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A Mixed Method Approach to Post-Awakening Cortisol Research: Exploring Associations with Post-Awakening Melatonin, Primary Open-Angle Glaucoma and Experiences of Participation

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A Mixed Method Approach to Post-Awakening Cortisol Research: Exploring Associations with Post-Awakening Melatonin, Primary Open-Angle Glaucoma and Experiences of Participation

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A thesis submitted in partial fulfilment of the requirements of the University of Westminster for the degree of Doctor of Philosophy

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Abstract

The non-invasive, ecologically valid, measurement of post awakening cortisol secretion (PACS) in saliva tissue has greatly informed literature within the psychoneuroendocrinological discipline. Measured in the first 45 minutes of awakening, Indices of PACS include the rise in cortisol (cortisol awakening response: CAR) and total cortisol concentrations as measured by the area under the curve with reference to ground: AUCg. Via melanopsin containing retinal ganglion cells (mRGCs), upon awakening the hypothalamic suprachiasmatic nucleus (SCN) enhances cortisol secretion at wake, in response to light. The same projection of stimuli to the SCN also suppresses melatonin production, inducing sleep. The current work of this programme of research firstly investigated the relationship between post awakening cortisol secretion (PACS) and post-awakening melatonin secretion (PAMS). Loss of mRGCs have been implicated in those with primary open-angle glaucoma (POAG). The second study therefore explored the relationship of PACS with primary open-angle glaucoma (POAG); a previously unstudied group. In the final study, an exploratory qualitative, investigation of individual experiences in participation in salivary cortisol research was conducted.

In study I, analysis of data from healthy males and females demonstrated for the first time the post awakening pattern of melatonin and its relationship with PACS.. Results displayed a typical cortisol awakening response (CAR) profile, with a substantial increase in cortisol over the 45-minute post-awakening period. The decrease in post awakening melatonin secretion (PAMS) however was not statistically significant, as there was no main effect of sampling. Further, there were no associations between the hormone's composites.

In study II, participants with POAG displayed the usual peak in cortisol following morning awakening over a two-day study period. This finding indicated preservation of the CAR amongst individuals with POAG. Overall post-awakening cortisol secretion of individuals with POAG was higher in comparison to a reference data (RD) group providing support for studies which have suggested an elevation

of cortisol in those with high intraocular pressure. Furthermore, self-reported visual function in the POAG group, was negatively associated with the CAR, indicating that participants who reported a lower level of visual function displayed a greater rise in cortisol following awakening. In addition, on observation of the adherence data of the POAG group, findings also revealed that participants delayed the first morning sample (> 8 minutes) more often than the RD group. These findings indicated that participants with POAG experienced difficulties adhering to the sampling protocol in comparison to the RD group with no known visual difficulties. The findings of this study prompted questions surrounding the methodologies of PACS research. With successful measures of PACS taking place in a variety of sample groups, little is known about the overall experiences of being a participant in these studies. Furthermore, to achieve a successful first morning sample, a crucial element of this research is the assumption that participants have fundamentally the same understanding of "morning awakening".

The aim of the final study was to understand the experiences of volunteers taking part in a PACS research study and to explore the understanding of the moment of morning awakening. A thematic analysis of the interview transcripts led to the development of three overarching themes. The first theme involved the motivations for taking part, for example, participants reported 'a sense of duty to research'. The second overarching theme encapsulated the experiences of participation. Here, participants recounted on the study's impact on sleep, personal apprehensions, heightened cognition, disruptions to morning routine and overall habituation after initial study day. The final theme identified the understanding of the moment of morning awakening. Participants discussed the difficulty in both explicitly describing and identifying the actual point of morning awakening. PACS research studies often take for granted the 'self-collection' instructions provided to participants. Through qualitative analyses of interviews study III emphasise the ambiguity of morning awakening and the difficulties participants experienced during the PACS study period and the requirement for PACS researchers to consider this when recruiting participants.

Overall, the unique contribution of this programme of research is threefold. Firstly, this was the first study to assay salivary melatonin in the UK and consider the

relationship between PACS and PAMS in healthy participants. Secondly, the investigation of the patterns of PACS in those POAG in comparison with a RD group is also conducted for the first time. And finally, this programme of research uses a qualitative methodology for the first time to explore the experiences of participants taking part in PACS research studies. This gives rise to several methodological recommendations and informs current best practice methodology for PACS research:

i. At the participant recruitment stage, researchers should account for sleep differences in sleep patterns.
ii. Researchers should enter an active conversation surrounding the subjectivity in the understanding of the first point of morning wakefulness.
iii. A recommendation of a minimum repeated study days (3 days) is necessary to habituate to initial participant responses.

Declaration

The work presented in this thesis is the work of the author. The reference group data for study II were obtained from a study published in 2019 in the Social Sciences department at the University of Westminster (Smyth et al., 2019). Dr Sanjay Joban is an academic member of the University of Westminster who aided in interviewing staff members for Study III.

List of Abbreviations

ACTH	Adrenocorticotropin
AUCg	Area under the curve with respect to ground
CAR	Cortisol Awakening Response
CRH	Corticotrophin-releasing hormone
DLMO	Dim Light Melatonin Onset
FAS	Fatigue assessment scale
GABA	Gamma-aminobutyric acid
HPA	Hypothalamic-pituitary-adrenal
HTA	Human Tissue Act
MnInc	Mean increase
mRGCs	Melanopsin containing retinal ganglion cells
NEI-VFQ	National Eye Institute – visual function questionnaire
MAC	Members of the academic community
PACS	Post awakening cortisol secretion
PAMS	Post awakening melatonin secretion
PIPR	Post-illumination pupil response
PNEC	Psychoneuroendocrinology
POAG	Primary open-angle glaucoma

PRGCs	Photosensitive retinal ganglion cells
PVN	Paraventricular nuclei
PSS	Perceived stress scale
RD	Reference data
RGCs	Retinal ganglion cells
S1	Sample 1
SAM	Sympathetic adrenomedullary system
SCN	Suprachiasmatic nucleus
SEM	Standard Error Mean
SQRT	Square root
WEMWBS	Warwick and Edinburgh Mental wellbeing scale

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List of Publications and Presentations

Peer-reviewed paper:

Ramachandran, N., Smyth, N., Thorn, L., Eardley, A., Evans, P., Clow, A., 2016. Relationship between post-awakening salivary cortisol and melatonin secretion in healthy participants. Stress 19, 260–263.

Conference Poster presentations:

Ramachandran N, Smyth, N, Thorn, Clow A. The Cortisol Awakening Response: Vision and the sleep wake cycle. Psychology, University of Westminster Annual Research Conference, London, May 2017.

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A special thank you to my amazing friend – Roopa. Thank you for keeping me going with nights out and evenings in, filled with food, wine, champagne and laughter. I would also like to remember my close friend Simrit Sandhu who lost her short and sudden battle with cancer during my studies. I take this moment to remember the care and support she brought me and the amazing times we shared.

I have been blessed with a very loving and supportive little family (inclusive of furry–friends - my cats Max and Bob). I am so grateful to my husband – Roysan. My gratitude is beyond words because it is so boundless. He is my biggest cheerleader, my best friend and the most amazing husband and father. Thank you for believing in me and encouraging me to believe in myself. You are my rock. Finally, I would like to thank my little boy Reeyan (8 years old) and my little girl Leela (7 years old) for being the most wonderful and happy children. They have brought such ‘meaning’ to my PhD journey and I am so grateful to be their mumma.

Roysan, Reeyan and Leela have shared this entire PhD journey with me, for this, I dedicate this piece of work to you.

Chapter 1 Introduction

Salivary measurement of hormonal circadian rhythmicity and the light dark cycle

1.1. Overview

This Chapter begins by providing a brief introduction to the literature behind the neuroendocrinological systems that mediate hormonal function. An elaborate examination of the function of the hypothalamic suprachiasmatic nucleus (SCN) and the influence of the light-dark cycle is also discussed. Light entering the eyes, projecting through the retinohypothalamic pathway activates the Hypothalamic Pituitary Adrenal (HPA)-axis which ultimately leads to the secretion of an essential human glucocorticoid - cortisol. The HPA-Axis is activated in response to both internal and external triggers. However, an additional pathway (innervation of the adrenal gland via the splanchnic nerve) has been associated with basal, non-stress activity. It highlights the critical role of cortisol as a master hormone that is responsible for regulating physiological function around the 24-hour light-dark cycle. The SCN is also responsible for the secretagogue function of the pineal gland. Thus, this initial introductory section examines the function of the pineal gland and its secretory hormone- melatonin, the circadian hormone responsible for sleep.

The measure of hormonal circadian rhythmicity in saliva samples and emphasis on the relation to the light-dark cycle forms the latter part of the Chapter. Whilst the light-dark cycle is an important ambient factor that mutually influences these circadian hormones, previous literature is lacking when attempting to understand post awakening cortisol secretion (PACS) in relation to post awakening melatonin

secretion (PAMS). In addition, conditions characterised by visual deficits, like primary open angle glaucoma (POAG) have been implicated in photosensitive retinal ganglion cell (pRGC) loss which suggest there may be an impact on circadian cortisol secretion, thus merits further investigation. Previous literature, again, is limited to observing melatonin suppression in POAG and there exists a gap in understanding the patterns of the PACS in this formerly unstudied group.

The penultimate section of this Chapter centres on the methodological practices of post awakening cortisol secretion (PACS) research studies. The established objective measures of participant adherence to the timing of the first morning saliva sample of the PACS is critical to the researcher. PACS researchers are therefore reliant on participants' self-reported wake time and commitment to conduct a timely first morning sample. To date, there is a lack of literature which has endeavoured in qualitatively exploring motivations, experiences of taking part in PACS research and participants' understanding of the timely moment of awakening.

The primary objective of this programme of research therefore, is to expand on previous literature by firstly exploring post awakening melatonin secretion in relation to PACS. Secondly, this research investigates PACS in participants with POAG, a previously unstudied group. Following expert consensus when investigating PACS, both studies in this thesis will adopt a strict monitoring of sampling adherence. Finally, with aim to inform methodological practices, this programme of research is interested in understanding the motivations and experiences of participants that have taken part in PACS research studies. Particular attention is given to capture participants understanding of the moments of morning awakening, an essential element of PACS research.

1.2. Psychoneuroendocrinology

The studies within this thesis fall under the area of Psychoneuroendocrinology (PNEC), which incorporates the research endeavour aimed at understanding functional links between nervous and endocrine system activity and their relation

to human behaviour, health and disease. This relatively new science encompasses knowledge from a wide range of disciplines including psychology, neurobiology, endocrinology, immunology, neurology, psychiatry, and medicine. For example, a recent paper by Lucchetti et al., (2013) uses a systematic methodology to search the terms “pineal gland” and ‘epiphysis cerebri’. The authors identified several themes associated with the pineal gland which included mental health, reproductive function, endocrinology, relationship with physical activity and spiritual connection - all of which can be applied to the multidisciplinary approach which form the basis of psychoneuroendocrinology today.

Researchers and clinicians have taken an active role in identifying the neural correlates of environmental acute and chronic stressors in relation to progressive disease and mortality (Agorastos et al., 2019; Mariotti, 2015; Mikkelsen et al., 2017). More so, it has been well established that psychosocial factors such as anxiety and depression have strong links with overall adverse health outcomes and increased mortality risk (Gassen et al., 2022; Juster et al., 2010, 2011). For example, childhood abuse, social disadvantages, social isolation in adulthood have all been associated with higher risks to cardiovascular morbidity (Barth et al., 2004; Suls, 2017).

Studies conducted in the 1980s have suggested that the positive correlation between cardiovascular responses and acute psychological stressors predict a high predisposition to later hypertension (Light, 1981; Orbist, 1981). Subsequently, a more recent meta-analysis conducted by Turner et al., (2020) examined 1720 papers and consisted of approximately 33,000 healthy participants. Authors attempted to understand the scale of the response to acute psychological stress in relation to prospective health and disease outcomes. They confidently identified that stress reactivity as a predictor for future health and disease outcomes included an increased predisposition for cardiovascular disease. Besides associations with chronic stress and depression, circadian disruption is also a known factor to be associated with adverse health conditions (Abbott et al., 2020; Hou et al., 2020; Sorensen et al., 2020; Wong et al., 2022).

Recent literature surrounding the study of insomnia and ill health suggests that individuals with this sleep disorder have an increased risk for dementia, particularly Alzheimer's disease (Hoile et al., 2019; L. Shi et al., 2018). However, even circadian disruption in early life can impact growth, development, and other health outcomes. During pregnancy, disruptions to circadian rhythms due to shift work have been associated with increased risk to premature birth and metabolic diseases in adult offspring (Abbott et al., 2020; Hou et al., 2020; Sorensen et al., 2020; Wong et al., 2022).

The pathways mediating the responses mentioned above, particularly that of the stress response - is the activation of the neuroendocrine systems. In conjunction with the endocrine system, hormones are secreted into circulation, providing a basis for cellular communication. The principal link between the nervous system and the endocrine system is via the hypothalamus of the brain and the adrenal gland, anterior pituitary also known as the Hypothalamic Pituitary Adrenal (HPA) axis.

The regulation of circadian rhythmicity in physiological function is controlled by an endogenous biological clock. The SCN part of the hypothalamus is the body's endogenous pacemaker. It largely controls the circadian rhythm of the neuroendocrine system by two pathways to the adrenal cortex: via the Hypothalamic Pituitary-Adrenal axis (HPA-axis) and the splanchnic nerve of the sympathetic nervous system. The function of the SCN and the methodology used to measure it, are the focus of the present thesis as well as its physiologically important end-product cortisol and melatonin detailed further in Sections 1.5 and 1.7 below.

1.3. The SCN and the light-dark cycle

Situated in the hypothalamus, the SCN is the body's 24-hour endogenous oscillator. The SCN switches from an active to an inactive state by using transcription and translation feedback loops within its deoxyribonucleic acid (DNA). The resultant neuropeptide communicates with surrounding cells in turn regulating brain and body function (Clow et al., 2010; Ma & Morrison, 2021). The cells of the

SCN synchronise with each other and external environmental cues. Such cues that entrain an organism's biological rhythms are called zeitgebers. Due to the strategic positioning of the SCN over the optic chiasma, light is the most powerful zeitgeber, resulting in light messages transmitting along the optic nerves via melanopsin-containing retinal ganglion cells (mRGCs) and projecting to the day and night cells in the SCN.

Melanopsin is a photo pigment contained in intrinsic photosensitive retinal ganglion cells (pRGC) of all mammals. These specialised cells detect environmental brightness (associated with a non-imaging function) integrating direct light responses with signals from rod and cone cells. There is a direct projection of fibres from the melanopsin-containing pRGCs to the SCN via the retinohypothalamic tract, synchronising the circadian pacemaker to the 24-hour solar cycle (Benarroch, 2011). The circadian pattern of cortisol is driven by the hypothalamic suprachiasmatic nucleus (SCN).

1.4. The HPA-Axis

The glucocorticoid, steroidal hormone – cortisol is the final product following activation of the HPA axis. As well as psychological stress, triggers for the activation of the HPA axis include pain, smoking, immune system activation, food consumption, vigorous exercise, changes in cardiovascular tone, respiratory distress, and awakening.

The cascade of physiological regulation of the HPA axis, commences with the stimulation of the neurosecretory parvocellular neurons in the paraventricular nuclei (PVN) of the hypothalamus to produce corticotrophin-releasing hormone (CRH; See Figure for illustration 1.1). CRH is transported to the anterior pituitary and the corticoptroph cells are stimulated to synthesis and release of adrenocorticotropin (ACTH) into the general circulation. ACTH circulates peripherally to arrive at the adrenal glands, which are situated above the kidneys. The adrenal cortex produces various steroid hormones. Cortisol is the main glucocorticoid in humans that is produced mostly by the zona fasciculate and at a lower rate by the inner zona reticularis.

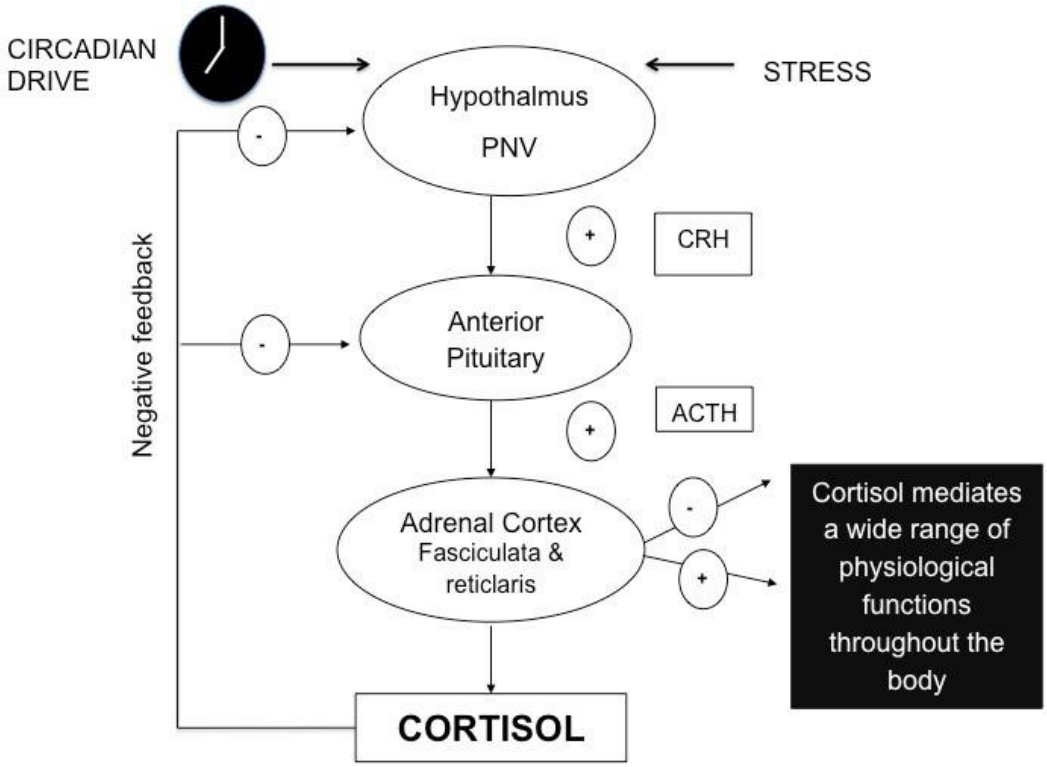

Figure 1.1 The hypothalamic-pituitary-adrenal (HPA) axis

Illustrates the physiological cascade involved in the HPA axis in humans. Corticotrophin releasing hormone (CRH) is transported to the anterior pituitary via the hypothalamic-pituitary portal blood vessels. Adrenocorticotrophic hormone (ACTH) is released into the general circulation. The '+' and '-' symbols indicate activation and inhibition respectively; cortisol exerts negative feedback as indicated to regulate the on-going cascade

1.5. Actions of cortisol

Essential for life, cortisol plays a fundamental role in maintaining regular physical function (Baxter, et al., 1995). A major responsibility of cortisol lies with its metabolic function and the mobilisation of energy reserves (Sapolsky et al., 2000). Cortisol is responsible for the utilisation of physiological resources (e.g., energy release) and enabling recovery from sympathetic adrenal medullary (SAM) activation (Nelson, 2011; Thau et al., 2021). By elevating the circulation of glucose concentrations, cortisol promotes the breakdown of protein and fat stores in the body. The overall outcome is a release of energy reserves that allow

for sufficient metabolic functioning (Dickerson & Kemeny, 2004; Sapolsky et al., 2000). Thus, cortisol's essential role of freeing energy stores provides fuel to promote and sustain physical activity in response to the activation of the HPA axis as a result of a psychological or social stressor.

Cortisol has several physiological functions which extend beyond its principal role in energy mobilisation. It is responsible for the regulation of blood pressure, cardiovascular function, metabolism of carbohydrates, body-fat distribution, serum calcium concentrations and immune function. There have also been tremendous effects associated with cortisol and the influence it has on the bones via calcium absorption. Excess cortisol caused by disorders such as Cushing's disease, can lead to a decrease in bone density resulting in osteoporosis. Cortisol also increases appetite and calorie intake, therefore, those suffering with Cushing's disease often deal with obesity due to the uneven distribution of fatty tissue around the abdomen, trunk and face (Clutter, 2011).

Glucocorticoids influence neural activity, mood and behaviour. Both chronic hypercortisolism and chronic hypocortisolism observed in Cushing's disease and Addison's disease respectively present with negative psychological symptoms revealing the role of cortisol in affective processes (Godoy et al., 2018). Cortisol also regulates a variety of developmental events in the brain; high levels can have detrimental effects on brain development. It has effects on cognitive performance, but this relationship is complex and unclear. For example, administration of cortisol has been shown to enhance (Krekeler et al., 2021) and impair (Kirschbaum et al., 1996; Law & Clow, 2020) cognitive performance, such as, memory. Table 1.1 details the actions of cortisol, each of which are synchronised around the 24-hour dark/light cycle by its circadian pattern of secretion.

