation purposes, the statistical maps are thresholded at $2.3 < Z$-score $< 5$ and overlaid using Freesurfer onto the inflated cortical surface of the left and right hemisphere average brain. The red-yellow colour bar shows positive correlations, and the blue-light blue shows negative correlations (anti-correlations).
Figure 42 The Visual Network for all subjects at the Oxford (left) and Manchester (right) sites

Panel A shows brain areas that contribute to the Visual Network in all four experimental conditions (“Ear”, “Head”, “Knee”, “No Light”). For visualisation purposes, statistical maps are overlaid using Freesurfer onto the inflated cortical surface of the left and right hemisphere average brain. The colour bar shows the P-value range used to display significant functional connectivity, calculated using permutation testing and threshold-free cluster enhancement ($P < 0.05$). Panel B shows a contrast of each light condition (“Ear”, “Head”, “Knee”) > “No Light” condition. For visualisation purposes, statistical maps are overlaid onto the MNI-152 standard brain using FSLview. Since clusters are small and do not survive cluster correction, uncorrected t-statistics are reported ($T > 2.3$).

Prepared by HB and GC.
Figure 43 The Default Mode Network for all subjects at the Oxford (left) and Manchester (right) sites

Panel A shows brain areas that contribute to the default mode network in all four experimental conditions (“Ear”, “Head”, “Knee”, “No Light”). For visualisation purposes, statistical maps are overlaid using Freesurfer onto the inflated cortical surface of the left and right hemisphere average brain. The colour bar shows the P-value range used to display significant functional connectivity, calculated using permutation testing and threshold-free cluster enhancement (P < 0.05). Panel B shows a contrast of each light condition (“Ear”, “Head”, “Knee”) > “No Light” condition. For visualisation purposes, statistical maps are overlaid onto the MNI-152 standard brain using FSLview. Since clusters are small and do not survive cluster correction, uncorrected t-statistics are reported (T > 2.3).

Prepared by HB and GC.
6.4 Discussion of evidence of Extraocular Photoreception in Humans

This study did not find any valid brain activity during any of the extra-ocular light conditions; behind the ear, head or under the knee in congenital and acquired anophthalmics or sighted controls. These data also show that the brain activity does not differ between light conditions in congenital anophthalmia, acquired anophthalmia or control participants, a finding consistent with previous studies (Herbert, 1994; Lockley et al., 1998; Eastman, Martin and Hebert, 2000; Rüger et al., 2003).

In our study, we used an objective measurement by reporting on the effect of light stimuli at different locations on brain activity. This information was captured by using functional MRI scanning, and did not rely on self-reporting
from participants (Timonen et al., 2012), which can be subject to bias. Multiple analyses were carried out on the data to distinguish any inherent changes in brain activity from activity evoked by the extraocular light stimulation. Applying a 15 second model instead of 30 seconds produced clusters of activity in various study conditions even though this period did not correspond to any external light stimulation.

Starck et al reported increased functional connectivity of the lateral visual cortex on BOLD fMRI imaging when 24 subjects were exposed to extraocular light compared to 26 subjects who received no light stimulation (Starck, Nissilä, Aunio, Abou-Elseoud, et al., 2012). Starck et al also mentioned the sensation of ‘subjectively perceived change in the visual function’ in some subjects which may be resulting from leakage of light activating a visual response (Starck, Nissilä, Aunio, Abou-Elseoud, et al., 2012).

Participants with bilateral congenital and acquired anophthalmia were chosen for the study design to determine whether any light responses in sighted participants could be due to scattered light reaching the retina. However, neither sighted subjects nor subjects with anophthalmia showed any consistent response to the extraocular light.
6.4.1 Adverse effects

No patient suffered from any serious adverse effect as a result of the fMRI scan. One participant complained of vertigo following the scan but this adverse effect has previously not been reported in the Oxford centre of functional MRI of the Brain (FMRIB).

6.5 Limitations of the FMRI Study

There are several limitations of the study. Firstly, the study numbers are relatively low. However, for light stimulation in sighted participants, there is evidence of neural activity even at a single subject level. In this study, no individual participant showed a consistent response to the extraocular light that exceeded the response to ‘no light’. However, the light measurements through the cosmetic shell showed no light passed through the cosmetic shell. A useful measure would have been to conduct the study without the cosmetic shells in place in the anophthalmic group to check if any vestigial tissue’s in the eye help to illicit responses in the brain. Secondly, the location of the light sources had to be adapted to work in the MRI scanner. The ‘ear’ stimulus was actually presented behind the ear, rather in the inner canal. This was a practical solution because participants had to wear ear plugs to protect their hearing in the scanning (particularly important for visually-impaired participants). Lastly, experiments have shown that near infrared light can penetrate a cadaveric skull and soft tissue (Young et al., 2000; Jagdeo et al., 2012), whilst this study used a bright white light source. Using an infrared source, which is known to traverse...
the skull would have been a better method of conducting the experiment to compare the other regions studied.
7 Structural MRI changes in Anophthalmia Results & Discussion
7.1 Introduction

The previous chapter investigates the evidence of extraocular photoreceptors in subjects with anophthalmia using fMRI. MRI can also be used to detect pathological changes in the brain resulting from cell loss (Whitwell, 2009). This can be manifest as loss of brain tissue or atrophy, which can be detected on a structural MRI (Whitwell, 2009). Traditional techniques of analysing atrophy include visual assessment by experienced radiologists and manual measurements of structures of interest (Whitwell, 2009). Automated techniques allow the assessment of atrophy across large groups of subjects without the need of time consuming manual measurements or subjective visual assessments (Whitwell, 2009).

Voxel-based morphometry (VBM) is an automated technique introduced by Wright et al and Ashburner and Friston (Wright et al., 1995; Ashburner and Friston, 2000). It is relatively easy to use and has provided biologically plausible results. Grey matter volume can be measured using VBM to detect group differences in brain anatomy (Bridge et al., 2014).

7.2 The absence of the eyes impacts upon the optic nerve

Structural T1-weighted MPRAGE images were used to assess the integrity of the optic nerve in congenital and acquired anophthalmia. Figure 45 shows axial
slices for all congenital (A) and acquired (B) anophthalmic cases where the optic nerve is normally located. For comparison, a sighted subject from each site is shown with red arrows pointing to the optic nerve. The optic nerve was almost entirely absent in all congenital cases, whilst most acquired cases showed residual optic nerve, as shown by the mean volume for all four subject groups (C). Optic nerve volume for each individual subject is plotted against percentage of life spent blind in D. Optic nerve volume was significantly lower in both blind groups compared to their respective control groups (independent-samples t-test; congenital group $t = -10.03$, $p < 1 \times 10^{-7}$; acquired group $t = -4.081$, $p = 0.001$). Furthermore, optic nerve volume was significantly lower in the congenital compared to the acquired anophthalmia group (independent-samples t-test; $t = -4.03$, $p = 0.013$). Finally, a plot of optic nerve volume for each acquired anophthalmic case against age of blindness onset (in years) (Figure 45E) revealed a significant positive correlation (Pearson’s $R = 0.8$, $p = 0.017$).

### 7.2.1 No evidence for increased grey matter in acquired anophthalmia

There were no brain areas in the acquired anophthalmia group showing increased grey matter volume compared to the sighted control group. Figure 46 shows the cortical regions with increased grey matter in the congenitally anophthalmic, including a right dorsal occipital region corresponding to visual area V2-V3 and a left lateral occipital region corresponding to visual area V5 (according to the Juelich Histological Atlas). Increased grey matter volume in
the congenital group was also found bilaterally in the parahippocampal gyrus (according to the Harvard-Oxford Cortical Structural Atlas). The peak coordinates, t-statistics and cluster sizes are reported in Table 21.

7.2.2 Decreased grey matter in both congenital and acquired anophthalmia

Figure 47 shows decreased grey matter volume in the congenital (A) and acquired (B) anophthalmia groups compared to sighted controls. Peak coordinates and t-statistics are reported in Table 21. Both non-sighted groups show reduced grey matter volume in the occipital region corresponding to primary ‘visual’ cortex (Juelich Histological Atlas). This reduction is bilateral in the acquired subjects and in the left hemisphere only in the congenital subjects. In the congenital group only, grey matter is also reduced in right extrastriate cortex corresponding to ‘visual’ area V4 (Juelich Histological Atlas). In addition to these occipital changes, grey matter volume is considerably reduced bilaterally in the occipito-parietal cortex of the acquired subjects.

Next, we investigated whether changes in grey matter volume in acquired anophthalmia (increases or decreases compared to sighted controls) are related to age of blindness onset. This was done with an additional VBM group comparison looking at negative effects of blindness years (measured as demeaned percentage of total lifetime spent blind, in order to account for age differences between the groups). This yielded no significant results at a group
level. In order to look at both blind and sighted groups, grey matter volume in ‘visual’ areas V1, V2, V4 and V5 was extracted in all subjects (Juelich Histological Atlas definitions; V1, V2 and V4 thresholded at 40%, V5 thresholded at 15%). No significant correlations were found between percentage lifetime spent blind and grey matter volume in any regions (left and right hemisphere together or separately). Additional comparisons were made with age of blindness onset and number of years of blindness, which also yielded no significant correlations.

7.2.3 Increased V1 cortical thickness in congenital, but not acquired anophthalmia

Cortical thickness in primary visual cortex (V1) has consistently been shown to be greater in congenital blindness compared to sighted controls. In the congenital anophthalmia group, regions corresponding to both V1 ($t = 3.7; p < 0.005$) and V2 ($t = 3.3; p < 0.005$) were thicker than in the sighted control group. This was not the case for any of the other regions shown in Figure 48A. Furthermore, there were no significant differences between the acquired anophthalmia group and the relevant sighted controls. Given the wide range in the duration of anophthalmia in this group, a second analysis was undertaken in which cortical thickness in V1 and V2 was correlated with both the age of blindness onset and the proportion of life spent blind. Using only the acquired anophthalmia group there was no correlation with percent life spent blind (Figure 48) in either V1 ($r = 0.48; p = 0.23$) or V2 ($r = 0.46; p = 0.26$). The age of onset of blindness had a stronger relationship with cortical thickness, but
was not significant in either area, particularly when Bonferroni correction was applied (V1: \( r = -0.69; \ p = 0.058 \); V2: \( r = -0.68; \ p = 0.064 \)).

### 7.2.4 White matter microstructure is altered in both congenital and acquired anophthalmia

There were no significant increases in fractional anisotropy (FA) (see methods) in either the congenital or acquired anophthalmia groups compared to the sighted control groups. Figure 49 shows the regions of white matter showing reduced FA in the congenital (A) and acquired (B) anophthalmia groups compared to the relevant controls. Results are thresholded at \( p<0.05 \) after correction for multiple comparisons (Threshold Free Cluster Enhancement).

FA was significantly reduced bilaterally along the optic radiations in both congenital and acquired anophthalmia, although this reduction appears primarily in the left optic radiations in the congenital group. In the acquired group, FA reductions in the optic radiations overlap with and extend to the longitudinal fasciculus (inferior and superior) and fronto-occipital fasciculus (according to JUH White-Matter Tractography atlas; MNI coordinates \( X = +25 \) to \( +35 \), \( Y = -31 \) to \( +19 \), \( Z = -14 \) to \( +26 \)).

In the acquired anophthalmia group only (Figure 49B), FA is also reduced in the thalamus (MNI coordinates \(-22, -30, +39\)). This region near the end of the anterior thalamic radiations is known to project to the occipital, posterior-parietal and temporal cortices (according to the Oxford Thalamic Connectivity
Probability atlas) and corresponds to where the pulvinar is normally located (Leh, Chakravarty and Ptito, 2008). Finally, FA in the congenital group only (Figure 49A) is reduced in a small right parietal region matching the location of the corticospinal tract (according to JUH White-Matter Tractography atlas; MNI coordinates +22, -20, +46) as well as the corpus callosum in both hemispheres (see Figure 49A at MNI coordinate Z = +2).

To confirm that different FA results in the congenital anophthalmia versus acquired groups were not due to different group sizes (five congenital cases as opposed to seven acquired cases), an additional TBSS analysis was performed for the Manchester data with only the five youngest acquired anophthalmic cases (to match the group size and age of the congenital cases). Reduced FA (compared to sighted controls) in this smaller acquired group did not differ from the larger group.

A second analysis was performed on the acquired anophthalmia group to determine the regions of white matter that exhibited a change in FA related to the percentage of life spent blind. Figure 50A shows the white matter tracts in the brain that show an inverse relationship with the percentage of life spent blind, that is that the longer the period spent blind, the lower the FA. This correlation is restricted to the optic radiation. Indeed, when the FA extracted from the entire optic radiation is correlated with percentage of life spent blind across all participants (Figure 50B) there is a significant negative correlation (Pearson’s R = -0.73, p < 0.00001). However, there is no significant correlation.
in the blind groups alone, so this result is likely driven by overall lower FA in both blind groups compared to sighted controls.
Figure 45 Optic nerve analysis using T1-weighted MPRAGE images.
Axial slices show the expected location of the optic nerve in all congenital (A) and acquired (B) anophthalmic cases. For comparison, the optic nerves from two example controls (red arrows) are also shown. At the thickest/most intact part of the optic nerve, left and right volume was extracted along an axial slice. Mean optic nerve volume for each group (C) as well as optic nerve volume for each individual subject (D) is plotted. Error bars represent standard error of the mean. Finally, optic nerve volume for each acquired anophthalmic case was plotted against age of blindness onset, revealing a significantly positive relationship (E).

Prepared by HB

**Figure 46 Increased grey matter volume in congenital anophthalmia compared to sighted controls (in MNI 2 mm standard space)**

For visualisation purposes, statistical maps are thresholded at t>3 (uncorrected). Arrow 1 points to left occipital cortex (V5), arrow 2 to right occipital cortex (V2/V3) and arrow 3 to bilateral parahippocampal gyrus.

Prepared by HB
Figure 47 Reduced grey matter volume in congenital (A) and acquired (B) anophthalmics compared to sighted controls (in MNI 2 mm standard space).

For visualisation purposes, statistical maps are thresholded at t>3 (uncorrected). In panels A arrow 1 points to bilateral occipital cortex (V1), arrow 2 points to right occipital cortex (V4) In B arrow 1 points bilateral occipital cortex, and arrow 2 points to bilateral occipito-parietal cortex.

Prepared by HB
Figure 48 Cortical thickness in visual regions of interest.

A; shows the cortical thickness measures in the congenital anophthalmia group and controls. Cortical thickness in V1 and V2 of the congenital anophthalmia groups was significantly greater than sighted controls. B; shows the same data for the acquired anophthalmia group. There are no significant differences in cortical thickness in any areas in the acquired anophthalmia group. C; shows the correlation between % life spent blind and V1 cortical thickness. D; shows the correlation between age of blindness onset and V1 cortical thickness. Asterisks indicate $p < 0.005$.

Prepared by HB
Figure 49 Reduced FA in congenital (A) and acquired (B) anophthalmia groups compared to sighted controls (in MNI 2 mm standard space).

The mean FA skeleton across both groups is shown in green. Blue regions indicate significantly lower FA in the anophthalmia groups. For visualisation purposes, all statistical maps are thresholded at $p<0.05$ (after TFCE correction for multiple comparisons).

Prepared by HB
Figure 50 A shows the white matter in which the reduction in FA correlates inversely with the percentage of life spent blind across the acquired anophthalmia group. Mean optic radiation FA (both hemispheres) plotted against percentage of lifetime spent blind for the congenital anophthalmics (black circles, all 100%), acquired anophthalmics (grey squares), congenital controls (white circles, all 0%) and acquired controls (white squares, all 0%).
Prepared by HB
Table 21 Peak coordinates (in MNI standard space) of increased and decreased grey matter volume in the congenital and acquired anophthalmic subjects compared to controls.

<table>
<thead>
<tr>
<th>Location</th>
<th>Left</th>
<th>T-stat</th>
<th>Right</th>
<th>T-stat</th>
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<tbody>
<tr>
<td>Increases in grey matter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Congenital</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parahippocampal gyrus</td>
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<td>26, -26, -20</td>
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<tr>
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<tr>
<td>Decreases in grey matter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Congenital</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occipital cortex (V1)</td>
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<tr>
<td>Occipital cortex (V4)</td>
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</tr>
<tr>
<td><strong>Acquired</strong></td>
<td></td>
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<td></td>
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<tr>
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<td>3.88</td>
<td>14, -78, -4</td>
<td>4.36</td>
</tr>
</tbody>
</table>

Results are thresholded at t>3 (uncorrected).
Prepared by HB

7.3 Discussion - Structural brain changes in congenital and acquired bilateral anophthalmia

While the structural reorganisation in congenital anophthalmia includes some increases in grey matter, people who became anophthalmic later in life do not appear to show this pattern, presumably reflecting the greater cross-modal plasticity found in congenital blindness  (Bavelier and Neville, 2002; Amedi et al., 2003; Burton, 2003; Lewis, Saenz and Fine, 2010; Renier et al., 2010;
Bedny et al., 2011; Collignon et al., 2011; Voss and Zatorre, 2012; Watkins et al., 2012).