Table 1.1 A summary of the actions of the hormone cortisol (adapted from Genuth, 1998)

Actions of the hormone cortisol
Support of glucose availability drawing upon protein and fat reserves (gluconeogenesis)
Inhibition of inflammatory processes and modulation of immune responses
Modulation of emotional tone
Promotion of wakefulness
Maintenance of cardiac output; increased arteriolar tone; decreased endothelial permeability
Increased glomerular filtration and free water clearance
Regulation of muscle function; decreased muscle mass
Decreased bone formation; Increased bone reabsorption

1.5.1. Circadian patterns of cortisol

All life on earth is reliant on the energy of the sun. The Earth's predictable 24-hour cycle of light and darkness results in most aspects of physiology and behaviour displaying circadian variations that are fundamental for survival (Golombek et al., 2010). Humans present daily physiological and behavioural rhythms with marked variations. The main feature which defines a circadian clock is the ability of a peripheral organ to maintain self-sustained oscillations with a period approximating 24-hours (Oster et al., 2017b). These cellular mechanisms are therefore more specifically sensitive to entrainment from light stimuli and reflect the rhythms observed in a wide range of functions including sleep, body temperature, urinary excretion of potassium and plasma concentrations of growth hormone and cortisol.

Cortisol has one of the most distinctive circadian rhythms in the healthy individual. Figure 1.2 illustrates the peak levels in the morning following awakening (the cortisol awakening response: CAR) and a declining pattern across the day, reaching nadir in the late evening and early part of sleep, before gradually increasing during late sleep prior to subsequent morning awakening (O'Byrne et al., 2021; Oster et al., 2017a; Thau et al., 2021).

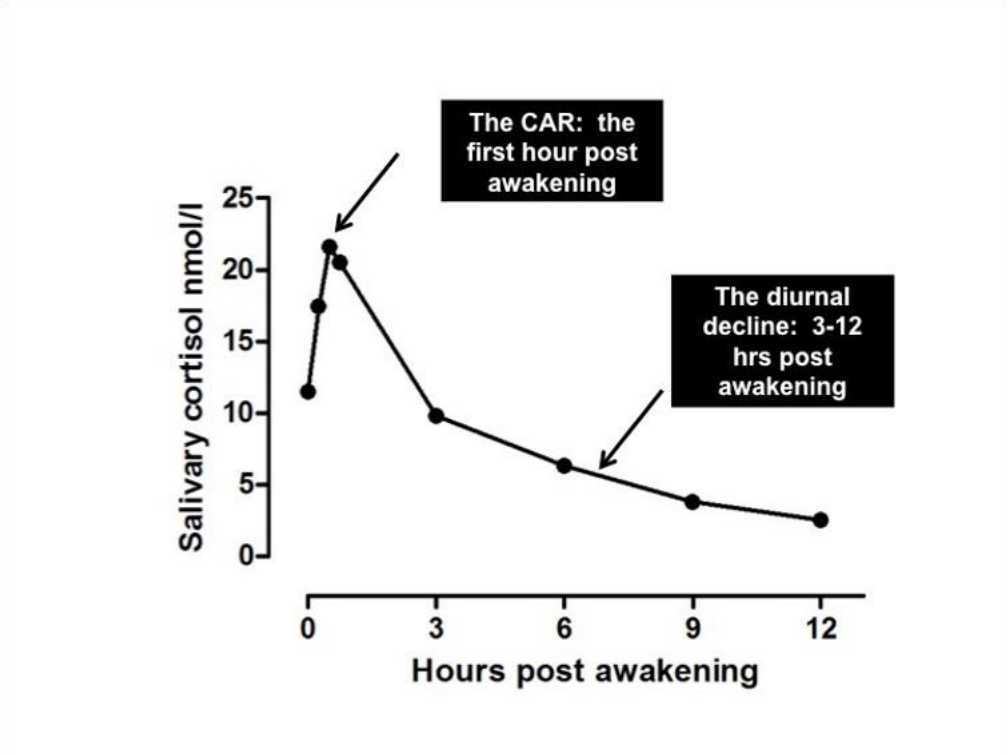

Figure 1.2 The diurnal pattern of cortisol secretion in healthy adults plot (Clow et al., 2010)

illustrates the cortisol awakening response (CAR) and diurnal decline. Data derived from a composite of studies conducted at the laboratory at the University of Westminster and indicate the typical values across the day

An additional pathway has been suggested to be more specifically associated with basal, non-stress cortisol secretory (Buijs et al., 2003a; Kalsbeek et al., 2012). This secondary, non-HPA axis dependant mechanism, is faster as it innervates the adrenal gland via the splanchnic nerve, increasing adrenal sensitivity to ACTH (Bornstein et al., 2008; Engeland, 1998; Ulrich-Lai & Engeland, 2002). The latter route has been suggested to be involved in facilitating light induced effects on the CAR (see review by Clow et al., 2010) and has particular importance with respect to the fine tuning of circadian influences ((Buijs et al., 2003a; Ulrich-Lai & Engeland, 2002). It is the role of this pathway which will be further explored in the following Chapters in relation to post awakening cortisol secretion (PACS). The mechanism however, by which the SCN imposes circadian rhythmicity on cortisol secretion may involve both stimulatory and inhibitory components.

Cortisol secretion is thus the product of either direct or indirect activation of the HPA axis cascade which generates its secretagogue, adrenocorticotrophic hormone (ACTH), from the anterior pituitary into to circulation. In addition, the release of melatonin is the product of a more complex multi-synaptic neuronal pathway from the SCN to the pineal gland via spinal nuclei of sympathetic neurons (Claustrat et al., 2005). These hormones perform complementary roles with cortisol dominant during daytime activities and melatonin dominant during night-time sleep.

1.6. The Pineal Gland

This highly vascularised neuroendocrine organ has been considered 'the seat of the soul' by the great philosopher Rene Descartes. Situated in the mid-line of the brain, the main function of the pineal gland is to receive and convey information about current environmental light-dark cycles, successively secreting melatonin.

Retinal ganglion cells containing melanopsin detect environmental brightness (associated with a non-imaging function), send neural signals to non-image forming areas of the brain including the pineal gland. Photic information is sent to the SCN in which positive light signals initiates the production of gamma-amnio-butyric acid (GABA) which inhibits the neuronal communication between the paraventricular nucleus (PVN) and the pineal gland. This in turn prevents the synthesis of melatonin. Conversely, during times of darkness, the SCN secretes glutamate which in turn stimulates the PVN to communicate with the superior cervical ganglion of the spinal column. Here, the superior cervical ganglion communicates with the pineal gland and melatonin is synthesised via the activation of pinealocytes.

1.7. Actions of melatonin

Often described as the 'hormone of darkness', melatonin is secreted into central and peripheral circulation. Like cortisol, melatonin has several vital roles in the human body. Commencing from early foetal development, melatonin has a part to play in regulating placental functions and the development of the central nervous

system (Tordjman et al., 2017). Out of the womb, melatonin is involved in several other physiological functions as outlined in Table 1.1 below.

Table 1. 1 A summary of some of the actions of the hormone melatonin (adapted from (Tordjman et al., 2017)

Actions of the hormone melatonin
Antihypertensive effect; rise of melatonin during sleep contributes to a low blood pressure
Enhances innate and cellular immunity, stimulating production of granulocytes and macrophages
Body mass regulation by preventing the increase in body fat with age
Increased bone mass by promoting osteoblast cell differentiation and bone formation
Physiological effects on reproduction through down-regulation of gonadotropin-releasing hormone (GnRH)

1.1.1. Circadian rhythm of melatonin

Melatonin also has a distinctive circadian rhythm and is secreted almost exclusively during the night (in both nocturnal and diurnal species). In humans, the daily rhythm of melatonin commences soon after dusk, reaching a peak in the middle of the night between 2 and 4am, gradually declining slowly during the second half of night-time sleep (see Figure 1.3). The daily rhythm of melatonin disperses chronological cues to a host of target tissues expressing melatonin receptors. The greatest density of melatonin receptors in humans is in the SCN. Melatonin is thus, representative of an internal zeitgeber, which boosts synchrony within the circadian system (Oster et al., 2017a; Shuboni et al., 2016; Yanovski et al., 1990).

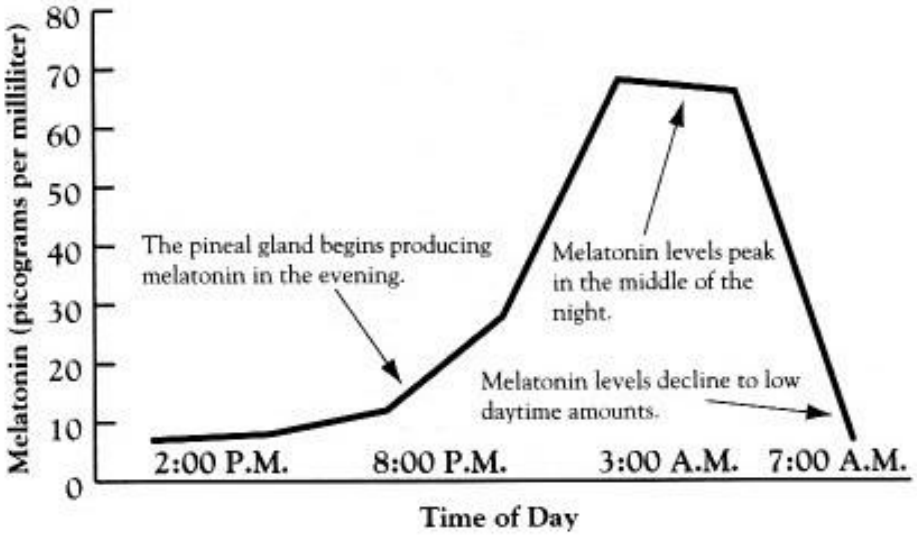

Figure 1.3 Melatonin concentration (pg/mL) between 2:00pm and 7:00am

1.8. Salivary measures

A fundamental purpose of saliva in research, is its function of 'objectivity'. The wide spectrum of molecules present in saliva provides valuable information for clinical diagnostic application. Applications include diagnostics in autoimmune diseases, cardiovascular markers, monitoring of drug level and many more (Malathi et al., 2014; Pedersen et al., 2005)

Within the last two decades, the evolution of salivary measures as both a diagnostic and detection of biological markers is representative of the dynamic field of clinical research. Saliva sampling has been used to detect several diseases including oral cancers (Ishikawa et al., 2017, 2020), neurodegenerative/neuropsychiatric diseases (Orive et al., 2022; Pawlik et al., 2021) and gastric cancer (Aqeel Aslam et al., 2020; Herrera-Pariente et al., 2021). It has a number of additional advantages over the determination of several biomarkers from blood samples. Unlike blood sampling, saliva sampling is convenient, non-invasive and can be carried out by any individual without being medically trained staff. Saliva sampling also provides scrutiny of the dynamics of the secretory activity in question during repeated sampling. Furthermore, the salivary sampling method is non-stressful which is

essential in any investigation of basal secretory activity. The following sections will now look at this in more detail.

1.9. Diurnal pattern of salivary cortisol secretion

Biologically active 'free' cortisol accounts for only 5-10% as the majority is bound to large proteins (cortisol binding protein and albumin) in plasma. Subsequently, blood cortisol is often measured as 'total' cortisol which encompasses both bound and unbound elements. However, methods of analyses of the free, active cortisol component in blood have been observed as being both time-consuming and expensive (Vining et al., 1983). Over three decades have passed since the validation of the assessment of cortisol in saliva samples by Clemens Kirschbaum and Dirk Hellhammer working within the laboratories based at the department of Clinical and Physiological Psychology in Germany (see (Kirschbaum et al., 1989). The revolutionary moment in research opened opportunities to investigate the dynamically changing state of biologically relevant HPA axis activity from repeated sampling of easily accessed saliva. The assessment of salivary cortisol measures only the biologically active "free" component of cortisol as the bound component is too large a molecule to pass through the saliva glands. Described as *a non-invasive window on the brain* (Clow & Smyth, 2020), the basal patterns of cortisol secretion provide insight about brain processes involved in circadian regulation, which are vital for brain health (Karatsoreos, 2012; Oster et al., 2017a)

As detailed in Section 1.5 above, diurnal patterns of cortisol can be separated into two essential components: the cortisol awakening response (CAR) and the daily diurnal cortisol decline. Repeated saliva sampling enables measurement of the diurnal pattern of cortisol secretion under naturalistic settings, providing an account of real-life exposure to stress and may be more relevant to long-term health outcomes (Adam et al., 2017a; Mikkelsen et al., 2017; Ryan et al., 2016). Although the diurnal decline is an important aspect of cortisol rhythmicity, the following section and the basis of this thesis is principally interested in discussing salivary measures of the CAR.

Typically, under basal conditions, a healthy HPA-axis is characterised by a distinctive circadian pattern of cortisol secretion, whereby salivary cortisol rises to a peak within 30-45 minutes of waking then falls to a nadir during sleep at approximately midnight. The major measurable parameters of this diurnal rhythm are the cortisol awakening response (CAR), which is the rise in cortisol during the first 30-45-minute following awakening (Clow et al., 2004). In general, an abnormal CAR, both abnormally large and small, appear to be consistent markers of HPA axis dysfunction (Adam & Kumari, 2009)

1.9.1. Measurement of post awakening cortisol indices

Measured in the first 45 minutes of awakening, indices of PACS include the rise in cortisol (cortisol awakening response: CAR) and total cortisol concentrations as measured by the area under the curve with reference to ground. Sample 1 refers to time 0 minutes post awakening, sample 2 is 15 minutes, 3 is 30 minutes and 4 is 45 minutes post awakening. The AUCg is calculated using the following formula sample2 + sample3 +(sample1+sample4)/2 (see Figure 1.4 below). The AUCg provides a good measure of overall cortisol secretion and has been previously demonstrated to be relatively stable across days and weeks in the absence of a significant stressor (Edwards et al., 2001a; Pruessner et al., 1997a; Wust et al., 2000b). The mean increase (MnInc calculation: sample2 + s3 + s4)/3-s1) however has been closely associated with measures of the dynamic change in cortisol following awakening. Thus, it has been argued that CAR is characteristically a ‘response’ to awakening (Clow and Thorn, 2010),

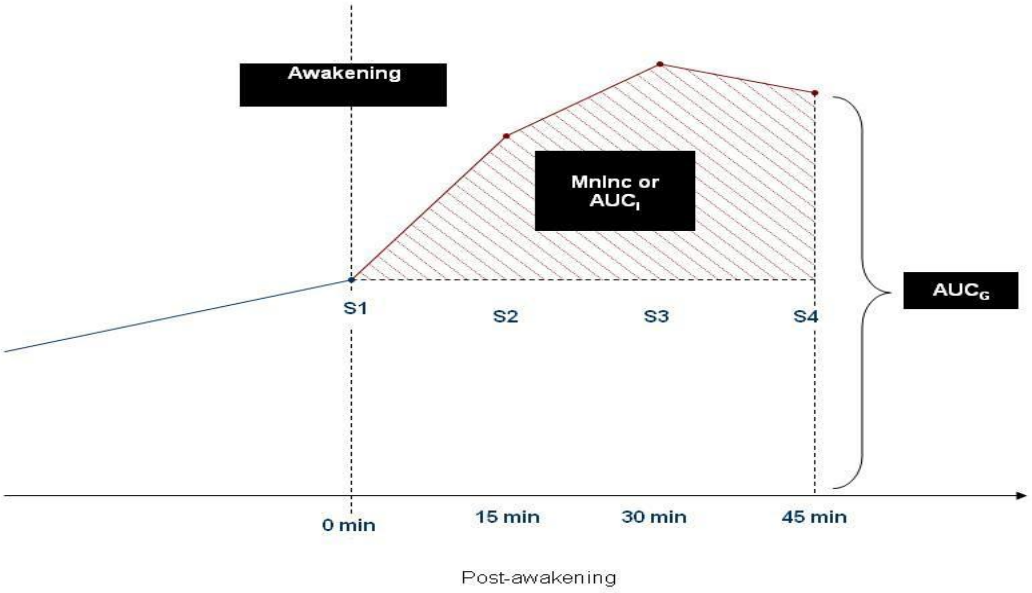

Figure 1.4 Measurement of the CAR, AUCi and AUCg

MnInc (mean increase) and AUCI (area under the curve relative to increase) provides an estimate of CAR magnitude. AUCg provides an estimate of total post-awakening cortisol secretion. Formulae: MnInc = (s2 + s3 + s4)/3 – s1; AUCI = s2 + s3 + [(s4 – s1)/2] - 2s1; AUCG = s1 + s2 + s3 + [(s4 – s1) / 2]; or AUCI = AUCG - 3s1 (s1 collected immediately on awakening, s2 collected at 15 min, s3 at 30 and s4 at 45 minutes post-awakening. These formulae all assume equal time intervals, arbitrarily denoted at unity, between all samples).

The ultradian rhythm of cortisol is determined by the pulsatile function of the HPA-axis, which results in a dynamic, fluctuating pattern with transitory secretory bursts. The CAR has recently been suggested to be the first pulsatile event of the day and a response to waking (Evans et al., 2019). The process of awakening is thought to synchronize the start of the ultradian rhythm so that the CAR, even when measured in saliva in multiple participants, is apparent as the coordinated initial pulse of the day.

Throughout the awakening process there is a dynamic change in salivary cortisol concentration, peaking at 30-45 minutes post-awakening. It has been approximately 3 decades since research has been able to successfully establish

the distinction between the secretion of cortisol levels across most of the day and the CAR (see above Figure 1.2). Pruessner and colleagues were the first to reveal the pattern of the CAR in healthy participants (Pruessner et al., 1997a). The same researchers later provide a more detailed clarification of the CAR demonstrating the time course and intra-individuality constancy of the CAR over three consecutive days and three consecutive weeks in children, younger and older adults (Pruessner et al., 1997b).

Stalder et al., (2013) describes the ability of all infants examined, to achieve a CAR. Initiated in response to waking, the CAR is subsequently evident in those as young as two months old and considered a relatively distinct aspect of circadian rhythm (Wilhelm et al., 2007). The CAR is relatively more sensitive to a range of psychosocial and health outcomes than the diurnal profile (see Chida & Steptoe, 2009; Fries et al., 2009). Disruption of the CAR can be considered therefore an early marker of dysregulated brain function and brain health (Adam et al., 2017b). Moreover, disruption has been associated with a number of other health and trait psychosocial variables such as mood (Thorn et al., 2009). As such, study of biologically active 'free' cortisol in saliva samples provides a useful pre-clinical indicator of the links between mind and body in currently healthy and clinical populations (Law & Clow, 2020; Smyth et al., 2013). Similar to salivary cortisol, there has been continuing interest in the potential role of melatonin in health and disease as well as the role of circadian rhythms in overall physiology.

1.10. Basal pattern of salivary melatonin

Arriving at the jugular vein of the neck, melatonin leaves the pineal gland and is secreted in the blood. Total melatonin is a protein-bound low molecular weight biomarker that is present in low concentrations and is amongst numerous potentially interfering 'larger' structure counterparts such as serotonin and N-acetylserotonin. Blood plasma concentrations can quickly surge to maximum levels at 0300 hours from barely detectable levels - estimated to be lower than 5 picograms per millilitre (pg/mL) during the day. Plasma melatonin concentration

then declines from 0300 to nadir between 0700-0800 hours. Thus, due to its dynamic nature, there should be caution when sampling melatonin.

Significant melatonin concentrations in saliva were identified by Vakkuri et al., (1985). Despite concentrations being reported as being 40-70% lower than serum melatonin, melatonin levels followed a similar circadian pattern when collected at similar intervals. Similar to salivary cortisol, salivary melatonin is represented by the unbound portion of circulating melatonin. This was confirmed by Kennaway & Voultsios, (1998) after directly measuring total and free plasma melatonin and saliva melatonin taken at the same times. Approximately two decades later similar findings were evident in a study conducted by van Faassen et al., (2017). Here, authors compared total melatonin, free melatonin and salivary melatonin. They established that total plasma melatonin positively correlated with free plasma melatonin, with approximately 75% of melatonin suggested to be protein bound to plasma. It has since emerged that salivary measurements of melatonin should not be used to deduce production of melatonin from the pineal gland but rather be "valid indicators of the 24-hour rhythmicity of the hormone" (Kennaway, 2020, p.4).

1.11. The light-dark cycle, salivary cortisol and melatonin

The circadian patterns of cortisol and melatonin are both driven by the hypothalamic SCN pathways, however, from the SCN to the adrenal (in the case of cortisol) and pineal (for melatonin) are quite different. Cortisol secretion is the product of activation of the HPA axis cascade which generates its secretagogue, ACTH from the anterior pituitary into to circulation. In contrast release of melatonin is the product of a more complex multi-synaptic neuronal pathway from the SCN to the pineal gland via spinal nuclei of sympathetic neurons (Claustrat et al., 2005). These hormones perform complementary roles with cortisol dominant during daytime activities and melatonin dominant during night-time sleep.

The natural occurrence of night and day or light and darkness on mammalian eyes – more specifically the retinas, positions the timing of the SCN. Melanopsin is a photo pigment contained in photosensitive retinal ganglion cells (pRGC) of all

mammals. These specialised cells detect environmental brightness (associated with a non-imaging function) integrating direct light responses with signals from rod and cone cells. There is a direct projection of fibres from the melanopsin-containing pRGCs to the SCN via the retinohypothalamic tract, synchronising the circadian pacemaker to the 24-hour solar cycle (Benarroch, 2011a). Cortisol synthesis is thus closely related to the photic role of the species. Peak concentrations in humans occur in the morning, whereas peak levels occur in the evening for nocturnal species. Light-activated neuronal input from the retina to the SCN (via the retinohypothalamic tract) has been observed to stimulate morning cortisol secretion (Leproult et al., 2001).

In contrast to cortisol production, the light of day and dark of night in turn impedes the timing of melatonin synthesis to the dark phase of the 24-hour cycle (night). Light-activated neuronal input from the retina to the SCN (via the retinohypothalamic tract) therefore mediates a suppressant effect on melatonin secretion (Cajochen et al., 2005; Gooley et al., 2011a; Moore Ry Fau - Lenn et al., 1972). Across the mammalian species, melatonin concentrations peaks at night regardless of the photic role of the animal e.g., crepuscular (e.g., rabbits, deer, possums), nocturnal (bats, badgers, dormice) or diurnal (humans, primates, birds and reptiles). Melatonin synthesis is distinctively robust such that neither environmental factors (e.g., diet or exercise) nor endogenous factors such as sleep, or illness has a major impact. The excluding factor here however is the influence of light on the production of melatonin.

1.11.1. Dim light melatonin onset (DLMO)

Light-activated neuronal input from the retina to the SCN (via the retinohypothalamic tract) mediates a suppressant effect on melatonin secretion (Cajochen et al., 2005; Gooley et al., 2011a; Moore Ry Fau - Lenn et al., 1972) and stimulates morning cortisol secretion (Leproult et al., 2001). One of the most applied measures of the timing of the central circadian clock in humans is the onset of the evening melatonin production measured in dim light, i.e., dim light melatonin onset, DLMO (Lewy et al., 1980a). The timing of evening melatonin onset has been

considered to be normal or ‘in-phase’ if melatonin concentration rises to > 4 pg/ml between the hours of 19:30 and 22:00 hours. DLMO has been of particular interest for several reasons. First, the DLMO is believed to accurately represent the timing of the SCN. When conducted under conditions of low light levels, salivary measurement of melatonin can provide insight into the phase position of the SCN. Second, the melatonin rhythm is less easily masked by environmental factors (apart from light) and, indeed, has been shown to produce more reliable circadian phase markers than the core body temperature rhythm (Benloucif, Guico, Reid, Wolfe, L’Hermite-Baleriaux, et al., 2005; Klerman et al., 2002). Third, the obvious convenience of saliva sampling has allowed DLMO to be a suitable phase marker. The ease of sampling has subsequently led to the widespread use of salivary melatonin measurements to establish the DLMO (see Figure 1.5 below).

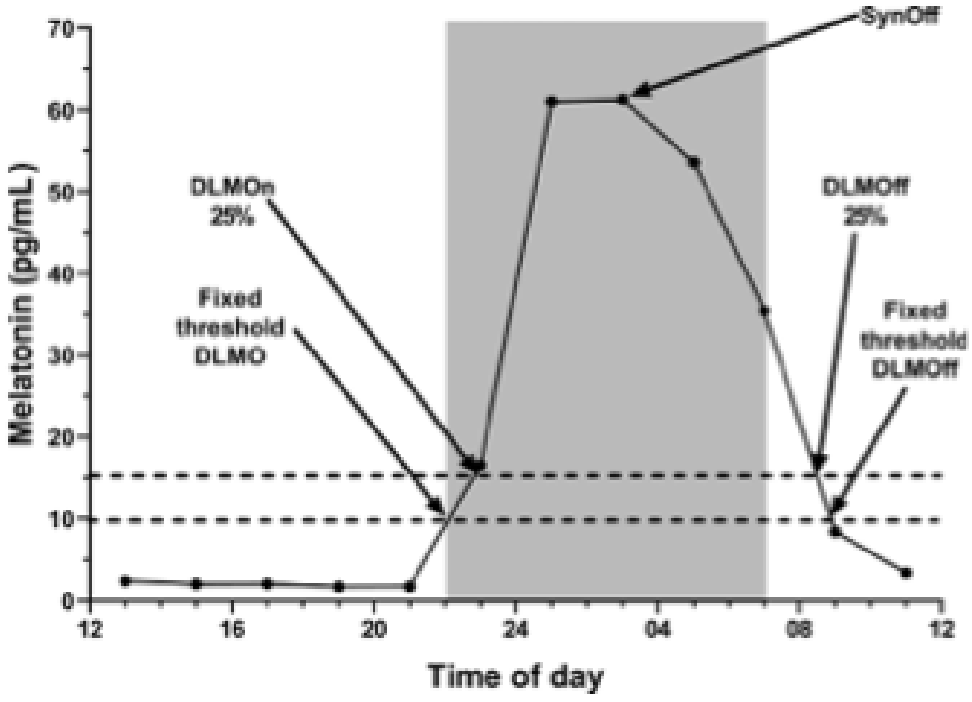

Figure 1.5 Schematic diagram of phase markers of DMLO

Illustrates the various phase markers for the plasma melatonin rhythm. Note the threshold for DMLO in plasma is 10pg/mL and 3 0r 4 pg/mL for saliva. SynOff refers to the time of night that active melatonin production ceases (see Kennaway, 2020**)**

1.12. Covariates and methodological implications of salivary cortisol and melatonin

Intra-individual studies in healthy participants have provided evidence that the CAR is associated with a range of situational/psychosocial state and trait variables. Such state variables include awakening time, sleep duration, sleep quality and ambient lighting including seasons, all of which have been shown to impact on the CAR (Edwards et al., 2001a; Petrowski et al., 2019; Violanti et al., 2017). In addition, in healthy individuals the CAR is strongly influenced by state psychosocial variables such as, anticipation of significant workload or challenges faced during the day ahead and negative experiences during the previous day (Adam et al., 2006; Bäumler et al., 2014; Desantis et al., 2010; Elder et al., 2018; Stalder et al., 2010; Wetherell et al., 2014) Some theories extend on the psychosocial state covariates, for example Adam et al., (2006) provides a functional interpretation of the CAR as an adaptive response designed to provide individuals with the “boost” needed to meet anticipated demands for the upcoming day. The hypothesis is supported by the notion that a higher CAR is more prevalent on weekdays due to occupational demands compared to weekends (Kudeilka & Kirshbaum, 2003; Kunz-Ebrecht et al., 2004; Stalder et al., 2009).

Excluding light intensity and time of awakening, production of melatonin is less affected by state factors and more influenced by trait-like covariates (See Table 2.1). Trait factors which have been associated with both melatonin and post awakening cortisol production include, age, sex, ethnicity, socioeconomic status, alcohol consumption, BMI and oral contraceptive use. However, studies examining the relationship between the CAR and post awakening melatonin are somewhat lacking. Nevertheless, several studies have endeavoured in observing the behaviour of DLMO in relation to both diurnal patterns of cortisol and the CAR. (Edwards et al., 2001; Garton, Vargas, Galecki et al., 2012; Fekedulegn, Innes, Andrew, et al., 2018; Petrowski, Schmallbach, Bjarne et al., 2019).

Table 1.2 State and Trait covariates and general findings for the CAR and melatonin concentrations

Factor	CAR		Melatonin	
State covariates	General findings	Citation	General findings	Citation
Time of awakening	Moderate stability of the CAR across two sampling days, with early waking participants showing a larger CAR ii. Earlier morning awakening is associated with a more pronounced CAR compared to late awakeners.	i. Edwards et al., (2001b); Kudeilka & Kirshbaum, (2003) ii; (Federenko et al., 2004; Smyth et al., 2013, 2015b, 2016; Zeiders et al., 2011)	i. Earlier occurrence of the onset of melatonin secretion is associated with an earlier wake time ii. Post awakening melatonin secretions are associated with later awakening.	i. Burgess & Fogg, (2008) ii. Ramachandran et al., (2016)
Sleep duration	i. Those with shorter sleep duration have lower cortisol levels at awakening and a faster rate of cortisol increase following awakening. ii. Longer sleep duration predicts a smaller CAR	i. Kumari et al., (2009); Vargas & Lopez-Duran, (2020); Wust et al.,(2000) ii. Anderson et al., (2021)	Later and shorter sleep is associated with a later melatonin circadian phase.	Danilenko et al., (2021)
Sleep quality	i. Poor sleep quality is associated with diminished awakening cortisol levels and dysregulated cortisol patterns over time ii. Nightmares compared to neutral dreams account for variances in the CAR.	i. Lasikiewicz et al., (2008); Williams et al., (2005) ii. Fekedulegn et al., (2018); Hess et al., (2020)	High melatonin (DLMO) associated with fewer arousals during sleep	Dubberke et al., (2019)
Habitual smoking	Higher CAR in smokers compared to non- or ex-smokers	Cohen et al., (2019); Kumari et al., (2009); Steptoe & Ussher, (2006)	Night-time serum melatonin levels unaffected by cigarette smoke	Ursing et al., (2005)
Alcohol consumption/ heavy drinking	Preliminary evidence that alcohol consumed the night before is associated with a decreased CAR the following morning). More chronic consumption of alcohol is associated with an increased CAR and a reduced slope of cortisol decline	Adam et al., (2006); Badrick et al., (2008); Stalder et al., (2015)	Alcohol intake alters melatonin secretion on those dependent on alcohol. Data suggest a lack of daytime secretion in heavy drinkers	Daniel & Touitou, (2006)

Ambient light levels	i. Bright light (800 lux) for the hour following awakening produced a significant increase in CAR magnitude at 20 and 40 minutes post-awakening. ii. increased AUCg magnitude over 45 mins when participants exposed to approximately 250 lux light for 30 mins on awakening.	i.Scheer & Buijs, (1999); Thorn et al., (2004) ii; Figueiro & Rea, (2012); Petrowski et al., (2019)	High intensity light presented between 0200-0400 hours suppresses melatonin. Blue light has the biggest impact on the inhibition of melatonin production during the night.	(Brainard et al., 2001, 2008; Cajochen et al., 2005; Gooley et al., 2011b; Hanifin et al., 2006; Lewy et al., 1980a; Thapan et al., 2001) Adamsson et al., (2016); Lockley et al., (2003); Phillips et al., (2019); Stothard et al., (2017); Tähkämö et al., (2019); Wright et al., (2004)
Season	In winter the CAR is reported to be significantly attenuated in SAD participants in comparison to the healthy control participants, but no difference observed in summer.	Thorn et al., (2011)	i. Melatonin production is longer during short days/long winter nights in comparison to long days/short summer nights ii. A later melatonin circadian phase is associated with increased sleepiness levels during Winter.	Adamsson et al., (2016); Burgess & Fogg, (2008); Stothard et al., (2017) ii. Danilenko et al., (2021)
Weekday vs weekend	i. Steeper rise in the CAR on weekdays, not accounted for by differences in waking time, state stress or perceived stress over the previous month. Such associations may be a product of participants failing to comply with the sampling protocol on weekends.	Thorn et al., (2006)	-	-
Prior day experiences	i. Prior-day feelings of loneliness, sadness, threat, and lack of control is associated with a higher cortisol awakening response the next day, but morning awakening responses did not predict experiences of these states later the same day ii. Prior day feelings of loneliness is associated with an increased CAR. Prior day feelings of nervousness/stress is associated with lower average wake-up levels of cortisol.	i. Adam et al., (2006); Desantis et al., (2010); Stalder et al., (2010) ii. Stalder et al., (2010) iii. Anderson et al., (2021)	-	-

	iii. Higher levels of prior day physical activity augments the CAR			
Posture	Smaller CARs are associated with a greater deterioration in postural sway when presented with moving stimuli.	Smyth et al., (2021)	Higher melatonin associated with the sitting position. Changes from lying to standing associated with elevated melatonin secretion	Deacon & Arendt, (1994); Kozaki et al., (2013); Nathan et al., 1998)
Anticipation of day ahead/prospective memory load	Relationships found between psychosocial state variables and the CAR, including an inverse relationship with the level of prior- day happiness and a positive relationship with study-day anticipations of the level of obligations/no leisure.	Bäumler et al., (2014); Elder et al., (2018); Griefahn & Robens, (2011); Stalder et al., (2010); Wetherell et al., (2014)	-	-
Mindfulness	i. Following intervention of daily visualisation of best possible self, improved mood and reduced worrying lowered the CAR	i. Nicolson et al., (2020)	i. Reflexology associated with reduced state anxiety however no changes observed with salivary melatonin secretion.	i. Mc Vicar et al., (2007)
	ii. Yoga, meditation and mind-body health increased the magnitude of the CAR and related to dynamic physiological arousal	ii. Cahn et al., (2017, 2022)	ii. Senior, more, experienced meditators have a generally higher melatonin levels in comparison to non-meditators	ii. Daube & Jakobsche (2015); Tooley et al., (2000)
Medication	Medication use should be recorded - numerous and ever-changing range of medications impact on salivary cortisol	Granger et al., (2009)		
Trait-like covariates				
Age	Attenuated CAR associated with increasing age	Almeida et al., (2009); Bäumler et al., (2014); Evans et al., (2007); Kudeilka & Kirshbaum, (2003); Oskis et al., (2012); Stalder et al., (2015, 2022); Wright & Steptoe, (2005)	Melatonin production is lower in older people, but the change occurs very early in life – around 20-30 years of age.	Kennaway et al., (1999); Mahlberg et al., (2006); Zisapel (2018)
Sex	Females have a greater CAR magnitude compared to males	Pruessner et al., (1997b); van Dam et al., (2018); Stalder et al., (2015, 2022); Wright & Steptoe, (2005); Wust et al., (2000b)	Females associated with higher melatonin than males.	Cain et al., (2010); Gunn et al., (2016); Obayashi et al.,(2015); van Reen et al., (2013)

Ethnicity	Black and Hispanics associated with lower CAR levels compared to White participants.	Hajat et al., (2010)	Light suppression of melatonin secretion is significantly higher in light-eyed Caucasians than in dark eyed Asians. Asians are also identified as having lower melatonin production than Caucasians.	Bhatti et al., (2013); Higuchi et al., (2007); Hsing et al., (2010); Jeong et al., (2019); Smith et al., (2009)
Socioeconomic status	i. Low socioeconomic status (SES) groups associated with lower levels of post awakening cortisol secretion.	Desantis et al., (2015); Hajat et al., (2010); Kunz-Ebrecht et al., (2004); Wright & Steptoe, (2005); Zhu et al., (2019)	Reduced levels of employment associated with increased amplitude of melatonin secretion.	Burgess & Fogg, (2008)
	ii. Low SES associated with both low and high CAR levels	Deer et al., (2021)		
BMI/Obesity	Greater abdominal fat is associated with greater responsivity of the HPA axis, reflected in morning awakening	Donoho et al., (2011); Incollingo Rodriguez et al., (2015); Shearrer et al., (2016)	Individuals with a lower BMI associated with increased amplitude of melatonin secretion	Burgess & Fogg, (2008)
	Greater BMI associated with a flatter CAR in children.	Miller et al., (2018).,	Significantly lower melatonin levels found in women with a higher BMI in comparison with healthy controls	
Oral contraceptive use	Oral contraceptive users display slightly blunted cortisol responses after awakening	Bouma et al., (2009); Pruessner et al., (1997a); Wust et al., (2000b)	Females taking contraception associated with higher levels of salivary melatonin concentrations and a longer duration of measurable melatonin secretion during the night.	Burgess & Fogg, (2008); Kostoglou-Athanassiou et al., (1998); Reinberg et al., (2009); st. Hilaire & Lockley, (2022)

1.1.2. The relationship between salivary cortisol and melatonin

It is evident that light plays an imperative role in the synchronisation of the internal biological clock to the environmental day and night schedule. A recent study conducted by Aubin et al., (2017) controls for this variable by comparing the 24-hour profiles of both salivary cortisol and melatonin in blind (light perception blindness, n=11) and normally sighted individuals (n=11). Despite a relatively small sample size, the authors successfully identified an increased presence of abnormal timing of melatonin onset in blind individuals in comparison to normal-sighted individuals. This timing was associated with the dormancy of cortisol, confirming an occurrence of an abnormal circadian pattern in blindness. Furthermore, authors identified that subjects demonstrated a greater overall melatonin concentration throughout the 24-hour period. Interestingly, cortisol profiles including post awakening cortisol secretion did not differ between blind and sighted individuals. Like their predecessors (Lewy & Newsome, 1983; Sack et al., 2009), Aubin et al., (2017), found that only half of their blind sample displayed abnormal patterns of melatonin secretion. They explain that the absence of retinal light input results in the absence of circadian rhythm entrainment which in turn gives rise to a 'free-running circadian rhythm'. It is, therefore, imperative to understand that even without light input, it is possible for the presence of a normal circadian marker. Free-running circadian rhythms can be environmentally entrained by non-photic, secondary zeitgebers (Mistlberger & Skene, 2004) such as food consumption (Challet, 2019; Mistlberger, 2011), exercise ((Aoyama & Shibata, 2020; J. Wang et al., 2022; Xu et al., 2022) and other social zeitgebers including bedtime, mealtimes, and the beginning of work (Boland et al., 2016). Aubin et al., (2017) monitors the circadian markers for only 24hrs where the presence of free running circadian rhythms can go undetected. The natural endogenous rhythms run at a non-24-hr pace phasing in and out of synchrony with the environmental 24-hr period. Therefore, it is likely that normal melatonin patterns were free-running and 'in-phase' during the sampling period.

One method of investigating the relationship between the CAR and free running or 'in-phase' melatonin patterns, is to observe the association of DLMO and the CAR. An earlier study by Rea et al., (2012) aimed to investigate that very notion. Twenty-five, healthy participants were instructed to maintain their usual sleep schedule for one week. In the second week the authors randomly assigned participants to two groups. Following awakening, the first received blue light (225 lux) and the second group received dim light (<1 lux). Participants were then required to attend a sleep lab for a night in which saliva samples were collected. Here, authors predicted baseline DLMO calculated from self-reported sleep schedules and melatonin saliva samples were taken every half hour from 3.5 hours before DLMO. Salivary measures for the CAR however, were taken every hour from awakening, for 4 hours. The overall findings determined that the later the onset of melatonin, the greater the CAR. Thus, higher concentrations of 'in-phase' melatonin were reflective of a higher CAR. Despite identifying a weak association between the CAR and DLMO (r= .28), the validity of the findings is questionable. The authors measured both DLMO and the CAR over a single day, discounting the recommendations of experts from CAR studies who explain that the CAR is influenced by state-like as opposed to trait-like covariates (Edwards et al., 2001b; Stalder et al., 2015, 2022; Wust et al., 2000b) and thus measurements over multiple days are essential. The authors also fail to employ the standardised protocol to measure the CAR in which saliva samples are required, every 15 minutes from awakening for 45 minutes. Furthermore, the researchers do not mention whether the sampling study day was measured over a weekend.

In studies controlling for the predisposition of weekend CAR to state variables, there have been marked day differences observed in the size of the CAR particularly in relation to ambient light and psychological state (Clow et al., 2010; Stalder et al., 2009). In addition, studies have identified that light-activated neuronal input from the retina of healthy individuals to the SCN (via the retinohypothalamic tract) mediates a suppressant effect on melatonin secretion, stimulating morning cortisol secretion (Cajochen et al., 2005; Gooley et al., 2011a; Leproult et al., 2001; Moore Ry Fau - Lenn et al., 1972).

It has since been suggested that melatonin and cortisol are differentially affected by night-time light exposure. Rahman et al., (2019) used a cross-sectional design in which participants were exposed to either a continuous or intermittent light during nocturnal sleep for five consecutive days. Employing a high frequency blood sampling technique, twenty-one healthy participants were randomly assigned to 3 conditions – either intermittent bright light (~9500 lux, IBL), continuous bright light (CBL) or continuous dim light (CDL, ~ 1 lux). Participants were exposed to their randomly assigned condition for 6.5 hours during the biological night, this occurred approximately 6 hours before wake time, between midnight and 0600hrs. The authors identified that melatonin suppression was relatively rapid, occurring within the initial 5 minutes of exposure to IBL. Melatonin concentrations also recovered more slowly between IBL stimuli. Although, not as quick as the IBL condition, the suppression of melatonin was also relatively rapid under CBL.

On the contrary, cortisol concentrations increased linearly between the start and end of each IBL. The authors also noted 'trimodal changes' in cortisol concentrations under CBL. Here, cortisol levels linearly increased and was reflected as an 'activating phase', followed by a 'rapid inhibitory phase' which finally entered an exponential 'recovery phase'. The overall summary of the study was that depending on type of light exposure during nocturnal sleep, circadian driven hormones produce a different outcome. The pattern of the reported trimodal changes, also appears to be similar to the patterns of the CAR i.e. the rise and fall of cortisol levels on awakening. The SCN regulates cortisol secretion via two separate pathways; as described earlier, the first involves input to the paraventricular nucleus and the HPA-axis cascade. The second, more rapid non-HPA-axis dependent pathway exerts autonomic input to the adrenal cortex via the splanchnic nerve (see Clow et al., 2010).