### 7.3.1 Occipital cortex shows reduced grey matter volume in both anophthalmia populations

The loss of input to V1 in both groups is likely due to the loss of major input via the optic radiations. In acquired anophthalmia the loss is likely to be due to a combination of trans-synaptic degeneration and reduction in usage as the LGN is no longer activated. In contrast, data from the group with congenital anophthalmia may be consistent with a change in the dominant subcortical-cortical projection. Previous work in the same congenital anophthalmia group suggests that auditory information may project directly to motion area hMT+ rather than via V1. Furthermore, in this population there appears to be little auditory activation in the posterior regions of the calcarine sulcus V1 suggesting that auditory information does not following the geniculo-striate pathway to get to the occipital lobe (Watkins et al., 2012).

### 7.3.2 Greatest changes in grey matter are in areas related to spatial navigation in both anophthalmia groups

In the absence of vision to provide a spatial reference for the world, it is necessary to rely on memory to know the location of objects. These increased
demands on memory may lead to the increased grey matter in the hippocampus and parahippocampal gyrus, structures known to be involved in spatial processing (Bird and Burgess, 2008; Wolbers et al., 2011). This would be comparable to the increased hippocampal volume found in taxi drivers who have very good spatial memory (Maguire, Woollett and Spiers, 2006). Since the hippocampal volume correlates with driving experience in taxi drivers, it is likely to be a change that occurs in adulthood (Maguire, Woollett and Spiers, 2006). It is perhaps surprising therefore that the cases of acquired blindness do not increase hippocampal volume with duration of blindness as the ability to navigate without vision is likely to increase.

The parahippocampal gyrus is activated in congenitally blind people while using a sensory substitution device (tongue display unit) to perform a virtual navigation task (Kupers et al., 2010). While the congenitally anophthalmic participants show this increase in grey matter related to spatial navigation, the acquired anophthalmia group show the largest decrease in volume in the precuneus, also implicated in spatial navigation (Cavanna and Trimble, 2006). This area also has strong functional connectivity with the visual regions (Margulies et al., 2009), so the loss of vision may lead to a significant loss of input and output targets.
7.3.3 **Structural changes in anterior visual regions greater in congenital anophthalmia and related to duration of blindness when acquired**

The considerable changes in the anterior visual system, previously shown in the congenital group using detailed MRI of the cranial nerves (Bridge et al., 2012) is not surprising when taking into consideration difference in time of onset of blindness. In congenital cases, the absence of the globe will invariably affect development of the optic nerve, leading to the total absence or extreme hypoplasia. Where the globes have been removed, as in the case of acquired anophthalmos, the previously intact optic nerves become deafferented, and the ganglion cells will undergo anterograde (Wallerian) degeneration. That this is a progressive degeneration is reflected in the correlation between optic nerve and the age of blindness onset. While the correlation is significant, there is also a reduction in optic nerve volume in those who had their eyes removed in the 6th or 7th decade. This is likely to reflect, in part, the age-related decrease in retinal ganglion fibres noted previously (Johnson, Miao and Sadun, 1987). It is perhaps surprising how small the reduction of optic nerve volume is in the participants whose eyes were removed in adulthood. Given the direct damage to the cell bodies and consequent loss of activity, greater volume loss might be expected.
7.3.4 Consistent white matter changes in optic radiation in both congenital and acquired anophthalmia

As in the majority of previous studies of blind populations (Shimony et al., 2006; Bridge et al., 2009; Wang et al., 2013; Dietrich et al., 2015; Nina L. Reislev et al., 2016; Nina Linde Reislev et al., 2016), changes in the white matter microstructure of the optic radiations represent the most prominent difference between both anophthalmia groups and sighted controls. This consistency has been shown previously by Reislev et al (Nina L. Reislev et al., 2016) for congenital and late acquired blindness. Since much of the optic radiation shows a significant inverse correlation with the proportion of life spent blind in the acquired anophthalmia group, it is likely that the change is due to degeneration of these deafferented fibre bundles. This finding is consistent with that of Reislev et al. in acquired blindness, due to heterogeneous causes, although there was no such correlation in the study of Wang et al. (Wang et al., 2013).

The reduction in FA in the optic radiations seen in the congenitally anophthalmic group presumably reflects the reduction in usage of this normally dominant tract. The atrophy of the LGN in this population (Bridge et al., 2009) and other congenitally blind populations (Cecchetti et al., 2016) is consistent with a loss of major input along the optic radiations.
7.3.5 Conclusion

Changes in structure of the occipital cortex are similar in both congenital and acquired anophthalmia, but beyond the occipital lobe, it is clear that there are different patterns of structural change between these two groups.

7.4 Limitations of the Structural MRI Study

The groups were scanned at different sites and therefore cannot be directly compared. Bilateral anophthalmia is a rare condition and therefore our study numbers are small.

There are limitations with the VBM analysis used. The methods used compare grey matter images on a voxel wise basis. (ref fsl.fmrib.ox.ac.uk) The method involves transforming images into a standard space using non-linear regression. In order to compare images voxel by voxel all the structures across subjects must match. If they are matched too much, you would lose seeing the true difference. Also, sometimes it is not possible to determine if the results found are due to an effective reduced thickness or atrophy in the grey matter or an indirect result of a different gyrification pattern ((ref fsl.fmrib.ox.ac.uk). There errors were limited as all the scan analysis completed by RM/GC was reviewed and checked by HB to ensure there was an agreement with the analysis process.
8 Final Discussion and Future Work
8.1 Role of Circadian Rhythms in Human Disease -

What this work adds to current literature

This study investigated the role of two diseases, DR and anophthalmia and sleep/wake disturbance (See Aims 2). In Summary:

1) There was no evidence of disruption in subjective sleep quality among varying severity of diabetic retinopathy.

2) Subjects with anophthalmia all showed the presence of free-running rhythms and a higher incidence of subjective sleep/wake disruption.

3) The functional MRI studies found that there is no evidence of EOCP in blind or sighted blind-folded individuals.

4) Changes in structure of the occipital cortex are similar in both congenital and acquired anophthalmia. The acquired anophthalmic group did not show increase in hippocampal volume (memory areas) or in the precuneus (spatial navigation).

There has been limited previous work in the field of the impact of DR and anophthalmia in sleep/wake problems. This study did not support the findings by Jee et al (the largest study on DR and sleep to date), where the amount of sleep in men was associated with DR (Jee et al., 2017). This is the first study assessing sleep/wake problems, circadian rhythms and brain activity in a homogenous group of completely blind subjects with no eyes. The results on the abnormal sleep/wake cycles and circadian rhythms are in keeping with current literature on the topic (Lockley et al., 1997; Tabandeh et al., 1998;
Lockley, Arendt and Skene, 2007; Flynn-Evans et al., 2014). However, this work also confirms the absence of EOCP in humans refuting previous work (Campbell, Murphy and Suhner, 2001; Starck, Nissilä, Aunio, Abou-elseoud, et al., 2012; Timonen et al., 2012; Persinger, Dotta and Saroka, 2013; Jurvelin et al., 2014; Sun et al., 2016). The changes in the brain re-wiring contribute to current knowledge as the acquired blind group has not previously been studied in such detail (Shimony et al., 2006; Bridge et al., 2009, 2012; Wang et al., 2013; Dietrich et al., 2015; Cecchetti et al., 2016; Nina L. Reislev et al., 2016; Nina Linde Reislev et al., 2016).

In summary, anophthalmia (the congenital or acquired absence of one or both eyes) is a rare condition which has significant implications as the individual is not only blind but often is troubled by sleep/wake disruption combined with depression and anxiety (Leger et al., 1999). Sleep/Wake problems represent a substantial emotional and social burden on this group of totally blind subjects. The evidence of the impact of circadian de-synchrony on health is increasing especially in people subject to shift work and jet lag. The International Agency for Research on Cancer (IARC) classified shift-work with circadian disruption as a probable carcinogen (Straif et al., 2007; Fritschi, 2009). It is known to cause prolonged fatigue, insomnia, appetite changes, mood changes, impaired alertness and performance and increased risk of hypertension and cardiovascular disease. Patients suffering from psychiatric disorders such as schizophrenia are also known to have a greater incidence of sleep and circadian rhythm disruption (Wulff et al., 2012). The overall cost of sleep
disorders in Australia in 2004 for a population of 20.1 million was 7497 million dollars (Hillman, Murphy and Pezzullo, 2006). Using the 2016 Organisation for Economic Cooperation and Development purchasing power parity of United States 0.702 pounds sterling per US dollar and the 2016 UK population of 65.64 million the financial impact of sleep disorders in UK would be £17 billion (£17,154,402,599.11) (Calculation by CA). This emphasises the huge impact sleep disturbance has on productivity, economics, and gross domestic productivity and such studies such as ours have a key role in exploring the contribution of ocular disease to sleep/wake disturbance.

The aim of these studies are to help improve quality of life by developing strategies to improve the sleep/wake disturbance, including the use of exogenous melatonin. Where participants have blind eyes with NPL vision, previously eyes were enucleated if they were unsightly and phthisical. However, there may be a role to leave these eyes in place (if they are not painful), so that the residual pRGCs in the retina and ocular structures can help entrain the NIF functions (Zaidi et al., 2007). Once the eyes are removed and participants are anophthalmic, there are no remaining ocular or extraocular structures to input light signals to the SCN. Ocular prosthesis also blocks out all light.

Accidents caused by sleepiness are one of the major causes of litigation. The National Highway Traffic Safety Administration in US conservatively estimates 100,000 police reported crashes are as a direct result of driver fatigue each
year (*Facts and Stats: Drowsy Driving – Stay Alert, Arrive Alive*, no date). It is therefore important to identify ocular disease that can result in sleep/wake disturbance so that steps can be taken to avoid the precipitating serious health and safety consequences that may affect their livelihood.

### 8.2 Future work

The work for this thesis formed one part of a larger study investigating the impact of ocular sleep wake on ocular diseases (glaucoma, AMD, retinitis pigmentosa, inherited retinal degenerations and optic neuropathies). During my research attachment, I was also involved in data collection for these separate studies. The results of these studies are shortly to be published and will contribute to the previously sparse literature in this area.

Important future work in this field needs to assess the melatonin rhythms in participants with severe ocular disease together with potential treatments such as exogenous melatonin. A long-term prospective study comparing the stages of ocular disease and health of individuals would be invaluable however difficult to conduct due to confounders such as smoking, diabetes control, family history.

Regarding the study looking at extraocular photoreceptors, it would be interesting to perform the fMRI studies on the congenitally anophthalamic participants without the ocular prosthesis or eye mask in place to check if
vestigial ocular remnants remain photosensitive and whether this can be enough to help entrain the internal circadian clock for these individuals. The results of this could change the treatment of many individuals with anophthalmia as they may choose not to wear a cosmetic shell that blocks out all light or newer cosmetic shells can be developed with an artificial ‘pupil’.

It has been suggested that the pRGC is resistant to damage compared to rods and cones (Cui et al., 2015) and the DR study adds to this hypothesis. There are incurable blinding ocular diseases such as retinitis pigmentosa where there is a gradual degeneration of rods and cones. Perhaps, the resistance of the pRGC to injury, gives the hope of using the third photoreceptor for retinal gene or stem cell therapy in these blinding conditions.

Future work in this field includes investigating sleep/wake disorders of participants living in glass houses ‘Living by the light of a glass house’ 2016/2017 (KW), lighting in nursing homes (Ancoli-Israel et al., 2002), prisons and hospitals (IA, KW). Commercial companies are developing applications with blue light filters to reduce the impact of artificial light of the circadian rhythms in collaboration with the circadian biologists in Oxford (RF). This field is evolving rapidly and the importance of finding therapies for individuals with sleep disturbance is highlighted by the social, economic and personal burden associated with sleep disorders.

It is surprising that this small photosensitive retinal ganglion cell with such a sparse population has such a significant impact on the lives of so many people.
It is therefore important that more work is carried out in achieving a complete understanding of its role in health and disease and the potential therapeutic avenues.
Chapter 9

9 References


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10 Appendix
10.1 Study Proforma used for all studies recruiting under study title “Impact of Ocular disease on Circadian Rhythms”

**Effect of ocular disease on sleep and body clocks.**

<table>
<thead>
<tr>
<th>Site:.................................</th>
<th>Study Number:.................................</th>
</tr>
</thead>
</table>

**Diabetic Retinopathy:**
- R □ M □ P □
- R □ M □ P □
- ETDRS □ □

**AMD:**
- Early □
- Dry □
- Wet □
- Fovea involving: Yes □ No □

**Glaucoma:**
- Primary Open Angle Glaucoma □
- HVF MD: □ □

**Inherited Eye Disease:**
- Diagnosis (based on EDTS i.e. “cone-rod dystrophy”) .................................................................
- Diagnosis (Clinical i.e. “Stargardt”) ........................................ Electrophysiology report attached □
- Goldmanns visual field area (natural log of V4e in cm2)..............................

**Optic Nerve Disease:**
- Diagnosis .................................................................
- Visual Field done Yes □ No □
- HVF MD: □ □

**Visual Acuity (preferred logMAR):**
- Right □
- Left □

**Comments, including medications/ cataract/ previous laser/other:**
- ........................................................................................................
- ........................................................................................................
- ........................................................................................................
- ........................................................................................................
- ........................................................................................................
- ........................................................................................................
10.2 Participant Information Sheet for Anophthalmia Study

Participant Information Sheet

Version 1.2 06/06/2012

Effect of bilateral acquired anophthalmia and the impact of ocular disease on Sleep and ‘Body-Clocks’ (Circadian Rhythms).

REC Reference: South Central - Oxford B 11/SC/0093

Miss S Downes, Principal Investigator

Dr Holly Bridge, Academic Supervisor

Dr Rupal Morjaria, Research Fellow

Part 1

We would like to invite you to take part in a research study. The doctors working on this study will be carrying out research degrees (MSc, MD) undertaken at the University of Oxford. Before you decide whether or not to take part you need to understand why the research is being done and what it would involve for you. Please take the time to read the following information carefully. You may talk to others about the study if you wish.

Part 1 of this form tells you the purpose of this study and what will happen to you if you take part. Part 2 gives more detailed information about the conduct
of the study. Please ask us if there is anything that is not clear or if you would like more information. Take your time to decide whether or not you wish to take part. Your participation is completely voluntary, your care will not be affected by your decision. Thank you for reading this.

What is the purpose of the study?

Our sleep/wake cycles are controlled by a receptor situated at the back of the eye. These receptors are sensitive to blue light and are known as the photosensitive retinal ganglion cells (pRGC). Depending on whether it is day or night, different amounts of light pass into the eye. As light enters the eye, this receptor detects how much blue light is present, and sends a signal to a certain part of the brain. This part of the brain controls the sleep/wake cycle that ensures a person sleeps at night and is awake during the day.

The light input can be upset for a number of reasons. For example, certain eye conditions like glaucoma, inherited retinal degenerations or macular degeneration may cause reduced light perception due to the changes at the back of the eye where the pRGC system is situated. This in turn may have an impact on sleep/wake cycle. A significant proportion of patients with loss of both their eyes, or absent eyes have reported to suffer from sleep disturbances. We plan to develop an informed approach in the overall management of the various eye problems by assessing the impact of different eye conditions and impact of absent/small eyes on sleep patterns and sleep/wake cycle.
Recent research also suggests that children born with no eyes (anophthalmia) or congenitally small eyes (nanophthalmia) can have changes to the regions of the brain that would normally process visual information and there may be changes in the connections between different brain areas. However, we do not know whether this is something that happens in everyone who loses their eyes, or only if damage is present from an early age.

We want to compare the changes in the structure, connections and functioning of the brains of people who have bilateral enucleations at different ages to the brains of people with congenital anophthalmia/nanophthalmia and people with ‘normal’ eyes.

Why have I been invited to take part?

We are looking for people with various eye conditions and patients who have lost both their eyes (from trauma, eye problems or surgery) to take part to help us get the most out of the study. There are 4 parts in this study: A, B, C and D. Over the course of 5 years, we hope to recruit approximately 3000 participants in total for the overall study.

For part A, we need to ask individuals who are willing to fill in a set of questionnaires and have their pupil measurements taken on two different days with an interval of six months in between.

We plan to look further into the sleep / wake timing over a 3 week period and to do this we would like to invite people from part A who are willing to participate
further into Part B or C.

Part B of the study will involve filling in a 3 weeks ‘sleep diary’ and a mood questionnaire at home about a month later from your first visit. We are looking for a total of 200 patients with similar eye conditions to take part in part B of the study.

In part C, participants will be asked to wear a small watch (called the actiwatch) on their non-dominant wrist and fill in ‘sleep diaries’ for 3 weeks. Participants will also be asked to do a urine collection for 48 hours so that we can analyse the sleep hormone level and correlate it with the other findings.

We are hoping to recruit a total of 50 patients with similar eye problems to take part in part C of the study.

Part D Study Recruitment: At this point, selected part C participants (based on their sleep/wake pattern) will be asked if they would agree to have a blood test for genetic testing to identify any changes/mutations in the ‘body-clocks’ genes. If participants are happy to consent to this, they will be recruited to part D of the study and a blood sample will be taken.

Patients who have lost both their eyes, and have had bilateral enucleations, will be asked to take part in all four parts of the study and have one or two MRI scan sessions.

Do I have to take part?

No. It is up to you to decide whether you wish to participate in any parts of the
study or not. If you wish to take some more time to think about the study and discuss it with friends or family, please take the information leaflet with you today. If you wish to take part, and decide to do later then you can inform the researcher later about your decision by either calling the contact number provided or asking them to call you. If you decide that you do not wish to take part, your care will not be affected in any way.