The recent work by Rahman et al., (2019), highlighted how differently the circadian hormones behaved in response to the duration and pattern of light when exposed during nocturnal sleep. These findings by Rahman et al., (2019) is perhaps the most relevant study to date. It considers the patterns of melatonin and cortisol during sleep, whilst controlling for light. However, it remains, that studies observing

the patterns of the CAR directly in relation to post awakening melatonin is lacking. Despite being each other's biological circadian counterpart, there are surprisingly few studies which have observed the pattern of melatonin following 'SynOff' timepoint (the moment at which melatonin production ceases, see above Figure 2.2) in conjunction with the CAR. Furthermore, it is apparent that differences in duration of light exposure have a significant effect on the outcome of both melatonin and cortisol, with the circadian hormones behaving in distinct ways. So distinct, that even observing those with night blindness can display a regular melatonin and cortisol secretory pattern (Aubin et al., 2017). Consequently, there also remains a question regarding the functionality of the retinohypothalamic tract in those with difficulties of the optic nerve, specifically with less severe visual difficulties and the relation this has with the CAR – an indirect biomarker of SCN functionality via the retinohypothalamic tract.

1.1.3. Difficulties with vision and salivary cortisol

The critical role for both the rod-cone and melanopsin systems in mediating the effects of light on sleep and awakening, imply that humans with deficits in either retinal pathways could be particularly vulnerable to acute effects of light and dark on sleep and wakefulness.

Vision decreases with age (Lin et al., 2016), such that the aging eye is susceptible to detrimental effects on the lens, cornea, ocular surface, ocular adnexa and associated qualitative changes in retinal neurons. Aging also affects the ability of the eye to set the body's biological clock. As the lens age, it becomes worse at transmitting short-wavelength visible (blue) light, while retaining the ability to transmit longer-wavelength visible (red) light (Kessel et al., 2011; Lin et al., 2016). It is therefore important to correct certain diseases of the aging lens such as cataracts, as it not only restores visual function but reinstates the availability of light accessing the rod-cone and melanopsin photoreceptive pathways (Lin et al., 2016). The aging eye is also susceptible to diseases which effect retinal ganglion cells (RGCs) and this can have a damaging effect on retinal sensitivity to light.

Effecting approximately 2% of the UK population, one of the most prevalent of ocular diseases, where retinal sensitivity is compromised is POAG (Moorfields, NHS UK, https://www.moorfields.nhs.uk/). Patients with POAG gradually suffer the loss of retinal cells, leading to optic atrophy and if not treated, can result in a slow loss of vision and ultimately blindness. There are typically no symptoms of POAG as it is usually the peripheral vision which is initially affected. As the disease progresses, patients experience blurred vision and light sensitivity which is often encountered as seeing rainbow-like rings around bright lights (NHS UK., 2022).

The involvement of melanopsin containing (mRGCs) as well as regular RGCs has been hypothesised to be directly associated with POAG (Gao et al., 2022; la Morgia et al., 2011a). Two studies in animal models of POAG suggests that a loss of the melanopsin photopigment leads to the impairment of circadian rhythm regulation (Drouyer et al., 2008; H. Wang et al., 2013). Jean-Louis et al., (2008), explained that "glaucoma may be the primary ocular disease that directly compromises photic input to the circadian time-keeping system because of inherent ganglion cell death" (Jean-Louis et al., 2008, p.1). Furthermore, the researchers suggested that POAG provides an opportunity to explore the effects of light transmission to the circadian system, observing any disruption as a result of ganglion cell loss. This disruption can be studied by careful examination of secretory patterns of hormones from saliva samples.

One study examined the effect of retinal ganglion cell loss on non-visual functions related to melanopsin signalling. Munch et al., (2015) recruited 22 participants, 11 were patients with bilateral visual loss and optic atrophy from either hereditary optic neuropathy or POAG (N=11). Here, melatonin suppression, subjective sleepiness and cognitive functions in response to bright light exposure in the evening were measured and were compared to age-matched controls. Salivary melatonin was measured 11 hours before habitual wake time and on two occasions post-illumination pupil response to blue light stimulus was also analysed. The findings of the study identified that patients with visual loss from POAG in comparison to those with visual loss from hereditary optic neuropathy and the control group, were solely associated with reduced light effects. Those with visual loss and optic

atrophy from POAG also demonstrated a relatively attenuated pupil response and reported feelings of being sleepier with slower reaction times. Both groups however displayed similar melatonin suppression when compared to their age-matched controls. This particular study highlights the stages of mild to moderate POAG detection in pupil function which is not expressed via the retinohypothalamic tract in relation to melatonin suppression. Whilst this study supplements the literature examining the behaviour of salivary melatonin to light exposure mentioned above (Aubin et al., 2017; Rahman et al., 2019), there remains a question as to whether the same conclusions can be applied when observing salivary cortisol, more specifically the CAR in those with POAG. i.e., is the detection of mild to moderate POAG conveyed via the retinohypothalamic tract in relation to the CAR? In previous studies, cortisol secretion measured in plasma and aqueous humour were identified as being outside the normal range (Kasimov & Aghaeva, 2017; Østergaard Madsen et al., 2021). Furthermore, this study supports the findings of Feigl et al., (2011) who measured post-illumination pupil response (PIPR) in those with POAG. The authors identified that a smaller constriction of the pupil following a short wavelength of light was associated with increased severity of POAG. Thus, it can be conceived that severe POAG may be associated with poorer mRGC functioning.

POAG patients are also more vulnerable to depression than the general aging population (Agorastos et al., 2013; Tang et al., 2022) and there remains a worthy question of whether this can be linked to the extrinsic light-specific drive to the circadian rhythm and more specifically - light input to the CAR. There also merits a question of whether there is a difference in post awakening cortisol levels in those with POAG and those with healthy vision. As well as clinical measures, it is imperative to get a full understanding of subjective ‘quality of life’ which forms the umbrella of psychological trait factors. These include wellbeing, fatigue and perceived stress as well as the impact this may have on the CAR.

1.1.4. Methodological implications of PACS research

Depending on the research context of observing patterns of these circadian

hormones, it is important to control for potentially confounding state factors such as differences in awakening times and ambient light exposure mentioned above and highlighted in Table 2.2. Earlier findings on the CAR have suggested a moderate to high test-retest reliability, particularly in relation to trait-like covariates across repeated assessment days (Edwards et al., 2001b; Stalder et al., 2015, 2022; Wust et al., 2000a). However, these notions have changed in the last decade.

Using structural equation modelling, Hellhammer et al., (2007), observed CAR data obtained across six consecutive days per person. They identified that the CAR on a single day is influenced to a larger extent by state compared to trait-like factors (see Table 2.2). Hellhammer et al., (2007) goes further to recommend the use of a six consecutive day saliva sampling period, suggested to be necessary to achieve reliable trait data on the CAR. A consensus paper by Stalder et al., (2015, 2022) has however, refuted this idea, explaining that extending the sampling period does not necessarily protect against state-related confounding factors and has recommended a minimum of a two-day sampling period.

CAR methodology relies on participants carrying out the required saliva sampling regime where the most crucial element is to collect the first saliva sample ‘immediately upon the moment of awakening’ (Stalder et al., 2015, 2022). Awakening from sleep described by Clow et al., (2010) involves a rapid attainment of consciousness followed by the relatively slow re-establishment of full alertness, some 20-30 minutes later. The period between regaining consciousness (i.e. awakening) and the attainment of full alertness is described as ‘sleep inertia’.

Sleep inertia has been examined by a few studies in relation to the CAR. Supportive of the role of the CAR in the regaining of state arousal, a positive association has been suggested between arousal at 45-minutes post awakening, post awakening cortisol levels (Thorn et al., 2004b) and the dynamic of the CAR (Thorn et al., 2009). It has been proposed that the role of the CAR provides a recovery from the sleep inertia state as well as the provision of an ‘energetic boost’ in morning awakening and put simply - the restoration of alertness (Clow

et al., 2010).

It has been stated that the onus to explain precisely what is meant by the 'moment of awakening' to the participant is on the researcher during the initial face-to-face briefing (Stalder et al., 2015). The definition in a consensus paper, focuses on the 'regaining of consciousness'. Stalder et al., (2015, p.43) explains:

"*When you are awake, i.e. you are conscious: you know who and where you are; you are in a state that clearly is different from when you were sleeping even though you may still feel tired….*"

Study designs, however, lack researcher oversight such that participant non-adherence to requested saliva sampling regimes can lead to inaccurate CAR estimates. For example, simply delaying the collection of the first sample following awakening (S1) by more than 15 minutes has resulted in false-high estimates of S1 and false low estimates of the CAR (Desantis et al., 2010; Dockray et al., 2008; Okun et al., 2010). More recent research studies by Smyth et al., (2013, 2015) carefully controlled awakening and sample times in healthy participants, sampling at 5-minute intervals. Findings revealed that cortisol levels remained relatively unchanged over the first 5-10 minutes following awakening ('latent period'), with a significant increase first being detectable in the 15-minute sample. One implication of this research is that accurate CAR measures can only be determined from data with strict adherence to commencement of saliva sampling following awakening. Therefore, to achieve an accurate measure of the CAR, a participant is required to accurately and consciously be aware of the moment of their awakening. However, this can pose some complications for those who find it problematic in defining that point of wakefulness.

Smyth et al., (2016) using multi-level modelling, demonstrated that if participants delayed up to 15 minutes, they could still be included in the data analysis provided objective electronic measures (which include actiwatches and medication event monitoring systems) were available. Therefore, the authors highlighted the importance of electronic monitoring of awakening and collection of the awakening sample (S1) in order to obtain known sampling timings in the post awakening period.

Of the several measures taken within this intensive CAR protocol, researchers

can objectively monitor morning awakening via an electronic monitoring device such as the Actiwatch (Phillips, Cambridge). Sampling times have been determined by the use of a Medication Event Monitoring System (MEMS) which contain the cotton swabs required for saliva collection. Opening of each MEMS cap device records the date and time. Interestingly, the differences in self-reported wake time, wake time as measured by the Actiwatch and the recording of the first morning opening of the MEMS cap can inform researchers about the duration of participant sleep inertia (Stalder et al., 2015, 2022). This reduced state of cognitive and motor performance (Tassi et al., 2006), may increase the difficulty of adhering to the saliva sampling protocol, preventing the precise determination of the moment when one is fully awake (Clow et al., 2010; Smyth et al., 2013).

PACS research can therefore identify the timing of unconsciousness during sleep via muscle movement measured by the Actiwatch. However, of the objective measures established to ensure critical adherence to the timing of the first morning saliva sample, the most crucial aspect for a researcher, is to assume that a participant is aware of the moment they are awake from a night's sleep. To date, there is a lack of literature which has endeavoured to qualitatively explore previous PACS research participant experiences of awakening, conducting the sampling protocol as well as understanding motivations to take part.

Filling the gap in the existing literature, this research will focus on exploring the subjective meaning of the moment of morning awakening, a process which is highly subjective and occurs outside of the researchers' direct oversight. There also remain unanswered questions surrounding personal experiences of taking part in CAR research studies. This study will adopt a participant perspective lens and aim to 'give voice' to the participants by exploring the motivations for participation, the experiences of taking part in CAR research studies in relation to saliva sampling and ultimately understanding some of the challenges they face.

1.13. Aims and thesis summary

The primary objective of this programme of research is to gain an insight into the measures of salivary hormones of circadian function in relation to the 24-hour light-dark cycle. Following expert consensus when investigating the CAR (Stalder et al., 2015, 2022), two studies in this thesis will adopt a strict monitoring of sampling adherence where post awakening cortisol secretion remains the focus of this programme of studies. In consideration of this saliva sampling protocol, the programme of research in this thesis is also interested in understanding the experiences of participants that have taken part in PACS research studies, giving particular attention in capturing the experience of the moments of morning awakening and actual experiences of saliva sampling. An overview of the studies and how they interlink is provided in Figure 1.6 and a brief outline of the aims of each study is given below.

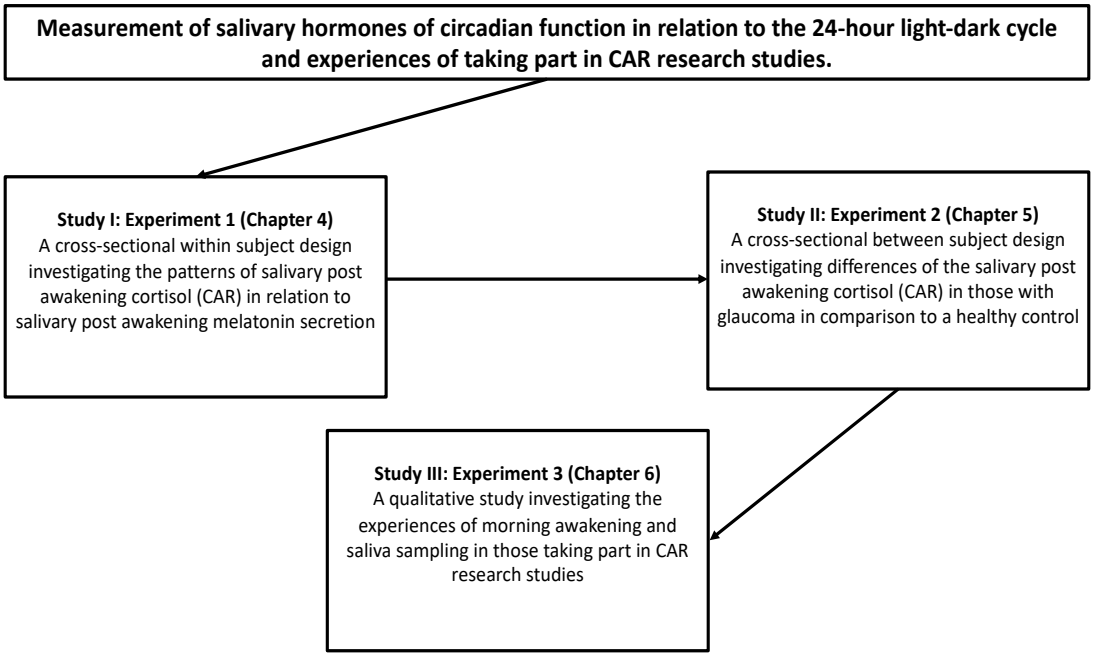

Figure 1.6 Flow diagram of the programme of research

1.1.5. Study I

The aim of this study was to explore associations between the pattern of salivary cortisol and melatonin following awakening. Previous research has identified weak associations between 'in-phase' salivary melatonin (DLMO) and the CAR (e.g., later onset of melatonin in relation to a greater CAR) in healthy participants. Prior research also demonstrates differences between circadian hormones in response to the duration and timing of light exposure during sleep. However, no study to date has explored the relationship between the rise in salivary cortisol following awakening and the decline in salivary melatonin. There is an indication that state and trait-based factors influence the CAR, therefore controlling for these factors, this study also aimed to understand whether state factors including ambient light, subjective anticipated demands and mood as well as trait factors including wellbeing, stress and fatigue are associated with post awakening cortisol and melatonin secretion. As sample timing accuracy is critical to the correct measurement of indices of PACS (Smyth et al., 2013) electronic monitoring of awakening and sampling times was employed.

1.1.6. Study II

Further extending the research suggesting associations between melanopsin containing RGCs and primary open-angle glaucoma (POAG), the aim of this study was to investigate differences in post awakening cortisol secretion in those with POAG in comparison to a reference data group with healthy vision. No study to date has observed PACS in those with POAG and compared with those with healthy vision. It is also imperative to obtain a full understanding of subjective 'quality of life' which forms the umbrella of psychological trait factors. Thus, in parallel with Study I, mental wellbeing, fatigue and perceived stress was also assessed in relation to PACS.

1.1.7. Study III

This study finally aimed to inform current methodological practices in PACS research. The aim of this study was to adopt a participant perspective lens with the intention to 'give voice' to the participants by exploring the experiences of taking part in PACS research - understanding some of the challenges they face. PACS research can identify the timing of unconsciousness during sleep via muscle movement measured by the Actiwatch. However, of the objective measures established to ensure critical adherence to the timing of the first morning saliva sample, the most crucial aspect for a researcher is to assume that a participant is aware of the moment they are awake from a night's sleep. The Actiwatch however, does not give any insight into the establishment of full alertness. No study to date has taken a qualitative approach in exploring the subjective meaning of morning awakening and experiences of taking part in PACS research.

In summary the overall aims of this research were:

a. To examine the association between post awakening salivary cortisol and melatonin in healthy participants using the well-established saliva sampling protocol. To explore the relationship between post-awakening hormone composites and psychological trait and state factors which include wellbeing, fatigue, perceived stress, ambient lighting and anticipation of mood and demand over the study period.

b. To investigate the differences in the CAR with those with POAG in comparison to a reference data group with healthy vision. To explore the relationship between those with POAG with trait and state variables. These include wellbeing, fatigue, perceived stress, visual function, ambient lighting and anticipation of mood and demand over the study period.

c. To Investigate the experiences of participants taking part in PACS research studies both from those with POAG and members of an academic community to:

I. inform best practice methodology for the ever-increasing number of studies in this area of research
II. explore the participants understanding of morning awakening

Chapter 2 Part I General Methods

2.1. Ethical considerations

The research presented in this thesis was conducted in accordance with the ethical guidelines for research with human participants established by the British Psychological Society (see Appendix 1 for ethics approval). Adherence to the standards and guidelines developed by the University of Westminster were also practiced. Participants were fully informed both verbally and via a written information sheet (see Appendix 2 & 3), which outlined the aims, rationale and procedure of the research studies. Participation was entirely voluntary and participants were advised to partake only if they felt at ease and confident to meet the requests of the study protocol. Rapport between researcher and participant was pivotal to ensure mutual respect and confidence before, during and after the study period. Participants provided written consent and were given every opportunity to ask questions. Participants were also made aware that they had the right to withdraw from the studies at any time, including withdrawing their data prior to publication. Upon completion of the study participants were fully debriefed and given the opportunity to discuss and issues or questions they had with the researcher.

For study II, apart from being diagnosed with POAG, all participants were healthy adults. There were no impairments in communication or understanding that prevented participants from providing full informed consent. Participants were not coerced to participate or remain in the study, despite some (from study I) being students of the researcher.

Participation in the studies meant there was no risk or harm greater than or additional to those encountered in normal daily routines. Minimal emotional

discomfort could have potentially arisen from completing the psychosocial measures relating to mental health. However, participants were reassured that they were under no obligation to disclose information that they did not wish.

Participants were reminded of their right to withdraw participation from the study, this included withdrawing their data prior to publication. Participants were also informed that should they be contacted about future research; they were under no obligation to participate. Participants were reassured that all information provided would be confidential and data used for publication would be collated such that no individual would be identifiable. The researcher took several steps to protect the confidentiality of the data provided:

Participant names were separately coded such that they were only identifiable to the researcher. All hard-copy data was stored in locked cabinets in secured offices at the Regent Street and New Cavendish campus of the University of Westminster. Saliva samples were stored in the Psychology and Stress Research Group (PSRG) laboratory at the New Cavendish campus of the University of Westminster. Saliva samples were handled in accordance with the Human Tissue Act (HTA; 2004). Stored samples were labelled using the appropriate participant code and were recorded on the HTA tracking form. Samples were finally destroyed following analysis, in accordance with the HTA.

There was no formal debrief, however, the researcher provided participants with the opportunity to discuss their experience of their study involvement. Participants were encouraged to give their feedback to ensure that any unexpected negative effects or misunderstandings were alleviated. They were also assured that they could contact the researcher via email if they wished to be informed about the findings of the research.

2.2. Participants and Researcher

The programme of research was conducted in the UK, with a process of on- going study design and recruitment from October 2012. The author of the thesis (NR: British Asian female aged 38) completed all aspects of each study including the study design, data collection, biological and statistical analysis.

For all studies, participants were recruited on the basis that they were not suffering from any serious medical or psychiatric illness which was ascertained by self-report. The exclusion criteria also encompassed any condition of adrenocortical dysfunction or illness that required treatment with corticosteroid medication. Females who were pregnant or had been pregnant within the year were also excluded due to subsequent changes in the HPA-axis following pregnancy after the third trimester (Obel et al., 2005).

For Study I, participants were drawn from the student body of the University of Westminster. Recruitment was conducted via the Psychology Department's research participation scheme (RPS). The RPS was developed by the Psychology department to encourage an active involvement of first year undergraduate students as participants in the research environment (see Appendix 4 for RPS ethics approval). Students had the option to participate in the RPS or write an essay detailing the ethical considerations for conducting research. Upon completion, participants were awarded credit towards their first-year undergraduate degree. Researchers within the psychology department and friends of the researcher also volunteered to partake in study I.

Study II required the participation of primary open angle glaucoma (POAG) sufferers. Details of the study were advertised on the International Glaucoma Association (IGA) newsletter, encouraging readers to get in contact with the researcher if they were interested in taking part. Recruitment relied on participants goodwill as no incentivisation were provided.