**What will happen to me if I take part?**

As mentioned before, the study has 4 parts. Please read carefully the information about all the parts and what is involved in each of them. A study flow chart is attached at the end of this information sheet for your convenience.

**Part A:**

This is a general part and all willing participants will be entered into the study through this part. At the time of your clinic visit we will ask you to fill in a set of questionnaires about your sleep and general health. Help can be provided by the researcher in filling the questionnaires, if needed. We will also take a measurement of your pupil size and its reaction to a dim light. These assessments will be done once again after about 6 months.

**Part B:**

In addition to the assessments as in part A, we will send a ‘3 weeks sleep diary’ and a mood questionnaire to willing participants about a month later from your first visit. You can fill these at home and return them to us by post. Stamped
addressed envelopes will be provided.

**Part C:**

In between the first and 2nd visits as in part A, we will call you for permission to bring the sleep diaries, actiwatch and urine collection bottles to your home. Alternatively, if you prefer, you can collect them yourself from us at the eye hospital. We can let you know how to do that (contact number at end of information leaflet). At the end of the 48 hours urine collection, the researcher will collect the bottles from your home. The actiwatch and diaries can be posted back to us. Stamped addressed envelopes will be provided.

**Part D:**

At the time of your participation in part C, you will be given the option of further participation in part D and if you agree, you will then come for one extra visit when a blood sample will be taken for testing and analysis to look for any genetic correlation in the ‘body-clocks’ genes for your particular condition.

**What will happen to me if I take part in the MRI scan?**

If you take part, you will come to either the FMRIB Centre or the OCMR Centre at the John Radcliffe Hospital in Oxford to have your brain scanned probably on either 1 or 2 occasions. When you arrive at the centre you would meet the researcher who will explain to you what would happen during the scan. Prior to your arrival, the researcher would go through an MRI Safety Screening Form with you. This makes sure that you do not have any metal in your body and that it is safe for you to enter the scanner.
Before the brain scan, you would be asked to remove any metal objects. This is because it is unsafe to take any metal objects into the scanner room where there is a strong magnetic field. The scanner is a large box with a tube running through the middle. You would lie on a bed, which moves into the centre of the tube, and a device shaped like a helmet would be put around your head. You would wear headphones to protect your hearing and so that you can listen to the researcher when spoken to. You will be able to see a screen at the end of the bed via a mirror on the helmet. You would also be given a call button to hold throughout the scan. You can use this to get the attention of the researcher who will be on the other side of a window just outside the scanner room. You can speak to the researcher via a microphone from inside the scanner. It is very important that you keep still during the scans and try not to move your head at all. We would make sure that you were comfortable by providing cushions around the head and under the legs and a blanket if necessary.

In the first scanning session, we would perform two types of scans, structural and functional. During the first scan, we would take pictures of your brain's structure. Then, we would take images showing connections between different areas of the brain. The functional scan may require you to listen to some sounds or to think about words while we measure your brain function. We would give full instructions and a practice run once you were in the scanner. All this lasts about an hour.

We may want to gain further information about the functioning of your brain. If this is
the case, we will ask you if you would be willing to return to the imaging centre at a future date for an additional scanning session. This session will last no longer than 1 hour.

**Expenses and payments**

Unfortunately, we are not able to pay you for taking part in the study. However, we will be able to reimburse any reasonable travel expenses you or person accompanying you might incur for any extra visits to the eye hospital.

**What will I have to do?**

If you decide to take part in the study you should let us know after reading this leaflet. We will then ask you to sign a consent form before you can participate in any parts of the study.

**What are the possible disadvantages and risks of taking part?**

The main ‘disadvantage’ of taking part in the study part A or B is filling out the questionnaires and sleep diaries. Help and advice will be provided in filling them if necessary. For taking part in part C, the ‘disadvantage’ is that you will need to fill in sleep diary at home and wear a wrist actiwatch (a watch-like device) for 3 weeks. You will also be asked to do a 48 hour urine collection at home. For part D, an extra visit may be necessary for a blood sample to be
taken. For the MRI scan, you will need to visit the MRI department. We can try and arrange this when it is most convenient for you.

**What are the possible disadvantages and risks of the MRI scan?**

It may not be safe to have a scan if you have metal or a pacemaker in your body. This is because of the strong magnetic field in the scanner room. Teeth fillings are safe but you should discuss other metal dental work (e.g. braces) with the researcher beforehand. For your safety, you will be asked to fill out a MRI Safety Screening Questionnaire, and to remove any metallic items (e.g. jewellery, coins) before you enter the scanner room.

Even though MRI does not use X-rays and the magnetic fields do not have any known harmful effects, it is our policy not to give an MRI scan to someone who is pregnant. If there is a possibility that you are pregnant, therefore, you should not take part in this study.

**What happens if something unexpected is found on your scan?**

In the unlikely event of us seeing any structural abnormalities on your MRI scan, a designated clinical specialist will contact you to discuss the implications with you and arrange for further investigations as necessary. However, it is important to note that we do not carry out scans for diagnostic purposes, and therefore these scans are not a substitute for a clinical appointment. Rather, our scans are intended for research
What are the side effects of any treatment received when taking part?

You will not be receiving treatment any different from any other person with the similar condition as yours. This is not a drug testing trial.

The pupil measurements take about few minutes to do, and involves dilating the eye, and then looking into a small machine that shines a dim light at one eye for 10-20 seconds. The reaction of your pupils to light is measured by the machine. There are no side effects anticipated from this measurement. The dilating drops are the same drops that you will have had put in at your pre-assessment visit. They can sometimes make your vision feel blurry for up to 4 hours, and we would advise you not to drive for this time.

What are the side effects of having an MRI scan?

There are no known side effects associated with having an MRI scan. Some people find that the scanner makes them feel uncomfortable (because they have to keep still for a long time) or claustrophobic (nervous in small spaces). Such feelings will go away once you are outside the scanner. If you know that you are claustrophobic, you may wish to discuss this with the researcher beforehand. When you have the scan, the researcher will move you slowly into the scanner tube, and constantly check with you that you feel comfortable. If you feel claustrophobic when you are put into the scanner then you will be immediately removed. You will not have to go in the scanner again if you do not want to. If you start to feel claustrophobic once the scan has
started then you can press the call button, the scan will be stopped and the researcher will speak to you immediately. You can then be quickly removed from the scanner and you will not have to go in the scanner again.

There are no after-effects of having a scan, so you are free to leave the centre when you are finished. It is safe to drive a car after an MRI scan.

**What are the possible benefits of taking part?**

It is unlikely that you will notice a direct benefit yourself from taking part in the study. We hope that the results of this study will help us to answer whether sleep patterns are affected by the various eye conditions and if changes in the brain occur as a result of having bilateral enucleations. Knowing the answers will give us an improved understanding of the eye diseases and help doctors in advising patients with various eye conditions in the future.

**What happens when the research study stops?**

We would not anticipate that you would require any further follow-up relating to this study after the study finishes. You will be followed up for your eye condition as appropriate in the outpatient clinic as for anybody else with similar condition.

**What if there is a problem?**
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

**Part 2.**

**What will happen if I don’t want to carry on with the study?**

You are free to withdraw from the study at any time without giving any reason. This will not affect your care in any way. The information already obtained from your tests will be kept and may still be used.

**What if there is a problem?**

If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact the researcher Dr R Morjaria on email: r.morjaria@doctors.org.uk in the first instance or Miss S Downes on susan.downes@ouh.nhs.uk or you may contact the University of Oxford Clinical Trials and Research Governance (CTRG) office on 01865 857939 or the head of CTRG, email heather.house@admin.ox.ac.uk.

The NHS Complaints Procedure will also be open to participants.
Do you have Insurance in case something goes wrong?

The University has arrangements in place to provide for harm arising from participation in the study for which the University is the Research Sponsor. NHS indemnity operates in respect of the clinical treatment with which you are provided.

Will my taking part in the study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. Medical notes pertaining to the study and data collected may be reviewed by NHS or University of Oxford staff involved in the study.

We will follow ethical and legal practice and all information about you will be handled in strictest confidence.

What will happen to any samples I give?

You will not give any samples for testing in part A or part B of the study.

In part C, the urine samples taken from you will be sent to a lab in the University of Surrey. The samples will be analysed for the hormone melatonin to correlate this with the actigraphy data.

In part D, the blood sample taken will be analysed here in Oxford.

MRI scans will be analysed in the FMRIB department at the John Radcliffe
Hospital.

**Will any genetic tests be done?**

Only in part D after consent, not in Parts A, B or C of the study. Selected patients from Group C (depending on their sleep wake profile/pattern) will be asked if they would be prepared to have an additional blood test done for genetic testing. All anophthalmic patients will be asked to take part in all the stages of the study.

**What will happen to the results of the research study?**

The results of the study will be published in scientific journals and presented at scientific meetings. No patient will be identifiable in any report or publication.

**Who is organising, funding and monitoring the research?**

The research is organised by the Nuffield Laboratory of Ophthalmology, University of Oxford. A Wellcome Trust Grant has provided funding for this research. Relevant data collected during the study may be looked at by individuals from the University of Oxford, for the purpose of audit and monitoring.

**Who has reviewed the study?**

All research in the NHS is looked at by an independent group of people, called
a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed by the Research Ethics Committee: South Central - Oxford B.

Further information and contact details

If you wish to be given any further information about this study or have any specific questions, please contact the researchers on 01865 234735 (leave a message if necessary and they will call you back). Alternatively, you can contact Dr R Morjaria on r.morjaria@doctors.org.uk or Ms S Downes on susan.downes@ouh.nhs.uk. If you are unhappy about any aspect of the study then you can contact the above number/email addresses.

10.3 Participant Information Sheet: Control Subjects

PARTICIPANT INFORMATION SHEET: Control subjects

Using MRI to investigate changes in connectivity, structure and function in patients with cortical visual damage

Oxford Research Ethics Committee B 08/H0605/156

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would
Please take time to read the following information carefully. Talk to others about the study if you wish.

Part 1 tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

Part 1

Why are we doing the study?

Recent research suggests that when damage occurs to the visual regions of the brain, there may be changes in the connections between different brain areas that could compensate for some of the damage. However, we do not know whether this is something that happens in everyone, or whether it depends on when the damage happened.

We want to compare the changes in the structure, connections and functioning of the brains of people who have suffered damage to the visual system at different ages to the brains of people without any damage.
Why have I been invited?

We have invited you to take part in this study because we need to scan people with normal vision, like you, so we can compare these scans to those taken from people with damage to their brain. In this study we want to take pictures of your brain to look at its size, shape and connections. Also, we are interested to see how the parts of your brain responsible for vision function together. In total we aim to recruit 36 participants, half of whom have damage to their visual systems, and half to act as controls subjects.

Do I have to take part?

It is up to you to decide. We will describe the study and go through this information sheet, which is yours to keep. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive.

What will happen to me if I take part?

If you take part, you will come to the OCMR Centre at the John Radcliffe Hospital in Oxford to have your brain scanned on either 1 or 2 occasions. When you arrive at the centre you would meet the researcher who will explain to you what would happen during the scan. The researcher would go through
an MRI Safety Screening Form with you. This makes sure that you do not have any metal in your body and that it is safe for you to enter the scanner.

Before the brain scan, you would be asked to remove any metal objects. This is because it is unsafe to take any metal objects into the scanner room where there is a strong magnetic field. The scanner is a large box with a tube running through the middle. You would lie on a bed, which moves into the centre of the tube, and a device shaped like a helmet would be put around your head. You would wear headphones to protect your hearing and so that you can listen to the researcher when spoken to. You will be able to see a screen at the end of the bed via a mirror on the helmet or via glasses. You would also be given a call button to hold throughout the scan. You can use this to get the attention of the researcher who will be on the other side of a window just outside the scanner room. You can speak to the researcher via a microphone from inside the scanner. It is very important that you keep still during the scans and try not to move your head at all. We would make sure that you were comfortable by providing cushions around the head and under the legs and a blanket if necessary.

In the first scanning session, we would perform two types of scans, structural and functional. During the first scan, we would take pictures of your brain’s structure. Then, we would take images to show connections between different areas of the brain. All this lasts about an hour and you could listen to a CD or just close your eyes and rest. The scanner is very noisy and vibrates a great
deal. We would give you earplugs to wear during the scan, but you can still hear the music. If you have a favourite CD, you are welcome to bring this with you. We have a small selection from which you are welcome to choose also.

During the other scan, we would measure your brain function while you perform different tasks. These may involve reacting to pictures presented on the screen at the end of the bed, while keeping your eyes still. We would give full instructions and a practice run once you were in the scanner.

We may want to gain further information about the functioning of your brain. If this is the case, we will ask you if you would be willing to return to the imaging centre at a future date for an additional scanning session. You do not have to do this, but if you would like to, we will perform some more scans to measure your brain function while looking at different types of pictures on the screen. This session will last no longer than 1 hour.

In addition to the scans, we would also like to measure how well you can see different objects. First, we will measure the region of the visual field in which you can see, which will require you to tell us when you can see lights flashed on a screen. We will then show you various pictures on a computer screen that may differ in their properties such as colour, shape or direction of movement. You would be required to tell us what you saw either verbally or by pressing a button. This session would take around an hour, and you would be free to have breaks as you wished.
One final test that we may perform is called Optical Coherence Tomography and allows us to look at the back of your eye. We can measure the thickness of the cells at the back of your eye (the retina) to serve as a control for the patients who will participate in this study. This will require you to sit still and look at a cross for up to 30 minutes.

**Expenses and Payments**

You will be paid £35 for each scanning session, which will last approximately 1.5 hours. We can also reimburse minor travelling expenses such as parking or bus tickets to get to the John Radcliffe Hospital. For sessions outside of the scanner you will receive £10 per hour.

**What are the side effects of having an MRI scan?**

There are no known side effects associated with having an MRI scan. Some people find that the scanner makes them feel uncomfortable (because they have to keep still for a long time) or claustrophobic (nervous in small spaces). Such feelings will go away once you are outside the scanner. If you know that you are claustrophobic, you may wish to discuss this with the researcher beforehand. When you have the scan, the researcher will move you slowly into the scanner tube, and constantly check with you that you feel comfortable. If you feel claustrophobic when you are put into the scanner then you will be
immediately removed. You will not have to go in the scanner again if you do not want to. If you start to feel claustrophobic once the scan has started then you can press the call button, the scan will be stopped and the researcher will speak to you immediately. You can then be quickly removed from the scanner and you will not have to go in the scanner again.

There are no after-effects of having a scan, so you are free to leave the centre when you are finished. It is safe to drive a car after an MRI scan.

**What are the possible disadvantages and risks of taking part?**

It may not be safe to have a scan if you have metal or a pacemaker in your body. This is because of the strong magnetic field in the scanner room. Teeth fillings are safe but you should discuss other metal dental work (e.g. braces) with the researcher beforehand. For your safety, you will be asked to fill out a MRI Safety Screening Questionnaire, and to remove any metallic items (e.g. jewellery, coins) before you enter the scanner room.

Even though MRI does not use X-rays and the magnetic fields do not have any known harmful effects, it is our policy not to give an MRI scan to someone who is pregnant. If there is a possibility that you are pregnant, therefore, you should not take part in this study.

**What happens if something unexpected is found on your scan?**
In the unlikely event of us seeing any structural abnormalities on your MRI scan, a designated clinical specialist will contact you to discuss the implications with you and arrange for further investigations as necessary. However, it is important to note that we do not carry out scans for diagnostic purposes, and therefore these scans are not a substitute for a clinical appointment. Rather, our scans are intended for research purposes only.

**What are the possible benefits of taking part?**

There is no benefit to you if you take part in this study. We hope that the information we get from this study may help us to better understand how the brain works.

**What if there is a problem?**

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

**Will my taking part in this study be kept confidential?**

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the centre
will have your name and address removed so that you cannot be recognised from it.

More details are provided in Part 2.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2.

What will happen if I don’t want to carry on with the study?

You are free to withdraw at any time, without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect you in any way.

What if something goes wrong?

If you have a concern or wish to complain about any aspect of the way in which you have been approached or treated during the course of the study, you can either contact Holly Bridge who will do her best to answer your questions (01865 222582) or contact the University of Oxford Clinical Trials and Research Governance office on 01865 743005.
Compensation for harm arising from an accidental injury, and occurring as a consequence of your participation in the study will be covered by the University of Oxford. If you are harmed and this is due to someone’s negligence then you may have grounds for legal action for compensation against the University of Oxford but you may have to pay your legal costs. The University maintains insurance to provide compensation in the case of both non-negligent and negligent harm.

**Will my taking part in this study be kept confidential?**

All information that is collected about you during this study will be kept strictly confidential and all data collected will be stored securely in an anonymous form such that that identification of participants will not be possible. If you join the study, some parts of your records and the data collected for the study may be looked at by authorised persons from the University, for the purposes of monitoring and audit.

The data collected in the study may be used for further meta-analyses by researchers at the University of Oxford. Additionally, if we are required to submit the data to databases of MRI studies, they may be available in countries outside the EU, where the laws do not protect your privacy to the same extent as the Data Protection Act in the UK. However, it will not be possible to identify you from these data, as your name and address will not be provided.
**What will happen to the results of the research study?**

We hope to publish the result of this study in a scientific journal. We may also present the results at a scientific conference or a seminar in a university. The result may also be published on our website. We would be happy to discuss the results of the study with you and to send you a copy of the published results. It will not be possible to identify you or images of your brain in any report or publication.