Study III employed a sample of participants with POAG recruited from study II (N=10). Members of the academic community (MAC) with normal vision at the University of Westminster (N=10) taking part in an on-going PACR study were also recruited. All Participants received no financial incentive to take part.

2.3. Salivary Cortisol and Melatonin

2.3.1. Saliva sampling protocol

Salivary free cortisol was measured for all studies detailed in this thesis and in addition, salivary melatonin was measured in study I. Salivary samples enabled collection of morning data within the participant's domestic setting. Dynamic changes in cortisol were captured based on the measurement of the cortisol awakening response (CAR), which required repeated sampling at regular time intervals following awakening. Sampling consisted of the following time points 0-, 15-, 30- and 45-minutes post-awakening (samples 1-4), which is the established time frame to capture the rise in cortisol (i.e., the CAR magnitude) (Stalder et al., 2015, 2022). For study I, the demonstration that melatonin is stable in saliva samples, with a circadian pattern reflecting serum (Vakkuri et al., 1985; Voultsios et al., 1997) together with the introduction of commercially available sensitive assay systems enabled repeated and simultaneous determination of both cortisol and melatonin in the same saliva samples.

Sampling on two study days enabled examination of the consistency of the PAMS (post awakening melatonin secretion in study I) for each individual across the two days. Participants were asked to awake in their usual way as studies have found that the CAR is independent to the mode of awakening (Pruessner et al., 1997a; Wust et al., 2000a).

During the 45-minute post awakening period, participants were asked to take nil by mouth and to avoid brushing teeth which may subsequently cause unavoidable abrasion and bleeding, contaminating the saliva sample. Participants were also asked to refrain from smoking, which acutely increases cortisol concentrations

(Steptoe & Ussher, 2006).

Cortisol remains stable at room temperature for up to seven days (and nine months if frozen at -20°C) and melatonin can be kept for up to six months once frozen (below -20°C) but should be refrigerated within thirty minutes and frozen within four hours of collection. Melatonin levels decrease by more than 20% after four days refrigeration at 2-8°C (Rzepka-migut & Paprocka, 2020). Therefore, participants for study I were asked to return their samples to the researcher on the morning of collection on the final study day. However, to avoid bacterial growth in the specimen, participants for all studies were asked to store saliva samples in their domestic refrigerator immediately following collection. The use of both salivettes for cortisol and eppendorfs for melatonin (see Figure 2.1 and 2.2) allowed for hygienic storage of the samples. Ziploc bags were provided, in which participants returned their samples to the researcher. Prior to the assaying, samples were subsequently stored at -20°C in the laboratory freezer at the New Cavendish campus of the University of Westminster.

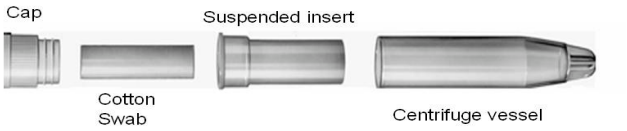

Figure 2.1 Cortisol salivette saliva sampling device (Sarstedt LTD)

Figure 2.2 Melatonin Eppendorf saliva sampling tubes (Eppendorf, UK)

2.3.2. Monitoring of adherence to the saliva sampling protocol and self-reported measures in the domestic setting

Successful measures of the CAR involve accurate and timely adherence to the saliva sampling protocol (Smyth et al., 2013). Non-adherence to the protocol can result in inaccuracy of the CAR magnitude and timing of the peak in cortisol concentration at 30 minutes post waking. A method of minimising the frequency of non-adherence is through active engagement between the researcher and the participant during the first initial meeting.

Fellow doctoral researchers and student participants for study I, individually attended a research induction session at the University of Westminster with the researcher (NR). During this session, (duration 20-30 min) participants provided informed written consent, received full verbal and written instructions on procedures. Participants were given a detailed illustration and example of the technique for saliva collection; they were also given time to practice and familiarise themselves with all devices included in the study

For study II participants with POAG attended a standardised one-to-one research induction over the telephone with researcher (NR). During a 25-30-minute phone call, participants provided verbal informed consent, received full detailed verbal instructions on completing the saliva sampling protocol and were given a summary of the electronic devices and questionnaires that would be included in the study pack (see Section 2.4 below. below).

All participants were provided with a study pack that included all equipment necessary for saliva sampling, questionnaires and electronic devices used to monitor awakening. This was either provided during the study induction at the university or sent via recorded delivery by Royal Mail postal service

The study pack contained study guidelines summarising all features of the study protocol. In the event of any arising issues, participants were also given the

researcher's full contact information (See Appendix 6 & 7).

2.3.3. Self-reported measures

Self-reported measures in the study diary (see Appendix 8 & 10) included a calculation of sampling times for the day comparative to the awakening time following the collection of saliva sample 1. Participants were also asked to record the time of actual collection of each saliva sample. This was used to determine potential deviations from the desired sampling time. Participants also rated their sleep quality on a five-point Likert scale, ranging from 'much better than usual' to 'much worse than usual'. The method of waking was also measured (e.g. alarm clock, somebody they asked to wake them, noises or they just woke).

2.3.4. Electronic measures of awakening and saliva sampling times

There are various resources that can measure indices of sleep quality, however strict adherence to protocol is necessary to avoid erroneous CAR estimates (Smyth et al., 2013, 2016; Stalder et al., 2015, 2022). Participant's self-reported awakening and saliva sampling times are inaccurate in comparison with electronic estimates which show that awakening times are typically earlier and sampling times are typically later than self-reports (Smyth et al., 2016). Electronic estimates of awakening and sampling times provide a proxy of an objective measure, and together they provide real-saliva sampling times (i.e., sample collation time relative to awakening time).

Sampling on awakening is crucial for accurate estimates of the CAR; delay in sampling on awakening will also delay subsequent sample points. To assess awakening time, participants wore an activity device on their wrist (see Figure 2.3) the night prior to each study day. The Actiwatch-score device (Actiwatch- Score, Cambridge Neurotechnology, Cambridge, UK) is a piezoelectric motion sensor recording physical activity.

Figure 2.3 The actiwatch score

Awakening times were estimated using the actigraphy software; it distinguished sleep and awakening periods by reduced and increased activity respectively. Figure 2.4 displays an example recording of awakening time from the actiwatch software. Actigraphy has been validated against the gold standard polysomnography (Lichstein et al., 2006) and is widely used in non-clinical and clinical studies (Lauderdale et al., 2006). Self-reported timings of awakening were used to guide the researcher during analyses of the actiwatch software. Awakening times were independently assessed by the human eye and agreed, by at least two members of the supervisory team following examination of actigraphy measures on each study day. This method has been suggested to be a more accurate measure of consistency compared to observing computer algorithms alone (Boyne et al., 2013; Smyth et al., 2016).).

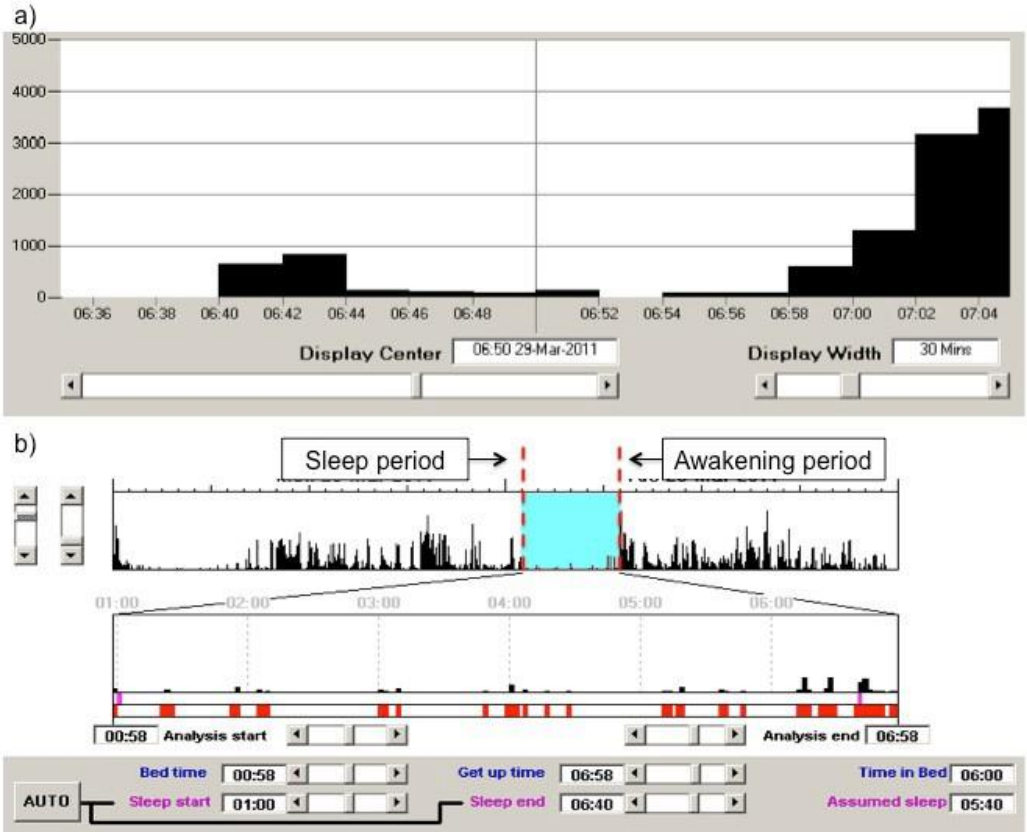

Figure 2.4 Actigraph analysis software (a) Actogram showing the awakening period (b) actigraphy software used to determine the point of awakening

In addition, sampling times were determined by track caps (e.g. Medication Event Monitoring: MEM Caps), the cotton swabs normally in the salivettes and used for saliva sampling were stored in the track caps (Figure 2.5) — participants were instructed to open this device only at sampling times. Following saliva collection, swabs were returned to the correctly labelled salivette for storage. Opening of each cap recorded the date and time and these timings were used as a proxy measure of participants' saliva sampling times.

Figure 2.5 Track cap device. Medication event monitoring system (MEMS) cap

2.3.5. Monitoring of adherence

Self-reported data on individual sleep time were used to guide the researcher to events on the actiwatch software. Timing of awakening was recorded as 'Electronic monitoring (EM) wake time' and this was used to assess the accuracy of the first morning sample. The MEMS provided an accurate time of the moment the device was opened for the first time on the study morning (MEM_1). The interval between EM waking and collection of saliva sample 1 were calculated as a difference and indication of sampling delay. A delay of more than 8 minutes (Smyth et al., 2013) was considered 'severe' and formed part of the analyses (See Table 2.1 for an example of an adherent and non-adherent participant).

For study I and II, Statistical analyses were conducted on all participants and repeated using only adherent participants. Any differences in results were documented.

Table 2.1 Adherent *(i)* and non-adherent (*ii*) examples of self-reported (*hh:mm)*, electronic monitoring (EM) wake times, MEMS monitoring of sampling time and caluclation of delay time (min),

(i) Adherent participant <8 minute delay (4 minutes)

Participant **self-report (**hh:mm)	Electronic Monitoring **Objective Measure** (hh:mm)	Delay (min)
Time of wake: *07:00*	EM Wake Time 06:59 Medication event monitoring system (MEMS) **Objective Measure**	EM Wake Time
Sample 1: 07:03	07:03	MEM 1 - EM wake time (07:03-06:59) = **4**
Sample 2: 07:18	07:18	MEM 2 - MEM 1 (07:18 - 07:03) = 15
Sample 3: 07:23	07:23	MEM 3 - MEM 2 (07:23-7:18) = 15
Sample 4: 07:38	07:38	MEM 4 - MEM 3 (07:38 - 07:23) = 15

(ii) Non-adherent participant > 8 minute delay (13 minutes)

Participant self-report (hh:mm)	Electronic Monitoring **Objective Measure** (hh:mm)	Delay (min)
Time of wake: 06.00	EM Wake Time 06:05 Medication event monitoring system (MEMS) **Objective Measure**	EM Wake Time
Sample 1: 06:02	06:18	MEM 1 - EM wake time (06:18-06:05) = **13**
Sample 2: 06:17	06:33	MEM 2 - MEM 1 (06:33 - 06:18) = 15
Sample 3: 06:32	06:48	MEM 3 - MEM 2 (06:48-06:33) = 15
Sample 4: 06:47	07:03	MEM 4 - MEM 3 (07:03 - 06:48) = 15

2.3.6. Short Messaging Service (SMS) protocol

Practical advice was provided either face to face or over the telephone to participants on preparing for their upcoming study days (e.g. prepare and place study materials next to their bed the night prior to their study day to prevent delays in the mornings due to having to search for the materials). As well as this, reminders were used to ensure that participants remembered to carry out certain tasks.

Text messaging is a useful and welcomed method of communicating with participants. It was crucial that participants remembered to wear the Actiwatch to bed the night prior to each study day, and during each sampling morning. Repeated saliva sampling prior to sleep and on awakening can be difficult for participants to remember to collect the samples. Text messages were used to remind participants to wear the Actiwatch to bed, prepare study materials the evenings prior to each study day and to collect the morning saliva samples (i.e. sample 1). SMS-messages were easily and securely sent using a cost-effective automated text messaging service (TextAnywhere, https://www.textanywhere.com). Participants had access to a mobile phone and this was utilised in order to remind participants about upcoming study days. Participants were sent a text message the evening prior to each study day reminding them to wear the Actiwatch to bed and to place sampling packs next to their bed. Text messages were used to remind participants to complete the morning diary. Use of text messages are a cost-effective method of increasing adherence to a range of health behaviours and completion of diary entries (Anhoj et al., 2004)) and have also been used in salivary cortisol studies to prompt participants to collect saliva (Oskis et al., 2012).

2.3.7. Determination of cortisol and melatonin in saliva samples

2.3.7.1. Cortisol

Saliva samples were assayed by both the researcher (author of this thesis) and Director of Studies (NS) in the PSRG laboratory at the University of Westminster. After saliva samples were thawed, clear saliva was released from the cotton swabs into the centrifuge vessel through centrifuging samples at 3500 rpm for 10 min. Samples were assayed using the Cortisol Enzyme Linked Immuno-Sorbent Assay (ELISA) developed by Salimetrics LLC (USA). All of the Salimetrics assay kits are commercially available. This assay uses immunological processes in the measurement of cortisol and is designed specifically to quantify the measurement of salivary cortisol. The assay is highly sensitive to low values of cortisol, with detection of cortisol as low as 0.16 nmol/l. Salimetrics report that the salivary cortisol assay correlates highly with those from a serum cortisol assay (r = 0.91, p < 0.001, n = 47 samples, see www.salimetrics.com). The amount of saliva required for the assay is just 25μL of saliva per test.

The assay kit contains a microtiter plate with 96 wells coated with monoclonal antibodies to cortisol; cortisol standards, which represent known cortisol concentrations (values in nmol/l: 82.77, 27.59, 9.19, 3.06, 1.02, 0.33). The standards and unknowns (saliva samples from participants) were pipetted into the wells in the microtiter plate, and an enzyme conjugate (cortisol labelled with horseradish peroxidase) was added. The test principle is that cortisol in standards and unknowns compete with the enzyme conjugate for antibody binding sites in the wells on the microtiter plate. Following incubation for one-hour unbound components are washed away using a phosphate buffered solution containing detergents. Bound cortisol peroxidase is measured by reaction with tetramethylbenzidine (TMB) solution, which produces a blue colour following 30 min incubation in the dark. The reaction is terminated by added sulphuric acid, which is a stop solution producing a change in the colour (blue to yellow). Within 10 min of adding the stop solution the optical density was read on plate reader with a 450nm filter. The amount of cortisol peroxidase present is inversely proportional

to the amount of cortisol, which visually follows that the more yellow the solution in each well, the less cortisol.

2.3.7.2. Melatonin

Saliva samples were also assayed by either the author (NR, who also developed the melatonin assay in the PSRG lab) or director of studies (NS) in the PSRG laboratory at the University of Westminster. After saliva samples were thawed, clear saliva was released from the cotton swabs into the centrifuge vessel through centrifuging samples at 3000 rpm for 15 min. Samples were assayed using the Salivary Melatonin Enzyme Linked Immuno-Sorbent Assay (ELISA) developed by Salimetrics LLC (USA). Similar to cortisol, this assay uses immunological processes in the measurement of melatonin and is designed specifically to quantify the measurement of salivary melatonin. The assay is highly sensitive, such that minimal concentration of melatonin that can be distinguished from 0 is 0.58 picograms per millilitre (pg/mL). Salimetrics report that the salivary melatonin assay correlates highly with those from a serum cortisol assay ($r = 0.81$, $p < 0.0001$, n = 47samples, see www.salimetrics.com). The amount of saliva required for the assay is 100µL of saliva per test.

Melatonin standards - in a Trizma buffered solution with stabilizer protein and a non- mercury preservative were serially diluted before use representing known melatonin concentrations (values in pg/mL: 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78). Akin to the cortisol ELISA kit, the assay contains a microtiter plate with 96 wells coated with rabbit monoclonal antibodies to melatonin; melatonin standards, The standards and unknowns (saliva samples from participants) were pipetted into the wells in the microtiter plate, and an enzyme conjugate (melatonin labelled with horseradish peroxidase) was added. The test principle is that melatonin in standards and unknowns compete with the enzyme conjugate for antibody binding sites in the wells of the microtiter plate. Following incubation for 3 hours at temperature 2-8^{o}C unbound components are washed away using a phosphate buffered solution containing detergents. Bound melatonin peroxidase is measured

by reaction with tetramethylbenzidine (TMB) solution, which produces a blue colour following 30 min incubation in the dark. The reaction is terminated by added sulphuric acid, which is a stop solution producing a change in the colour (blue to yellow). Within 10 min of adding the stop solution the optical density was read on plate reader with a 450nm secondary filter at 620 to 630nm. The amount of melatonin peroxidase present is inversely proportional to the amount of melatonin present, which visually follows that the more yellow the solution in each well, the less melatonin.

The researchers carefully followed the procedure provided by Salimetrics for all the salivary cortisol and melatonin assays performed. In addition to this, samples from one participant were assayed on the same day and each sample was assayed in duplicates. On the rare occasion that the percentage variation between the duplicate samples was greater than 10% the sample was re-assayed on another plate. In this way intra-assay variation was less than 10% for all studies. Other known concentrations of cortisol, the high and low controls, (27.6 nmol/l and 2.76 nmol/l, respectively) were treated like unknowns and used to determine inter-assay variability, which was below 10% for all studies. On average both intra and inter assay coefficients of variation were comfortably below 10%.

2.4. Demographic and state variables: Ambient light and diary measures

2.4.1. Demographic Variables

Demographic questions included age, sex, relationship status, smoking status, subjective health. Smoking status was assessed on a three-point Likert scale (regular smoker/occasional, ex-smoker, never smoked) and subjective health status was assessed on a single item scale ranging from 'do not wish to answer', poor to excellent. In addition, participants for study II were required to answer further questions regarding their experience of POAG including the duration of the disease, family history, pain and symptomology. Pain was assessed using a numerical rating scale, (0=no pain, 1-3 = mild pain, 4-6= moderate pain, 7-10= severe pain) and symptoms were assessed using a Likert Scale (redness of the eye, headache, eye tenderness, seeing halos/light distortion, misty vision, other, none).

2.4.2. Diary measures: Anticipated Demands and Mood

Self-reported measures were recorded on each study day using diaries (See Appendix 8 & 9). Prior to sleep and on awakening participants were asked to rate their anticipated demands for the day on a five-item Likert scale ranging from 1 = not at all busy to 5= very busy. A higher score is reflective of a higher level of anticipated demands for the day.

Anticipated mood was measured as participant's self-reported feelings for the day ahead (Stalder et al., 2009).This was recorded on a five-item Likert scale, ranging from 1= anticipation of a very negative feeling to 5= very positive. A higher score is therefore reflective of anticipations of a more positive and happier feel for the day ahead.

2.4.3. Ambient Light

Light data loggers (LDL; Tempcon Instrumentation, UK) recorded ambient light intensity ranging from 0 (overcast night sky) to 100000 lux (direct sunlight), see Table 2.1 for examples of typical light measures in lux (https://greenbusinesslight.com/resources/lighting-lux-lumens-watts/).

Participants were asked to place the LDL in their bedrooms, on a flat surface, away from direct electrical lighting. The LDL was activated from 18:00 for 48 hours on the first study day. Recordings of the LDL were analysed using HOBOware software (see Figure 2.6 and 2.7). Light data recorded on the HOBOware, was measured in 'lux' every 5 minutes from activation of the device. An average light intensity measured every 5 minutes one hour before sleep was calculated to give a mean evening light score (EL). This was repeated for morning light, where average light measured every 5 minutes for one hour after awakening was calculated to give a mean awakening light score (AL).

Table 2.2 Typical environmental light measures (Lux) (taken from https://greenbusinesslight.com/resources/lighting-lux-lumens-watts/)

Natural light conditions	Light Level (lux)
Direct sunlight	32,000 - 100,000
Ambient daylight	10,000-25,000
Overcast daylight	1000
Sunset/sunrise	400
Moonlight	1
No moon	<0.1

Figure 2.6 Light data logger

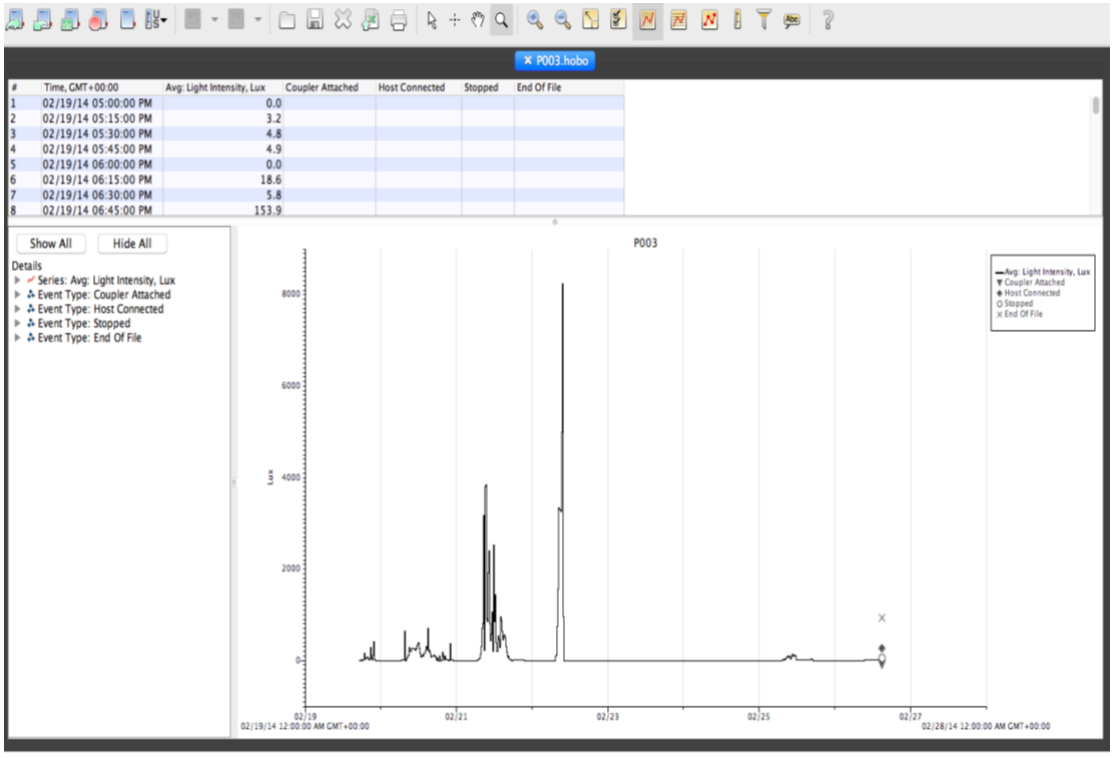

Figure 2.7 Light data logger analysis software

2.5. Psychosocial trait measures and the National Eye Institute Visual Function Questionnaire (NEI-VFQ)

Questionnaire booklets were included in the study packs. These contained all trait psychosocial measures detailed below (see Appendix 9 & 11).

2.5.1. Warwick and Edinburgh Mental wellbeing scale (WEMWBS)

. The researchers drew on findings from academic literature and qualitative research to select an effective scale to measure mental wellbeing. Mental wellbeing was measured using the Warwick and Edinburgh mental wellbeing scale (WEMWBS) developed by (Tennant et al., 2007). The WEMWBS is a 14-item scale that relates to an individual's state of mind (thoughts and feelings) in the previous two weeks. It comprises of both positive hedonic and eudemonic attributes to mental wellbeing. Examples include "I've been feeling cheerful', "I'm feeling optimistic about the future" and "I've been feeling loved". Responses are made on a 5-point Likert scale ranging from 1= 'none of the time' to 5= 'all of the time'. All items are positively loaded with equal weight therefore no reverse scoring is required. The overall score for the WEMWBS is calculated by summing the scores for each item. Therefore, scores range from the minimum of 14 to the highest score of 70. A higher score is reflective of a higher level of mental wellbeing.

2.5.2. Fatigue Assessment Scale (FAS)

Fatigue is a known symptom of several chronic illnesses and alongside back pain and headache, it is one of the most frequently reported symptoms in general practice (Petrie et al., 2014). Fatigue was measured using the Fatigue Assessment Scale (FAS) developed by (Michielsen et al., 2003). Characterising mental and physical fatigue, the FAS is a 10-item questionnaire comprising of the most semantically distinguishable statements taken from a pool of four fatigue questionnaires. Examples include "I get tired very quickly", "physically, I feel exhausted" and "when I am doing something, I can concentrate quite well".

Participants responded using a 5-point Likert scale ranging from ‘never’ to ‘always’. Items 4 and 10 are negatively loaded therefore requires reverse scoring. The overall score for the FAS is calculated by summing the scores for each item where possible scores range from 10 to 50. A higher score represents higher levels of fatigue.

2.5.3. Perceived Stress Scale (PSS)

The Perceived Stress Scale (PSS) was developed by (Cohen et al., 1983) and is used to measure the degree in which situations in an individual’s life are appraised as stressful. The researchers assessed the reliability and validity of the PSS on samples of students in high school education and a community smoking-cessation programme. A shortened 10-item PSS scale was used in the studies presented in this thesis due to better internal reliability than the full 14-item scale (Cohen & Williamson G., 1988). It comprises of 10-items referring to subjective appraisals of events occurring within a one-month time frame. Examples include “In the last month, how often have you felt nervous or stressed?” and “In the last month, how often have you felt that things were going your way?” Responses were measured on a 5-point Likert scale ranging from ‘never’ to ‘very often’. Items 4, 5, 7 and 8 are positively weighted therefore require reverse scoring. Subsequently all items are summed and an overall total score calculated. Scores range from 0-40 with higher scores indicating higher perceived stress. Scores ranging between 0-13 would be considered low stress, 14-26 would be considered moderate stress and scores between 27-40 is considered high perceived stress.

2.5.4. National Eye Institute Visual Function Questionnaire (NEI-VFQ)

Presented in this thesis, participants with POAG recruited for study II were asked to complete the 25-item National Eye Institute Visual Function Questionnaire (NEI VFQ-25). Developed by the National Eye Institute (Mangione et al., 1998), the full version of the NEI-VFQ comprises of 51 items relating to aspects of visual disability taken from qualitative analyses of focus groups. The validity and reliability of the NEI-VFQ-25 has been found to be comparable to the NEI-VFQ-51 and particularly

valid when assessing quality of vision of those with POAG (Mangione et al., 1998, 2001). The NEI-VFQ-25 consists of 12 subgroups (see Table 2.2), examples of items found in the NEI-VFQ-25 include “How much time do you spend worrying about your eyesight?” and “How much difficulty do you have driving at night?” For each subgroup, responses are made using either a 5 or 6-point Likert scale. A total composite score is calculated following the reverse scoring of several items (see Table 2.3). An average is then calculated by dividing the total score by the total number of items. Scores range from 0, (worst possible score) to 100 (best possible score). Therefore, a higher score signifies a higher level of self-reported visual function.

Table 2.3 Loading weight of each item in each of the 12 subscales of the NEI-VFQ-25 and descriptive examples (Mangione et al., 2001)

Subscale	Descriptive example	Number of items	Items to be averaged after recoding
General Health	Overall health rating	1	1
General Vision	Overall vision rating	1	2
Ocular Pain	Amount of pain	2	4,19
Near Activities	Reading normal newsprint	3	5,6,7
Distant Activities	Reading street signs	3	8,9,4
Vision Specific:			
Social Functioning	Visiting others, normal social functioning	2	11,13
Mental Health		4	3,21,22,25
Role Difficulties		2	17,18
Dependency	Worry, control	3	20,23,24
	Limited in endurance	3	15c, 16, 16a
	Requirement of help		
Driving	Difficult conditions		
Colour Vision	Difficulty matching clothes	1	12
Peripheral Vision	Seeing object in visual periphery	1	10

2.6. Approach to statistical analysis

All melatonin data presented in this thesis were positively skewed similar to cortisol data which is often the case (Edwards et al., 2001b). Square root transformations therefore, were required to optimally reduce the skew statistic. Successful transformation corroborates with the assumption of normality required for the use of parametric statistical analysis.

There were four separate saliva collection times to assess cortisol (and melatonin for study I) concentration. Saliva samples were collected at 0 =(s)ample 1, 15= (s2), 30=(s3) and 45=(s4) minutes post-awakening. Analyses for cortisol and melatonin were separate where composites of cortisol and melatonin were calculated for each participant and each study day. For both cortisol and melatonin, area under the curve with respect to ground (AUCg) over the 45 min post awakening period was computed to estimate total cortisol and melatonin secretion respectively. For cortisol, the CAR was calculated as the mean increase (MnInc) of subsequent samples (s2—s4) from the first sample (s1) taken at awakening ([s2+s3+s4]/3−s1).

In each study an overall mixed model ANOVA was initially performed to examine differences in the CAR. Raw results were plotted to provide a visual graphical illustration of the pattern of both the CAR and post awakening melatonin over time.

Other statistical analyses include t-tests to examine differences in demographic, situational and psychosocial variables between groups of interest. T-tests were used to analyse day differences in ambient lighting, anticipated mood and demand in both studies I and II. To understand the associative relationship between ambient light, diary (state) measures, psychosocial (trait) measures and post awakening cortisol/melatonin concentrations, Pearson's and Spearman's test of correlation were used for both study I and II. For all correlations, scattergrams were produced to check that any significant associations were valid and not unduly influenced by outliers etc. All given values of p are two- tailed. All statistical

analyses were performed using SPSS (IBM, Version 25-28)

Chapter 3 Part II Qualitative methods

3.1. Qualitative data generation

Face to face semi-structured interviews were initially conducted by Dr Sanjay Joban (SJ), following up on an ongoing study conducted by Smyth et al., (2021). The research questions addressed the experiences of academic staff (within the Psychology department) taking part in CAR research studies and exploring the subjective meaning of morning awakening. Face to face interviews took place in either the qualitative research laboratory, health laboratory or a research cubicle within the Psychology suite of the University of Westminster. In this instance, interviews occurred on the final day of cortisol sampling.

During the telephone screening, following the recruitment of participants with POAG taking part in study II, participants were asked if they would be interested in taking part in an extended study investigating the aims described above. Semi-structured interviews were conducted over telephone by the researcher (author of this thesis) and were completed with the quantitative research and analysis. The process of qualitative data generation, therefore, began while the researcher continued to collect quantitative data from other participants. Thus, here, interviews were conducted within a week of the last cortisol sample taken for study II. Telephone interviews have been considered less inhibiting for participants as the interview occurs within an environment familiar to participants (Ward et al., 2015).

3.2. Format of the semi-structured interviews

Semi-structured interviews for the academic staff sample were conducted by SJ over a 3-month period between January and March 2016. For POAG sufferers taking part in study II, semi-structured interviews were carried out by the researcher over 4-month period between February and June 2019.

The interviews in 2016 and 2019 followed a similar course, commencing with welcoming and thanking participants for taking part, they were reminded of the purposes of the study and were asked for permission to audio record the interviews. SJ used the recording application available on the Apple iPhone, series 6 which was effective in recording sounds within a private office space. Here, interviews took place in either the qualitative research laboratory, the health lab or a research cubicle within the Psychology laboratory suite of the University of Westminster. In contrast, telephone interviews were conducted in 2019 and recorded using an office telephone on loudspeaker using the recording application of an Apple, iPhone device, Series 6.

The indicative topics (see Table 3.1 below) was used to permit a focused, yet open approach to data generation, whilst ensuring the conversation did not veer from study objectives.

Table 3.1 Indicative topics

Topic (prompts, if necessary)
• What were your reasons for taking part in this study? • What were your initial thoughts? • What were your general feelings about participation? (e.g. providing saliva samples upon waking up?) • How did you feel the night before, when you were aware that you needed to provide saliva samples the following morning? • How did your routine/experience compare to your usual routine? • Did you do anything differently? • What was the experience like for you to collect the saliva samples over the two mornings? • How long was it between waking up and collecting the saliva samples? • What was it like collecting samples as soon as you woke?

- How did you find collecting samples every 15 minutes for 45 minutes after the first sample?
- How did you fit the morning saliva sampling in with your morning/daily routine?
- How did you find collecting the samples as soon as you woke?
- Tell me about your thoughts of the other aspects of the study e.g. wearing the watch, completing the diary etc.
- How did you determine this 'moment of awakening' as requested by the team?
- What does being awake mean to you? (What do you understand by the moment of waking up?)
- At what point, after gaining consciousness, do you normally feel fully alert?
- How would you describe your awaking process? What do you go through as you are waking up?
- How do you feel in the morning when you wake up?
- How long does it take you from waking up to getting out of bed and going about your daily business?

The interview introduces opening question which promoted a relaxed and comfortable environment for conversation to start. The aim was to ease any pre-existing anxieties participants may have had prior to the interview. Additionally, Galletta et al., (2016) explains that most exploratory concepts the majority of the exploration of the subject at hand with participants are constructed from the initial segments of an interview with the participant. Using open-ended questions creates an opportunity for participants to narrate their experiences, thus the focus of the initial question is quite deliberate, ensuring conversation stays close to the research topic.

To ensure minimum distraction and maintain an active listening stance, notes taken during the interview were kept to a minimum. Reflexive notes were either made after the interview or during analyses.

The overall interview duration ranged from 25-30 minutes and comprised of a series of 10 open-ended questions (see Appendix x). In accordance with the aims of this study, the participants were asked about the general experience of taking part in PACR. This was followed by open-ended questions around specific experiences of the saliva sampling protocol and examining any difficulties faced.

The final open question of the interview asked participants to give an account of what the moment of morning awakening feels like to them.

For the interviews conducted in 2016, recordings were transcribed and coded using thematic analysis by the author of this thesis. Coding and themes were checked by at least 2 other researchers from the team (NS & LT), forming initial themes (codes) and identifying the overarching themes using the six stages outlined by Braun & Clarke, (2014, 2021) within a qualitative paradigm.

3.3. Reflexivity of the researcher

Maintaining open mindedness in conjunction with the ability to conduct a qualitative research study without biases can be managed using the active role of researcher reflexivity. McLeod, (2001) explains this as “turning back one’s awareness on the self” (McLeod, 2012, p.195). The interest of NR in conducting this study arose from designing the initial studies of this thesis. However, prior to this, NR volunteered as a participant in many PACS research studies. Participation occurred without wholly understanding the demands the study protocol placed on usual activities during the study period and the researcher was initially keen to explore this concept. To keep an open mind, Rowley, (2014) and Gilham (2010) explain that the researcher should attempt to move beyond their assumptions, specifically when such views may exist. Reflexivity was conducted using two methods, the first was the use of taking part in active conversations with both the director of studies and SJ who also conducted the interviews in 2016. The second was a reflexive diary (see Appendix 12 for an excerpt), which was kept during the interviews and analysis of the content transcribed. These methods allowed for a more detailed examination of content with connecting the researcher’s assumptions and experiences.

3.4. Qualitative data analysis

A six-phase guide recommended by Braun & Clarke, (2014, 2021) allowed for the analysis of qualitative data produced by this study. The phases included:

- Familiarisation
- Generating initial codes
- Search for themes
- Review themes
- Define themes
- Write-up (interpretation)

3.4.1. Familiarisation

The basic concept of familiarisation is used from the outset of conducting the semi-structured interviews. The researcher then became further familiar with the data when listening to audio recordings and during the process of transcription. These initial stages in turn helped the dialogue with future semi-structured interviews and aided with rapport building. The most useful however, was to return to these notes of familiarisation which consisted of repetitive phrases and words and aided with the development of sub themes.

3.4.2. Generating initial codes

Braun & Clarke, (2014, 2021) later explain that thematic analysis is “a method for identifying themes and patterns of meaning across a dataset in relation to a research question”. (Pg 175). Here both an inductive and deductive process was used which initially aimed to commence analysis taking a data driven approach but also formed the structure of analyses for the interviews conducted in 2019. Rather than being rigid, this methodology of analyses is therefore flexible and dependent on the research question

3.4.3. Search for themes

The data analysis software programme NVivo 9/12 (2020) was used to both collate and organise the generated data. Repetitive words and quotes were coded using ‘nodes’ forming the initial sub themes and the development of the overarching themes. Using the NVivo software allowed for quick access to transcribed

interviews as well as convenient access to quotes for references in the results Section.

3.4.4. Review and define themes

The process of charting allowed for a clear illustration of the overarching themes in relation to sub-themes. This allowed the researcher to gain a broader understanding of the data generated. For this study two different charts were generated. This was then further analysed to form one principal chart. This diagrammatic illustration can be found in Chapter 6, Section 6.2 (see figure 6.1)

3.4.5. Write up (Interpretation)

Once overarching and subthemes were identified, all data was interpreted in relation to overall participant experience but more specifically to initial research questions. Braun & Clarke, (2014, 2021) explain that each theme should have a detailed and logical explanation. Here, interpretation of the sub themes and overarching themes is an opportunity of further assessment to establish recurrent explanations for attitude and behaviours of resultant experience

Chapter 4 Study I

Post-awakening exploration of cortisol and melatonin in healthy adults

4.1. Introduction

Circadian rhythm is characterised by the Earth's predictable 24-hour cycle and its shift between night and day. The resultant features of physiology and behaviour display circadian variations that are fundamental for survival e.g. body temperature, sleep and awakening (Alessandro et al., 2019; Golombek & Rosenstein, 2010). Disruption of the circadian rhythm is a common feature of aging, mental and physical ill-health (Coles et al., 2021; Jagannath et al., 2013; Wulff et al., 2010). These observations have led to detailed explorations of the mechanisms linking circadian function and health, especially with respect to the role of the HPA axis (Law & Clow, 2020; Menet & Rosbash, 2011; Pezük et al., 2012; Turner et al., 2020) and the pineal gland (Walker et al., 2020). Like the circadian rhythm in arousal and body temperature, hormone secretion exhibits distinct diurnal and nocturnal states with abrupt switch-like transitions between these two states (Benloucif, Guico, Reid, Wolfe, L'Hermite-Balériaux, et al., 2005; Wehr et al., 2001). Thus, it is imperative to not only understand the influence of the Earth's 24-hour axial rotation on the essential hormones of sleep and waking but to note the existence of any potential relationship.

Commencing with the composites of post awakening salivary cortisol secretion (PACS), the discrete CAR has been commonly investigated in relation to psychosocial, cognitive and health variables, with an elevated CAR being

associated with poorer health, increasing daytime demand and poorer cognitive functioning (Chida & Steptoe, 2009b; Clow et al., 2004; Fries et al., 2009; Kudielka & Wüst, 2010; Law & Clow, 2020; X. Shi et al., 2018; Smyth, Flynn, et al., 2019; Smyth, Skender, et al., 2019). Adam et al., (2006) provides a functional interpretation of the CAR as an adaptive response designed to provide individuals with the "boost" needed to meet anticipated demands for the upcoming day. The hypothesis is supported by the notion that a higher CAR is more prevalent on weekdays due to occupational demands compared to weekends (Kudeilka & Kirshbaum, 2003; Kunz-Ebrecht et al., 2004; Stalder et al., 2009).

Composites of PACS, particularly the CAR have been described as a useful biomarker in the measurement of trait and state psychosocial wellbeing (Boehringer et al., 2015; Chida & Steptoe, 2009b; Hänsel et al., 2010). For example, an elevated CAR has been associated with greater life stress and lower mood (Chida & Steptoe, 2009b; Refsgaard et al., 2022; Rnic et al., 2022). Yet, there have been inconsistent associations between PACS and psychosocial stress/wellbeing such that contradictory relationships have frequently been reported e.g. lower life stress and greater mood have been related to an elevated CAR (Juster et al., 2011; Stalder et al., 2010). Furthermore, lower CARS have been associated with anxiety (high stress) in those with chronic illnesses (Weber et al., 2022b). In addition, other studies have found no such statistical relationship with the CAR and wellbeing (Stafford et al., 2017).

Smyth et al., (2015a) provides an explanation behind the conflicting findings surrounding the CAR and trait psychosocial wellbeing. The authors highlight the importance of participant adherence to the post awakening saliva protocol. Via strict monitoring of adherence, authors conclude that no associations were identified between the CAR and trait wellbeing, however the authors identified that lower trait wellbeing was associated with higher post awakening cortisol AUCg. This further clarifies that simple delays in saliva sampling can result in erroneous findings between wellbeing and PACS.