**Who is organising and funding the research?**

This research study is organised by the University of Oxford and City University. It is funded by The Royal Society.

**Who has reviewed this study?**

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the Oxford Research Ethics Committee B 08/H0605/156.

**Contact for further information**
You should contact Dr Holly Bridge or Professor John Barbur for further information. You can contact Dr Bridge via email holly.bridge@clneuro.ox.ac.uk or by phone 01865 222582. Professor Barbur can be contacted by email johnb@city.ac.uk.
10.4 Effect of Anophthalmia and Sleep and body Clocks Consent Form

Effect of Anophthalmia (absent eyes) on Sleep and ‘Body-Clocks’ (Circadian Rhythms)
Reference: Version 1.0, 16/10/2012

Patient Identification Number:

**CONSENT FORM Part A / B**

Name of Principal Investigator: Miss Susan Downes
Please initial

box

1. I confirm that I have read and understand the information sheet dated 06/05/2011 (version 1.1) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the University of Oxford, or from the Oxford University Hospitals NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

4. I agree to take part in the above study.
5. I wish to be told the results of the study once available  

Y  N

________________  ________________  ________________
Name of Patient  Date  Signature

________________  ________________  ________________
Name of Person  Date  Signature
taking consent

10.5 Participant Invite Letter for Anophthalmia Study

Date:

Dear

Study Title: Effect of Anophthalmia on Sleep and ‘Body-Clocks’ (Circadian Rhythms)

REC Ref No: 11/SC/0093

We would like to invite you to join a research project investigating the effect of having no eyes (anophthalmia) on our sleep/wake cycle and the rhythms of our body clocks. Please find enclosed a Participant Information Sheet, which gives details of the study.

If you have any questions, in the first instance please contact our Researcher XXX on: XXX Bleep XX.
Thank you for your interest.

Yours sincerely
10.6 Pittsburgh Sleep Quality Index (PSQI)

Patient no: Questionnaire no:

The Pittsburgh Sleep Quality Index

Instructions:

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions on both sides of the paper.

During the past month,

1. What time have you usually gone to bed? __________

2. How long (in minutes) has it taken you to fall asleep each night? __________________________

3. What time have you usually gotten up in the morning? __________________________

4. How many hours of actual sleep do you get at night? (This may be different than the number of hours you spend in bed) __________________________

5. Please tick the appropriate box for each of the following questions:
During the past month, how often have you had trouble sleeping because you.....

<table>
<thead>
<tr>
<th></th>
<th>Not during the past month</th>
<th>Less than once a week</th>
<th>Once or twice a week</th>
<th>Three or more times a week</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Cannot get to sleep within 30 minutes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Wake up in the middle of the night or early morning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Have to get up to use the bathroom</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Cannot breathe comfortably</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. Cough or snore loudly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f. Feel too cold</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g. Feel too hot</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>h. Have bad dreams</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Have pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>j. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason(s):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. During the past month, how often have you taken medicine (prescribed or “over the counter”) to help you sleep?

7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?

<table>
<thead>
<tr>
<th></th>
<th>Very good</th>
<th>Fairly good</th>
<th>Fairly bad</th>
<th>Very bad</th>
</tr>
</thead>
</table>

9. During the past month, how would you rate your sleep quality overall?
10.7 Hospital Anxiety and Depression Score (HADS)

Hospital Anxiety and Depression (HAD) Scale

Patient no: Questionnaire no:

(Please tick the box that applies to you).

I feel tense or 'wound up':

<table>
<thead>
<tr>
<th>Most of the time</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A lot of the time</td>
<td></td>
</tr>
<tr>
<td>Time to time, occasionally</td>
<td></td>
</tr>
<tr>
<td>Not at all</td>
<td></td>
</tr>
</tbody>
</table>

I still enjoy the things I used to enjoy:

<table>
<thead>
<tr>
<th>Definitely as much</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Not quite so much</td>
<td></td>
</tr>
<tr>
<td>Only a little</td>
<td></td>
</tr>
<tr>
<td>Hardly at all</td>
<td></td>
</tr>
</tbody>
</table>

I get a sort of frightened feeling as if something awful is about to happen:

| Very definitely and quite badly |   |
| Yes, but not too badly          |   |
| A little, but it doesn't worry me|   |
| Not at all                      |   |

I can laugh and see the funny side of things:
Chapter 10

Worrying thoughts go through my mind:

<table>
<thead>
<tr>
<th>A great deal of the time</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A lot of the time</td>
<td></td>
</tr>
<tr>
<td>From time to time but not too often</td>
<td></td>
</tr>
<tr>
<td>Only occasionally</td>
<td></td>
</tr>
</tbody>
</table>

I feel cheerful:

<table>
<thead>
<tr>
<th>Not at all</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Not often</td>
<td></td>
</tr>
<tr>
<td>Sometimes</td>
<td></td>
</tr>
<tr>
<td>Most of the time</td>
<td></td>
</tr>
</tbody>
</table>

I can sit at ease and feel relaxed:

<table>
<thead>
<tr>
<th>Definitely</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Usually</td>
<td></td>
</tr>
<tr>
<td>Not often</td>
<td></td>
</tr>
<tr>
<td>Not at all</td>
<td></td>
</tr>
</tbody>
</table>

I feel as if I am slowed down:
<table>
<thead>
<tr>
<th>Frequency</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nearly all the time</td>
<td></td>
</tr>
<tr>
<td>Very often</td>
<td></td>
</tr>
<tr>
<td>Sometimes</td>
<td></td>
</tr>
<tr>
<td>Not at all</td>
<td></td>
</tr>
</tbody>
</table>
I get a sort of frightened feeling like 'butterflies' in the stomach:

<table>
<thead>
<tr>
<th>Not at all</th>
<th>Occasionally</th>
<th>Quite often</th>
<th>Very often</th>
</tr>
</thead>
</table>

I have lost interest in my appearance:

<table>
<thead>
<tr>
<th>Definitely</th>
<th>I don't take so much care as I should</th>
<th>I may not take quite as much care</th>
<th>I take just as much care as ever</th>
</tr>
</thead>
</table>

I feel restless as if I have to be on the move:

<table>
<thead>
<tr>
<th>Very much indeed</th>
<th>Quite a lot</th>
<th>Not very much</th>
<th>Not at all</th>
</tr>
</thead>
</table>

I look forward with enjoyment to things:

<table>
<thead>
<tr>
<th>As much as ever I did</th>
<th>Rather less than I used to</th>
<th>Definitely less than I used to</th>
<th>Hardly at all</th>
</tr>
</thead>
</table>
I get sudden feelings of panic:

<table>
<thead>
<tr>
<th>Very often indeed</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Quite often</td>
<td></td>
</tr>
<tr>
<td>Not very often</td>
<td></td>
</tr>
<tr>
<td>Not at all</td>
<td></td>
</tr>
</tbody>
</table>

I can enjoy a good book or radio or TV programme:

<table>
<thead>
<tr>
<th>Often</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sometimes</td>
<td></td>
</tr>
<tr>
<td>Not often</td>
<td></td>
</tr>
<tr>
<td>Very seldom</td>
<td></td>
</tr>
</tbody>
</table>

Date completed: ______________

10.8 General Health Questionnaire

General Health Questionnaire

Date completed: .........................

General Part

Please complete all questions about yourself

1. Weight .........................
2. Height: .........................

3. Current status: Please circle which describes you best.

- Single (never married)
- Married
- Partnership
- In a relationship
- Divorced
- Widowed
- Separated
Children: Yes/No

4. Current living situation: Please circle which best describes your living situation:

   Alone                     With Partner                   With Friends
   With Family           With Parents                    Other  .................

5. What type of accommodation do you live in:

   House          Nursing Home                        Supported Housing
   Flat              Shared Accommodation         Hospital

6. What is the highest level of education you have ever completed? Please circle one.

   Secondary school                   Polytechnic
   Apprenticeship                          Bachelor degree (BSc/BA)
   Grammar school                     Masters degree
   Doctoral degree

   Other, please describe:..............................................................

**Medical Part**

7. Have you ever been told that you have high blood pressure?

   Yes        No

   If Yes, please give details
   ........................................................................................................
   ........................................................................................................

8. Have you had any history of heart trouble?

   Yes        No

   If Yes, please give details
   ........................................................................................................
   ........................................................................................................

9. Have you a family history of heart disease/stroke?

   Yes        No

   If Yes, please give details
   ........................................................................................................
   ........................................................................................................