The composite of post awakening cortisol secretion (PACS) observing overall cortisol concentration (as measured by the AUCg) provides further insight into HPA-axis function and overall health outcomes (Evans et al., 2007; Smyth et al., 2013, 2015b; Stalder et al., 2015, 2022; Steptoe & Usher, 2006; Wust et al., 2000a). Unlike the CAR, the AUCg is correlated with the 12-hour diurnal mean of cortisol concentration (Edwards et al., 2001b) which is more consistently associated with measures of stress (Hernández et al., 2018; Liu et al., 2022; Nater et al., 2010; Schuler et al., 2017). However, in comparison to total diurnal cortisol (AUC), morning AUCg has been shown to be a more sensitive indicator of minor changes to HPA- axis activity (Stalder et al., 2015, 2022; Wust et al., 2000a). Nevertheless, the AUCg is recognised for being relatively stable across days and weeks in the absence of a significant stressor (Edwards et al., 2001b; Pruessner et al., 1997a; Wust et al., 2000a). For example, an elevated AUCg has been associated with poorer scores of wellbeing (Evans et al., 2007; Smyth et al., 2013) but more specifically, poorer trait wellbeing has been associated with being dependent on sample timing accuracy (Smyth et al., 2015b) and can be considered a marker of state stress. To further elaborate on the mechanics and function of the HPA-axis, greater emphasis should be made on the biggest influencer of the Earth's 24-hour axial rotation – the sun and thus lighting (both environmental and artificial).

Light-activated neuronal input from the retina to the SCN via the retinohypothalamic tract stimulates morning cortisol secretion (Scheer & Buijis, 1999; Figueiro & Rea, 2010; Leproult et al., 2001; Petrowski et al., 2019, 2020; Rea, Figueiro, Bierman, et al., 2012) such that exposure to a dawn simulator on awakening, with increasing brightness has been found to increase subjective alertness and elevate the CAR in healthy individuals (Thorn et al., 2004b, 2009). Furthermore, in comparison to the summer; those that have previously reported high scores of seasonality during the shortened photoperiod of the winter months have had a reduced CAR following awakening (Thorn et al., 2009) . It is somewhat expected therefore that ambient lighting has an activating effect on the adrenal glands, in turn stimulating the release of post awakening cortisol. Conversely, as night-time draws in and darkness commences, light-activated neuronal input

mediates a suppressant effect on melatonin secretion (Benarroch, 2011b; Cajochen et al., 2005; Gooley et al., 2011a; Moore Ry Fau - Lenn et al., 1972).

Pathways from the SCN to the adrenal (in the case of cortisol) and pineal (for melatonin) are quite different. Cortisol secretion is the product of activation of the HPA-axis cascade which generates its secretagogue, adrenocorticotrophic hormone (ACTH), from the anterior pituitary into to circulation. In contrast the release of melatonin is the product of a more complex multi-synaptic neuronal pathway from the SCN to the pineal gland via spinal nuclei of sympathetic neurons (Claustrat et al., 2005).

Melatonin secretion has important functions as an endogenous synchroniser in relation to sleep and is an excellent marker of circadian function (Aulinas, 2019; Claustrat et al., 2005). Typically, melatonin levels begin to increase in the 2–3 hours before the usual onset of nocturnal sleep. Nocturnal melatonin secretion, appears to be somewhat contradictory in the presence of psychosocial state variables. For example, a number of studies have attempted to understand the effects of holistic therapies such as meditation and reflexology in relation to melatonin secretion. Yet, discrepencies exist amongst the literature with some stating significant changes to melatonin secretion have been established (Carlson et al., 2004; Mc Vicar et al., 2007). Some studies have identified that senior, more, experienced meditators have generally higher melatonin levels in comparison to non-meditators (Nagendra, Sathyaparabha & Kutty, 2017and others not (Carlson et al., 2004; Daube & Jakobsche, 2015; Mc Vicar et al., 2007; Tooley et al., 2000).

The most prominent and influential environmental state variable to directly affect nocturnal melatonin secretions is ambient lighting. Its secretion is almost entirely inhibited by light, and, with the onset of darkness, it reaches a peak in the early morning hours, and decreases to daytime levels around usual waking (Molina & Burgess, 2011; Morris et al., 2012). Melatonin secretion has been identified as being suppressed by room lighting of less than 200 lux, in turn shortening the body's internal representation of night duration (Gooley et al., 2011a). To fully

understand the impact of light and its intrinsic influence on the secretion of melatonin, the following section addresses shift work.

A worker returning home after finishing a night shift, would usually draw their curtains to sleep in dim-light conditions and continue to do so until their night shift rota ends. Studies have evaluated the effects of light on daytime sleep after simulated night work. Following shift work and transitioning to a regular night-sleep/day-wake pattern, Nagashima et al., (2018) suggests that workers completing their night shift, should sleep during the day, under bright light conditions to enhance their nocturnal melatonin secretions before midnight, in turn resetting their circadian sleep rhythm. It is evident therefore that sleeping in a dark environment during daytime reduces nocturnal melatonin secretion and delays its onset. Melatonin, therefore, represents an important humoral signal modulated by the innate 24-hour environmental photoperiod. One of the most applied and standardised measures of the timing of the central circadian clock in humans is the onset of the evening melatonin production measured in dim light, i.e., dim light melatonin onset, DLMO (Lewy et al., 1980b).

Studies observing chronobiological features of illness whether psychological or physical, tend to observe the phase angle between peak diurnal cortisol secretion (cortisol acrophase) and DLMO. For example, a pilot study by (Buckley & Schatzberg, 2010) identified those with major depressive disorder exhibited a higher phase angle than their healthy counterparts. This was later corroborated by Krystal et al., (2021) who concluded that the phase angle between peak cortisol and DLMO was a suitable biomarker and preliminary indicator for depression as well as treatment efficacy.

Both hormones are regulated by the SCN and are responsive to light; melatonin secretion is suppressed, and the CAR enhanced (Morris et al., 2012; Scheer and Buijis, 1999). These well esteemed findings are advantageous to determine the chronobiological influences of illness. Furthermore, it is plausible that changes in post awakening melatonin may contribute to some of the effects attributed to post awakening cortisol secretion. However, only a few studies have observed the

patterns and relationship between cortisol and melatonin in a healthy population. More so, no study to date have observed PACS in conjunction with post awakening melatonin secretion (PAMS).

4.1.1. Aims

A detailed investigation of the relationship between post-awakening cortisol and melatonin secretion in healthy individuals is proposed. No study to date has explored the relationship between the rise in cortisol following awakening and melatonin concentrations. There is an indication that state and trait-based factors influence the CAR, therefore controlling for these factors, this study also aims to understand whether state factors including ambient light, subjective anticipated demands and mood as well as trait factors - wellbeing, stress and fatigue are associated with post awakening sleep/wake hormones. As sample timing accuracy is critical to the correct measurement of the CAR (Smyth et al., 2013) electronic monitoring of awakening and sampling times was employed. The specific aims and objectives are outlined below (see Table 4.1).

Table 4.1 Specific aims and objectives

Aims	Objectives
Explore relationship of PACS and PAMS in healthy individuals	Close monitoring of first morning sample to examine patterns of both post-awakening salivary cortisol and melatonin in healthy participants.
Explore relationships between PACS and PAMS with state and psychosocial trait factors	Exploring associations between post awakening cortisol and melatonin secretion alongside state (ambient lighting, anticipated mood and demands) and trait psychosocial variables (mental wellbeing, perceived stress, and fatigue)

4.1.2. Hypotheses

Using a cross-sectional design, it was hypothesised that melatonin concentrations would fall after awakening and that this fall would be related to the simultaneous rise in cortisol secretion. It was hypothesised that both the PACS and PAMS would be associated with psychosocial trait factors including wellbeing. A higher score in the WEMWBS will be associated with a higher PACS and specifically total post awakening cortisol AUCg. Notable associations between fatigue and stress and PACS and PAMS is also hypothesised. In addition, associations between measures of state variables including anticipated demand/mood, ambient lighting over the two study days and PACS and PAMS is hypothesised. To elaborate, it is hypothesised that greater anticipated demand will be associated with elevated PACS, particularly the CAR. With respect to ambient lighting, it is finally hypothesised that the brighter the light (greater lux) will be associated with a more elevated PACs and a lower PAMS.

4.2. Method

4.2.1 Participants

Fifty-one participants (41 female and 10 male) were recruited on the basis that they were healthy and were not suffering from any medical psychiatric illness (ascertained by self-report). Approximately half (54.9%) of the participants were students at the university of Westminster and the other half were either in full or part time employment. Ages ranged from 18-39 (21.6 ± 5.0) years. Participants rated their health on a scale 1-5 ranging from poor health to excellent health and on average they rated their health as 2.5 ± 0.9. Most participants (76.5%) did not smoke. Participants received no financial incentive to take part in the study but received course credit. The University of Westminster ethics committee approved the protocol. All participants provided signed informed consent

4.2.2 Materials and measures

Participants were provided with a study pack containing full standardised written instructions, a questionnaire booklet of psychosocial measures, a saliva sampling kit consisting of two Zipoloc bags labelled either day 1 or 2. Each bag contained four coded Eppendorf tubes (Eppendorf, Cambridge, England), labelled tube 1-4 (awakening – 45 min post awakening). Participants were provided with a record sheet to confirm their awakening and saliva sampling collection times. Also included were electronic devices, which monitored ambient light, participant awakening times (wrist-worn Actiwatch-score device) and the saliva sampling times (MEM cap), containing straws required for passive drool collection

4.2.1. Psychosocial trait measures

Demographic questions included age, sex and marital status. Participants also reported their smoking/drinking (current, occasional, ex-smoker/drinker, never smoked/drank alcohol) and health status (1=poor, 5= excellent). The questionnaire booklet included measures of subjective wellbeing and quality of life, assessed

using the 14-item Warwick-Edinburgh Mental Well Being Scale (Tennant et al., 2007; (WEMWBS; higher scores indicated greater mental wellbeing). The WEMWBS was designed to capture both hedonic and eudemonic wellbeing over the last two weeks. Also included in the questionnaire is a 10-item trait Fatigue Assessment Scale (Michielsen et al., 2003; FASS; higher scores indicated greater feelings of fatigue) and the 10-tem Perceived Stress Scale (Cohen et al., 1983; PSS; higher scores indicated greater feelings of perceived stress).

4.2.2. Environmental and Psychosocial state measures

Light was recorded using the light data logger (LDL) (HOBOware device). Recordings of the LDL were analysed using HOBOware software. Light data recorded on the HOBOware, was measured in 'lux' every 5 minutes from activation of the device. An average of light measured every 5 minutes one hour before sleep was calculated to give a mean evening light score (EL). This was repeated for morning light, where average light measured every 5 minutes for one hour after awakening was calculated to give a mean awakening light score (AL).

The Actiwatch and MEMs devices were used in combination to identify real sampling time from awakening. Electronically-monitored (EM) sampling times were determined by the actigraphy estimates of awakening and MEMs-verified sampling times. Awakening times were independently assessed, then agreed, by at least two members of the supervisory team following examination of actigraphy measures on each study day. The interval between EM waking and collection of saliva sample 1 were calculated as a difference score and indication of sampling delay. A delay of more than 8 minutes was considered 'severe' and formed part of the analyses.

Self-reported measures were recorded on each study day using diaries (See Appendix 8). Prior to sleep and on awakening participants were asked to rate their anticipated demands for the day on a five-item Likert scale ranging from 1 = not at all busy to 5= very busy. A higher score is reflective of a higher level of anticipated demands for the day.

Anticipated mood was measured as participant's self-reported feelings for the day ahead (Stalder et al., 2009). This was recorded on a five-item Likert scale, ranging from 1= anticipation of a very negative feeling to 5= very positive. A higher score is therefore reflective of anticipations of a more positive and happier feel for the day ahead.

4.2.3 Procedure

Participants individually attended a research study induction session at the University of Westminster with the lead researchers (NR & NS). During this session (duration 15-25 min) participants provided informed consent, received full verbal and written instructions on procedures, and practiced the techniques for collecting and recording times of saliva samples.

Participants were asked to place the LDL in their bedrooms, on a flat surface, away from direct electrical lighting. The LDL was activated from 18:00 for 48 hours on the first study day.

As strict adherence to protocol is necessary to avoid erroneous CAR estimates (Smyth et al., 2013, 2016; Stalder et al., 2015, 2022) participants were informed about the need to adhere to the strict sampling regime relative to awakening time and were informed that the electronic devices would be used to verify the accuracy of their saliva sampling. Prior to sleep and to verify awakening times, participants were asked to wear Actiwatch (Cambridge Neurotechnology, Cambridge, UK) or the Actiwatch-2 (Philips Respironics, UK) device. Participants were reminded to prepare for the study day (e.g., place samples next to their bed and wear the Actiwatch-score device to bed) via automated text messages.

Participants were instructed to collect saliva samples via method of passive drool before sleep, immediately on awakening (S1),15 (S2), 30 (S3), and 45 (S4) minutes post awakening. Miniature straws were stored in a medication event-monitoring (MEM) bottle where participants were instructed to open the device removing the straw for each specified sampling time.

Participants were instructed to awaken in their usual way either spontaneously or by an alarm clock. During the saliva collection period participants were asked to refrain from smoking, brushing their teeth, exercising and take nil by mouth except water.

Samples were initially stored in a domestic freezer until they were returned to the laboratory to be stored at - 20ºC until assayed. Participants were asked to complete a record sheet and evening/morning diary, entering their awakening times, their protocol-required saliva sampling times based on their awakening time that day, their actual saliva sampling times and scoring the anticipated demands (1=not at all busy-5=very busy) and anticipated mood (1=very negative feelings-5= very positive) for the following day. The study materials and the saliva samples were returned to the researcher at the end of the study.

4.2.4 Cortisol and melatonin assessment and assay

Salivary cortisol and melatonin assays were carried out at the University of Westminster. Saliva samples were thawed and centrifuged for 10-15 minutes at 3,000-3,500 rpm. Cortisol and melatonin concentrations were determined by enzyme linked immune-sorbent assays developed by Salimetrics LLC (USA). Intra- and inter-assay variations were below 10% for the analysis of both hormones.

4.2.5 Treatment of data and statistical analysis

Composites from the raw values of melatonin and cortisol were calculated. For both cortisol and melatonin, area under the curve with respect to ground (AUCg) over the 45 min post awakening period were computed to estimate total cortisol and melatonin secretion respectively. For cortisol, the CAR was calculated as the mean increase (Mninc) of subsequent samples (s2—s4) from the first sample (s1) taken at awakening ([s2+s3+s4]/3−s1).

Composites of PACS including the cortisol mean increase (Mninc), total post waking cortisol (cortAUCg) and total post waking melatonin concentration (melAUCg) were all positively skewed and again normalised by square root

transformations for inferential statistical analyses. State measures including evening and morning light data were also positively skewed. Mean evening light (EL) and awakening light data for day 1 data were normalised using square root transformations. An attempt was made to normalise light sample distributions for day 2 via root four transformations however data remained skewed. Prior/same day anticipated demands and mood data, were normally distributed thus statistical transformations were not performed.

To examine patterns of the sleep/wake hormones a paired t-test was used to analyse differences between sampling times. A two 2(day) x 4(sample) within-subject ANOVA was conducted on post awakening cortisol and melatonin data to further highlight the main effects of study day, sampling time and the interaction between factors. Where Mauchley's test of sphericity was significant Greenhouse-Geisser corrected degrees of freedom are presented. Paired t-tests were also used to determine differences between lighting measured in the evening and on awakening the following day. Similarly paired t-tests were used to determine differences in anticipated demands and mood measured before sleep and on awakening. In addition, bivariate correlational analyses were used to analyse relationships between sleep/wake hormones, psychosocial and state measures. As a result of some non-normalised distribution of data (awakening light data) and a small sample size, Spearman's Rho coefficient was also used to explore and analyse the data.

4.3. Results

Descriptive statistics for post awakening melatonin and cortisol composites are presented in Table 4.2 below.

Table 4.2 Means, standard deviations and ranges of sample 1, 2, 3, 4 and the mean increase (MnInc), total post awakening (cort/melAUCg) concentration for cortisol (nmol/L) and melatonin (pg/mg) (N=46)

	Mean (SD±)		Min-Max	Min-Max
	Day 1	Day 2	Day 1	Day 2
Cortisol				
Sample times (minutes)				
0	7.400(4.50)	7.67(4.59)	0.00-21.48	0.00-20.99
15	10.99(6.43)	10.57(6.37)	0.24-25.59	1.06-30.89
30	14.17(7.52)	12.80(7.12)	2.35-31.28	2.72-38.57
45	14.76(7.90)	13.67(8.27)	1.88-35.28	1.93-41.82
MnInc	5.91(5.13)	4.67(5.17)	-3.01-23.92	-8.88-22.53
cortAUCg	36.25(18.10)	34.05(18.06)	4.83-76.87	7.00(98.25)
Melatonin				
Sample times (minutes)				
0	35.41(20.37)	34.77(21.63)	8.344-105.46	8.00-95.06
15	35.30(20.99)	30.92(17.72)	7.021-92.55	3.36-82.50
30	35.04(20.87)	30.83(18.10)	6.72-114.92	6.08-77.93
45	33.87(19.86)	32.46(20.68)	2.58-116.21	4.17-84.02
MelAUCg	105.14(58.59)	95.37(52.09)	22.88-302.85	18.08-235.41

4.3.1. Patterns of post awakening salivary cortisol (PACS) and melatonin secretions (PAMS)

4.3.1.1. PACS

Awakening cortisol values ranged between 0.24 and 41.82 nmol/L. Analysis of the cortisol data revealed a typical CAR profile of substantial increase in cortisol over the 45-minute post awakening period $F_{1.84,79.25} = 49.585$, $p < .001$. Figure 4.1 shows salivary cortisol secretion over the 45-minute post awakening period. There was also no main effect of study day $F_{1,43} = .801$, $p = .376$. In addition, there was no significant interaction between sampling time and study day $F_{2.20,94.75} = 1.802$, $p = .167$.

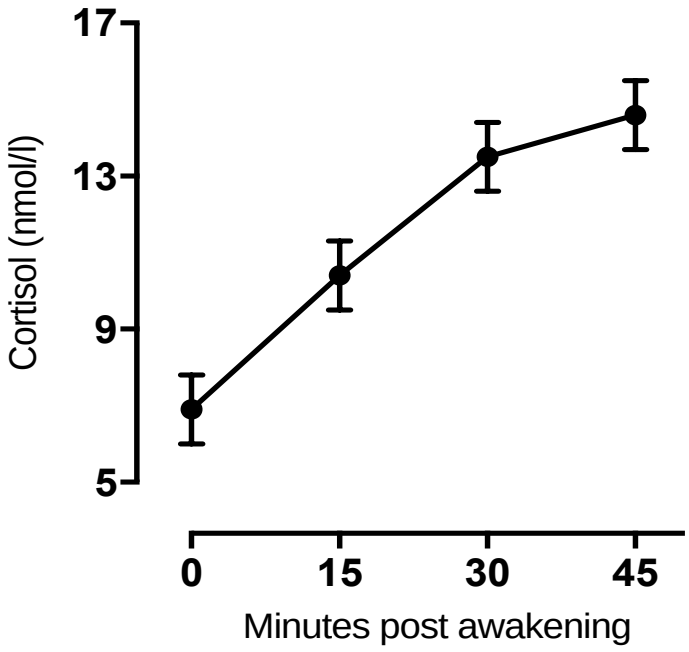

Figure 4.1 Mean (±SEM) cortisol at sampling time 0, 15, 30 and 45 minutes across the two sampling days

4.3.1.2. PAMS

Post-awakening melatonin concentration ranged between 2.58 and 116.21 pg/mg. There was no main effect of sampling time $F_{2.153,132}$ = 1.31, p = .330 nor effect of day $F_{1,44}$ = 2.318, p = .135. For illustration purposes raw data is presented in Figure 4.2 below. There was also no significant interaction between sampling time and day $F_{2.53,132}$ = .905, p = .428.

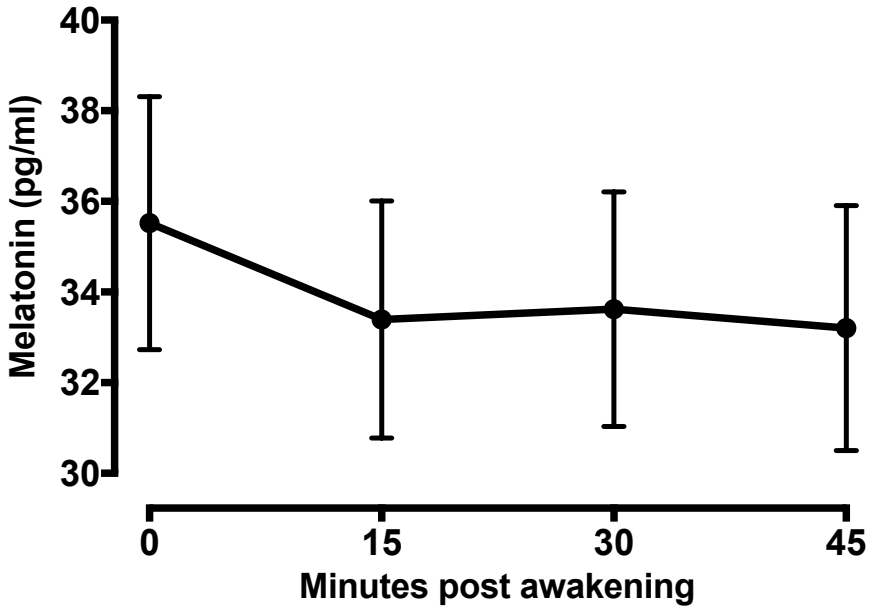

Figure 4.2 Mean (±SEM) melatonin at sampling time 0, 15, 30 and 45 minutes over the two sampling days

4.3.2. Relationship between composites of post awakening cortisol and melatonin secretion

Correlational coefficients for the analyses with cortisol and melatonin composites are presented in Table 4.3. Here, the usual relationship between post awakening cortisol composites is evident, of which a significant positive relationship between post awakening cortisol Mninc and AUCg can be identified r=.353, n=48, p=.014, thus the higher the CAR the higher the overall total cortisol concentration following morning awakening. There were, however, no significant relationships between composites of post awakening cortisol secretion and melatonin (see Table 4.3

below).