10. Have you ever been told by a doctor that you have asthma?

    Yes        No
11. Do you suffer from a wheezy chest?
   Yes  No

12. Do you ever have pains in your heart and chest?
   Yes  No

13. Do you ever feel faint or have spells of dizziness?
   Yes  No

14. Has a doctor ever told you that you have a bone or joint problem which could be made worse by exercise?
   Yes  No

15. Have you been in hospital at all in the last two years?
   Yes  No
   If Yes, please give reason and outcome
   …………………………………………………………………………………………………………

16. Have you had any operations or major illness in the last 6 months?
   Yes  No
   If Yes, please give details
   …………………………………………………………………………………………………………

17. Are you undergoing treatment or having any regular medication at the moment?
   Yes  No
   If Yes, please give details
   …………………………………………………………………………………………………………

18. Are you taking any pills or any other medicines, including inhalers, for any of the following:
   Heart trouble/angina? ………………………………. Yes/No
   Chest pains or blood pressure? ……………………. Yes/No
Asthma or other chest diseases?................................. Yes/No

Contraceptives?............................................................ Yes/No

Or for anything else?...................................................... Yes/No

If Yes, please give full details:
........................................................................................................
........................................................................................................
........................................................................................................

19. Do you have any physical disabilities of any kind?
   Yes    No

   If Yes, please give details
........................................................................................................
........................................................................................................

20. Have you ever suffered any complications from previous trials or treatments?
   Yes    No

   If Yes, please give details
........................................................................................................
........................................................................................................

21. Do you suffer from any allergies?
   Yes    No

   If Yes, please give details
........................................................................................................
........................................................................................................

22. Have you ever had an adverse reaction to a medicine or food?
   Yes    No

   If Yes, please give details
........................................................................................................
........................................................................................................

23. What is your average weekly intake of alcohol?
    .......................... units per week
    (One unit is half a pint of beer/one glass of wine/one measure of spirits)

24. Has there been a time when you regularly consumed more than 14 units of alcohol per week?
   Yes    No
25. How many cigarettes, cigars and/or tobacco do you smoke?

<table>
<thead>
<tr>
<th>Options</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
</tr>
<tr>
<td>1-5 a day</td>
<td></td>
</tr>
<tr>
<td>5-10 a day</td>
<td></td>
</tr>
<tr>
<td>10-20 a day</td>
<td></td>
</tr>
<tr>
<td>more than 20 per day</td>
<td></td>
</tr>
<tr>
<td>Less than 10 a month</td>
<td></td>
</tr>
<tr>
<td>Other (please specify)</td>
<td></td>
</tr>
</tbody>
</table>

If Yes, please give details

26. Have you, or a member of your family, ever been told by a doctor that you had epilepsy problems?

<table>
<thead>
<tr>
<th>Options</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

If Yes, please give details

27. Have you ever been diagnosed with a mental problem such as depression?

<table>
<thead>
<tr>
<th>Options</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

If Yes, please specify

28. Have you done any shift work in the last year?

<table>
<thead>
<tr>
<th>Options</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

29. Have you travelled across more than one time zone in the past 3 months?

<table>
<thead>
<tr>
<th>Options</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

30. *(Women only)* Do you have regular menstrual cycles? (ranging in length from 26-35 days with a maximum of three days variation month to month).

<table>
<thead>
<tr>
<th>Options</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>
31. How often, on average, do you consume caffeine containing beverages or food? (coffee, tea, cola beverages, chocolate etc.)

Never
Rarely (less than 10 times a month)
Occasionally (less than 1 time per day)
Sometimes (1-3 times per day)
Frequently (3-7 times per day)
Often (more than 7 times per day)

10.9 Sleep Diary

Patient no.

Day and date completed: ________________  Time completed_______

**How did you feel upon awakening?**
(Please put a vertical line on the lines below to indicate your preferences)

<table>
<thead>
<tr>
<th>Feeling</th>
<th>Extremely</th>
<th>Alert</th>
<th>Not at all</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extremely</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alert</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tired</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fully</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not at all</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rested</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extremely</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Happy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not at all</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

What time did you:

Turn off the light? ..................
Fall asleep?........................................
Wake up?........................................
How many times did you wake in the night?............... 
For how long (in total) were you awake during the night?..............
How many times did you nap during the day?......................
For how long (in total) did you nap during the day?......................
<table>
<thead>
<tr>
<th>Date:</th>
<th>Patient no:</th>
</tr>
</thead>
<tbody>
<tr>
<td>00:00</td>
<td>04:50</td>
</tr>
<tr>
<td>00:10</td>
<td><strong>05:00</strong></td>
</tr>
<tr>
<td>00:20</td>
<td>05:10</td>
</tr>
<tr>
<td>00:30</td>
<td>05:20</td>
</tr>
<tr>
<td>00:40</td>
<td>05:30</td>
</tr>
<tr>
<td>00:50</td>
<td>05:40</td>
</tr>
<tr>
<td><strong>01:00</strong></td>
<td>05:50</td>
</tr>
<tr>
<td>01:10</td>
<td>06:00</td>
</tr>
<tr>
<td>01:20</td>
<td>06:10</td>
</tr>
<tr>
<td>01:30</td>
<td>06:20</td>
</tr>
<tr>
<td>01:40</td>
<td>06:30</td>
</tr>
<tr>
<td>01:50</td>
<td>06:40</td>
</tr>
<tr>
<td><strong>02:00</strong></td>
<td>06:50</td>
</tr>
<tr>
<td>02:10</td>
<td><strong>07:00</strong></td>
</tr>
<tr>
<td>02:20</td>
<td>07:10</td>
</tr>
<tr>
<td>02:30</td>
<td>07:20</td>
</tr>
<tr>
<td>02:40</td>
<td>07:30</td>
</tr>
<tr>
<td>02:50</td>
<td>07:40</td>
</tr>
<tr>
<td><strong>03:00</strong></td>
<td>07:50</td>
</tr>
<tr>
<td>03:10</td>
<td><strong>08:00</strong></td>
</tr>
</tbody>
</table>
10.10 Publications and Abstracts

Manuscripts ready for submission

Structural brain changes in congenital and acquired bilateral anophthalmia compared to healthy controls
Rupal Morjaria, Gaëlle S L Coullan, Rebecca Trossman, Catherine E Warnaby, Russell G Foster, Susan M Downes & Holly Bridge.

Functional MRI imaging during extra-ocular light stimulation in anophthalmic and sighted subjects – No evidence for extra-ocular photosensitive receptors
Rupal Morjaria, Gaëlle S L Coullan, Stuart Pierson, Katie Warnaby, Carina Pothecary, Brian Leatherbarrow, Russell Foster, Susan M. Downes & Holly Bridge.

Manuscripts in preparation

Impact of Diabetic Retinopathy on Sleep and Mood
Rupal Morjaria, Iona Alexander, Ngai Victor Chong, Katharina Wulff, Russell G Foster, Susan M Downes
Circadian rhythm disorders in severe Sjogren’s Syndrome.
Iona Alexander, Rupal Morjaria, Jasleen Jolly, Katharina Wulff, Russell G Foster, Susan M Downes

Sleep/Wake disturbance in anophthalmia.
Rupal Morjaria, Iona Alexander, Brian Leatherbarrow, Katharina Wulff, Russell G Foster, Susan M Downes

<table>
<thead>
<tr>
<th>Presentations &amp; Posters</th>
<th>Titles &amp; authors</th>
</tr>
</thead>
</table>
| International           | **ESRS September 2016**  
  Alexander I, **Morjaria R**, Wulff K, Foster R, Downes S. Sleep quality in Anophthalmia. |
|                         | **ESRS September 2014**  
  Alexander I, **Morjaria R**, Wulff K, Foster R, Downes S. Sleep quality in Anophthalmia. |
|                         | **ARVO May 2014**  
|                         | **Royal College of Ophthalmologists 2014**  
<table>
<thead>
<tr>
<th>Event</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>National Presentations</strong></td>
<td>MOS (Midland Ophthalmological Society) Birmingham 2016 Impact of Anophthalmia on Circadian Rhythms. Oral Presentation; Won Travel Grant</td>
</tr>
<tr>
<td></td>
<td>MOS (Midland Ophthalmological Society) Birmingham 2016 Impact of Diabetes on Circadian Rhythms. Poster Presentation; Won Poster Prize</td>
</tr>
<tr>
<td></td>
<td>Impact of Ocular Disease on Sleep-wake and Mood meeting Oxford 2015. Sleep studies in ocular disease/anophthalmia</td>
</tr>
<tr>
<td></td>
<td>MOS (Medical Ophthalmological Society) London 2014 Impact of Diabetic retinopathy on Circadian Rhythms</td>
</tr>
<tr>
<td></td>
<td>OBCS (Oxford Bristol Cardiff Southampton) 2014 Impact of Ocular disease on Circadian Rhythms</td>
</tr>
</tbody>
</table>