Table 4.3 Pearson's correlation coefficients for PACS (MnInc, AUCg) of cortisol and melatonin AUCg (mAUCg) (N= 48)

	cortAUCg	melAUCg
MnInc	.353*	.172
cortAUCg		-.056

†, trend, *p<.005

Post-hoc, partial correlational analyses were conducted between total post awakening melatonin secretion as measured by melAUCg and controlling for indices of PACS. There were no significant correlations between melAUCg and cortisol MnInc *r= .162, n= 45 p=.28.* No significant correlation between melAUCg and cortAUCg was also observed *r=-.005, n= 45 p=.97.*

However, according to a power analysis conducted, a sample size of at least 263 participants is required to achieve 80% power at an alpha level of .05. This assumed effect size of .50 for the Pearson product-moment correlation coefficient (see Table 4.4 below)

Table 4.4 Pearson's correlation coefficients for PACS (MnInc, AUCg) of cortisol and melatonin AUCg (mAUCg) (N= 48)

	N	Actual Power	Test	
			Null	Alternative
Pearson's	263	.80	.172	.172

4.3.3. Relationship between salivary cortisol and melatonin with trait and state psychosocial variables

4.3.3.1. Psychosocial trait

Descriptive statistics of the psychosocial trait measures are outlined on Table 4.5 below.

Table 4.5 Means, standard deviations and ranges of psychosocial trait measures including the Warwick and Edinburgh mental wellbeing scale (WEMWBS), the Perceived stress scale (PASS) and the Fatigue assessment scale (FAS) N=45

	Mean (±SD)	Min-Max
WEMWBS	49.86(6.71)	35-63
PSS	15.3(3.91)	6-21
FAS	22.69(6.51)	13-41

Presented in Table 4.6 are the bivariate correlation coefficients of the trait questionnaire measures, melatonin and cortisol composites. There were no significant relationships identified between post awakening melatonin and psychosocial trait variables. Similarly, there were no significant associations between awakening cortisol composites and psychosocial trait variables.

Table 4.6 Pearson's correlation coefficients for, PACS (MnInc, cortAUCg), melatonin AUCg (melAUCg) and trait psychosocial measures (N=30)

	WEMWBS	FAS	PSS
Mninc	-.110	-.181	-.013
cortAUCg	.113	-.174	-.188
melAUCg	-.022	.011	.017

4.3.3.2. Environmental and psychosocial state variables

Table 4.7 below, presents descriptive data of psychosocial state variables for the two study. In this study, measures of anticipated mood and demand were analysed using paired t-tests to observe potential day differences.

Table 4.7 Means, standard deviations and ranges of psychosocial state variables with regards to prior-day and study day anticipated mood/demand.

Diary	Mean (SD±)		Min	Max
	Day 1	Day 2	Day 1	Day 2
Ambient light (lux)				
Evening light	85.17(134.70)	102.10(162.38)	0.00-587.70	0.00-660.10
Awakening light	362.63(582.35)	92.38(192.22)	0.00-2573.00	0.00-766.99
Prior study day				
Anticipated mood	3.50(0.83)	3.84(0.84)	1.00-5.00	2.00-5.00
Anticipated demand	3.90(0.98)	3.62(1.10)	1.00-5.00	2.00-5.00
Study day				
Anticipated mood	3.37(0.84)	3.51(1.00)	1.00-5.00	1.00-5.00
Anticipated demand	3.84(0.96)	3.71(0.95)	1.00-5.00	2.00-5.00

Evening light ranged between 0 and 660.10 lux and light measured an hour after awakening ranged between 0 and 926.97 lux. There were no significant day differences between average evening light t = -.098, df = 26, p = .923. Therefore, an overall mean evening light score (EL) was calculated. A paired t-test revealed significant day differences between average light, 60 minutes after awakening t = 2.321, df = 24, p = .029, with day 1 being significantly brighter than day 2 by an average of 68.79 lux.

A paired t-test revealed that there were no day differences between anticipated demands reported over the two study days t = 1.323, df = 49, p = .192. This was replicated when observing day differences between reported anticipated mood over the two study days t = -.846, df = 48, p =.402. Data remained normally

distributed upon calculating an overall average measure of ‘same day’ anticipated demands.

No significant associations identified between ambient light, anticipated demands or mood and patterns of post awakening cortisol/melatonin measured over the two study days (see Table 4.8 below).

Table 4.8 Pearson’s correlation for the melatonin, cortisol composites and the state variables.

	EL	AL1	AL2	Anticipated demands	Anticipated mood
melAUCg	.208	-.244	-.077	.121	.067
Mninc	-.037	-.197	-.069	-.118	-.001
cortAUCg	.-.003	.169	-.046	.098	.153

(EL= Evening light, AL1= Awakening light day 1, AL2= Awakening light day 2)

4.3.4. Sampling adherence

Electrically monitored awakening times (EM) and saliva sampling times were highly concordant with participant’s self-reported timings (90% matched timings). All self-reported (SR) awakening data were assessed with reference to EM awakening. A simple t-test revealed there were no significant differences between EM awakening and SR (*day 1: t* = -1.77*, df* = 43*, p* = .083*; day 2: t* = -.965*, df* = *41, p*=.340). Particularly the first waking sample was used to check adherence and the assumption of the 15-minute interval accuracy in providing post waking samples 2-4.

Three participants were identified as being non-adherent to sampling protocol due to a severe delay in the first morning sample for both study days (> 8 min). However, overall results were consistent after excluding these participants from the analyses.

4.4. Discussion

Using saliva samples collected within the domestic setting, the findings of this study revealed for the first time, the pattern of post awakening melatonin (PAMS) and PACS in healthy participants. Analyses of both post awakening circadian hormones suggest there were no significant study day differences. There was no effect of sampling time on post awakening melatonin, thus the decrease in melatonin following the 45-minute post awakening period did not change significantly in comparison to the fundamental characteristic of the CAR and its increasing cortisol levels, refuting the experimental hypothesis.

The findings of this study demonstrated that there were no significant main effects of sampling for post awakening melatonin secretion following awakening and no associations were identified with the composites of PACS. It is therefore evident that changes in PAMS did not contribute to any effects attributed to PACS in this sample.

In the case of state variables, ambient light, anticipated demands, and mood during the study period were not related to post awakening levels of either post awakening cortisol or melatonin. Psychosocial variables including wellbeing (WEMWBS), fatigue (FAS) and perceived stress (PSS) did not have any statistical association with post awakening melatonin. In addition, there were no statistical relationships between psychosocial variables and PACS.

Within the circadian pattern of neuroendocrine function, cortisol and melatonin are both regulated by the SCN and perform complementary roles as endogenous synchronisers with cortisol dominant during daytime activities and melatonin dominant during night-time sleep (Wehr et al., 2001). This study reports a detailed time course of immediate post awakening melatonin and cortisol secretion at 15-minute intervals from saliva samples collected within the domestic setting. The period of awakening from night-time sleep to awakening is a time of dynamic change within the brain and neuroendocrine system (Balkin et al., 2002). It was initially hypothesised that melatonin concentrations would fall after awakening and

that this fall would be related to the simultaneous rise in cortisol secretion. For this sample size, this emphasises the notion that PACS and PAMS are discrete and distinctive aspects of neuroendocrine function.

Furthermore, it was hypothesised that both PACS and PAMS would be associated with psychosocial trait factors including wellbeing, fatigue and stress. Yet, in this study, no statistically significant findings were identified for either hormone, accepting the null hypothesis. Previous studies have identified that the critical factor governing the relationship between PACS and wellbeing is participant adherence to the saliva sampling protocol (Smyth et al., 2015a). Usually considered as a marker of state stress, post awakening cortisol AUCg has also been identified as being associated with trait wellbeing (Smyth et al., 2015a)

Participants reported good mental wellbeing, with moderate perceived stress and fatigue. Despite a stringent monitoring of adherence to the saliva sampling protocol as suggested by Smyth et al., (2015a) the WEMWBS - a measure of trait wellbeing, was not associated with composites of PACS including the AUCg. This upholds findings from a study conducted in 2017 by researchers at University College London (UCL). Stafford and colleagues conducted a meta-analysis to test the hypothesis that cortisol patterns indicative of a dysregulated hypothalamic–pituitary–adrenal axis functioning would be associated with poorer wellbeing – as measured by the WEMWBS. The authors refuted their experimental hypothesis, wellbeing was not associated with a ‘dysregulated’ morning cortisol, diurnal slope or awakening response. This study supports the findings by Stafford et al., (2017), demonstrating that trait wellbeing measured by the WEMWBS in a healthy and younger sample is not associated with PACS.

Falling under the umbrella measures of trait wellbeing, fatigue and perceived stress measured by the fatigue assessment scale (Michielsen et al., 2003) and the perceived stress scale (Cohen et al., 1983) were not associated with either PACS or PAMS. Participants self-reported good health and were aged between 18-39. Most participants were of working status and did not smoke. All participants also displayed PACS with a typical CAR profile. Trait measures in this instance may not have been relevant to the timing of salivary sampling for the waking hormone as

identified by Stafford et al., (2017). Nonetheless, upon observing measures of ambient lighting, anticipated mood and demands, these psychosocial state variables were also not statistically related to either PACS or PAMS. This suggests no association between PACS/PAMS and fluctuations in the participant's state psychosocial and ambient environment. A limitation to this study was that post awakening hormones were only measured over a two-day period. This may not have been sufficient to detect both changes in PACS and PAMS as well as it's relation to psychosocial trait and state measures.

The most notable property of the cortisol circadian cycle is that bright light administered in the post awakening period can significantly enhance the CAR (Scheer & Buijs, 1999) as can gradually increasing illumination (dawn simulation) administered prior to awakening (Clow et al., 2004). In contrast melatonin secretion is inhibited by bright light (Claustrat et al., 2005; Wehr et al., 2001). Yet, in the case of state measures that included ambient lighting during the study period, no significant statistical relationships were identified with either post awakening levels of cortisol or melatonin. Whilst this study attempted to control several covariates for both cortisol and melatonin, some factors were overlooked. It has been suggested that individuals with light-eyes have a significantly higher suppression of melatonin in response to light compared to individuals with darker eyes (Bhatti et al., 2013; Higuchi et al., 2007; Hsing et al., 2010). This potential confounding variable may have impacted the results of PAMS. In addition, the influence of the postural state on melatonin secretions were also not considered.

Participants were requested to take their samples following the moment of awakening which would usually involve raising from a laying position to a seated position to conduct the saliva sampling protocol. A simple action like sitting up from laying therefore has been associated with elevated melatonin secretions (Nathan et al., 1998). Further studies monitoring melatonin at the point of awakening should therefore ensure to control for postural factors to achieve true melatonin secretory concentrations upon awakening. Furthermore, the constraint of the study being conducted over two days may have impacted the overall results. However, it has since been recommended that CAR data are obtained over two consecutive study days which are evenly distributed across the week and incorporating one weekend

in cross-sectional research (Hellhammer et al., 2007; Stalder et al., 2015, 2022).

On the contrary, despite measuring PACS and PAMS over only two consecutive days, each of the four samples taken every 15 minutes following awakening were electronically monitored. This study thus, took account of strict adherence to the sampling protocol and is a significant strength of the study, with only three participants not adhering to the sampling protocol (Smyth et al., 2013; Stalder et al., 2015, 2022).

Despite a low number of non-adhering participants, study I had a low sample. Assays were expensive and time-consuming. The incubation time of 3 hours (in comparison to 1-hour with cortisol ELISAs) limited the number of participants in the study. A power analysis indicated that a sample size of at least 263 participants is necessary to achieve 80% power. This suggests that the sample size of 51 participants was inadequate to detect the expected correlation between post awakening cortisol and melatonin secretions. The methodological implications here, suggest that a larger sample size would provide more precise estimation of the relationship between the two hormones following awakening, increasing the external validity of these results. Thus, methodological issues may have masked the predicted relationship between PAMS and PACS. Future studies interested in the exploration of PAMS and PACS should account for a larger sample size (>263).

In conclusion, this study showed for the first time, within these healthy males and females, post awakening pattern of hormones responsible for sleep - melatonin and waking - cortisol. Despite being regulated by the SCN, it has been demonstrated that the best estimates of association between PACS and PAMS are close to zero i.e. there were no relationships between post awakening cortisol and melatonin secretions. The exploration of PAMS for this thesis ceases here such that PACS remains the focus of this programme of studies. In this sample, the underlying changes in post awakening melatonin secretions are unlikely to be associated with effects linked to the CAR. It also highlights that PACS and PAMS remain a distinct characteristic of neuroendocrine function. Similarly, in this group, there were no relationships between either hormones and psychosocial variables and state factors, which included environmental light, anticipated demands, and

mood. Future studies incorporating a longer sampling period, with a greater sample would further strengthen these findings.

Chapter 5 Study II

Exploration of post-awakening cortisol secretion patterns in individuals with POAG

5.1. Introduction

Study I highlighted that there was no association between PAMS and PACS in a relatively small sample group. For study I, PAMS and PACS remain a distinct aspect of neuroendocrine function. The focus of this thesis remains with the observation of PACS and thus saving on overall cost and time.

The sleep and wake cycle are the most prominent circadian rhythm in humans. Moderated by the interaction between day and night-time, light signals are transmitted through the rod-cone and melanopsin photoreceptive pathways of the retinohypothalamic tract. Cortisol synthesis is therefore, closely related to the photic input, where peak concentrations in cortisol levels occur during the crack of dawn and following morning awakening (see Chapter 1, Section 1.5). For example, disturbances in sleep due to both short and long-term shift work, has been observed to disrupt the release of cortisol from the adrenal cortex and has subsequently been associated with negative health outcomes (James et al., 2017; Li et al., 2018). These disruptions have been attributed to a lengthy exposure to night-time lighting – however this is usually more specifically associated with the effects of bright light exposure and the suppression of melatonin (Cajochen et al., 2005; Chellappa et al., 2013; Gooley et al., 2011a). To fully understand the effects of the Earth's axial position on the circadian rhythm of morning wakefulness and the CAR, it is fundamental to take a further insight into the functions of the SCN.

There are two potential mediating pathways from the SCN. First, are the indirect/direct effects of the SCN on the PVN which leads to the standard activation of the HPA-axis. Second is a faster 'extra-pituitary', non HPA-axis dependent mechanism which innervates the adrenal gland via the splanchnic nerve, situated in the lumbar region, increasing adrenal sensitivity to ACTH (Bornstein et al., 2008; Engeland & Arnhold, 2005). The latter route has been suggested to be involved in facilitating light inducing effects on the CAR (see review by Clow et al., 2010) and has particular importance with respect to the fine tuning of circadian influences (Buijs et al., 2003a; Ulrich-Lai et al., 2006).

Composites of post awakening cortisol secretion (PACS) have previously been investigated in relation to the effects of lighting. A study conducted approximately two decades ago at the University of Westminster, identified that total cortisol concentration (cortisol AUCg) during the first 45 minutes post awakening as well as the CAR, were significantly higher when exposing healthy participants to a dawn simulator of increasing illuminance (to 250 lux) over 30 minutes prior to awakening (Thorn et al., 2004a). These findings are reiterated in other studies which have assessed the CAR in relation to light exposure. For example, participants exposed to blue/short wavelength light (40 lux) for 80 minutes following awakening in a sleep laboratory, demonstrated an elevated CAR in comparison to dim light exposure (Figueiro & Rea, 2012). The findings also replicate the conclusions of an earlier study conducted by Scheer & Buijs, (1999) in which authors identified higher total cortisol levels 20- and 40-minutes post awakening following light exposure of 800 lux in comparison to dim light.

The overall theme of the literature examining post awakening cortisol concentration supports a stimulatory effect of light exposure on the CAR. Moreover, consistent findings can be observed by the most recent study to explore the effects of light on the CAR by (Petrowski et al., 2019). Here, authors invited participants to a sleep laboratory at the German Sport University in Cologne. Following a randomised controlled trial, findings revealed that post awakening exposure to bright light (414 lux) increased the CAR by 76% in comparison to exposure to dim light (< 2 lux). Furthermore, Petrowski et al., 2021) later identified that specific areas of the light spectrum i.e., blue, red, and white light, impacts

HPA-activity accordingly, such that blue and bright white light may have a greater stimulatory effect on HPA-activity. The studies mentioned above are conducted in either ambulatory (Scheer & Buijs, 1999; Thorn et al., 2004a) or sleep laboratory settings (Figueiro & Rea, 2012; Petrowski et al., 2019, 2021) providing both an ecologically valid and experimentally controlled set of data. These consistent findings highlight the critical role of the melanopsin-containing retinal ganglion cells (mRGCs) in the retinohypothalamic pathway. It also implies that humans with deficits in retinal pathways may be more particularly vulnerable to either HPA-axis or splanchnic nerve disruption in response to the acute effects of light.

In the most extreme cases of visual disruption, one of the earliest case studies observed a free running 24.5-hour cortisol rhythm in a blind subject (Orth et al., 1979). The authors also highlighted that the subject displayed the notable peak in cortisol concentrations following awakening. Approximately 40 years later, Aubin et al., (2017) echoed similar findings, such that no differences were identified in cortisol profiles between blind and normally sighted individuals. In this instance, it can be interpreted that the mRGCs of those who are blind and without light perception are still very much intact and unaffected such that cortisol circadian rhythmicity is maintained. However, details of the methodology employed in these studies should be carefully considered. Participants were required to attend sleep laboratories within the research institute, and were woken for saliva sampling during night-time sleep, thus reducing ecological validity. Furthermore, overall day patterns of cortisol were measured and not specifically PACS. To broaden the knowledge of the function of the mRGCs/retinohypothalamic tract role in post awakening cortisol secretion, other areas of visual disruption – particularly diseases of the aging eye should be given careful consideration.

Patients with primary open angled glaucoma (POAG) gradually suffer the loss of retinal cells – particularly mRGCS (Gao et al., 2022; Jean-Louis et al., 2008; la Morgia et al., 2011b, 2018) and if not treated, it can result in a slow loss of vision (Kessel et al., 2011; Lin et al., 2016). Like Alzheimer's and Parkinson's disease, POAG has been described as a neurodegenerative disease due to the selective loss of neuron populations and transsynaptic degeneration (Ly et al., 2011).

Interestingly, a recent study conducted by (Smyth, Skender, et al., 2019) observed the notable peak in cortisol following morning awakening in those with Parkinson's Disease over a two-day study. Overall levels of post-awakening cortisol were lower refuting previous studies (Skogar et al., 2011). It was noted that these levels were in accordance with levels of healthy adults of similar age. These findings may be demonstrative of the boost hypothesis suggested by Adam et al., (2006). Here, authors provide a functional interpretation of the CAR as an adaptive response designed to provide individuals with the "boost" needed to meet anticipated demands for the upcoming day. To further elaborate on the perseverance of PACS on a clinical sample with similar neurodegenerative cell loss, with direct impact on the retinohypothalamic pathway, it is indeed worth observing PACS in individuals with POAG.

The aetiology of POAG remains the subject of continuing investigation. Exploration of the effects of circadian rhythmicity and the autonomic nervous system have often been queried as part of the diagnosis of POAG (Gherghel et al., 2004; Werne et al., 2008). For example, positive associations have been observed between POAG and vascular systemic factors which include the circadian rhythm of blood pressure, intraocular pressure, and aqueous humour (Gherghel et al., 2004). Interestingly, as mentioned in the introductory Chapter (see Chapter 1, Section 1.7) cortisol is an influencing factor in circadian rhythmicity of blood pressure and this has been specifically associated with an increased sensitivity to catecholamines release, triggering the body's stress response of those with POAG (He., 2011). Furthermore, higher levels of plasma cortisol have been associated with higher aqueous humour and consequently higher intraocular pressure in POAG patients in comparison to healthy controls (Kasimov & Aghaeva, 2017; Østergaard Madsen et al., 2021; Schwartz et al., 1987). Interestingly, an elevated diurnal salivary cortisol secretion has been observed in the summer months in those with POAG in comparison with healthy controls. (Østergaard Madsen et al., 2021). Whilst POAG is an inherently physiological disease, the psychological wellbeing of the individual also merits examination.

It has been suggested that individuals with POAG are more vulnerable to depression than the general aging population (Agorastos et al., 2013; Shin et al., 2021). A meta-analysis conducted by Wang et al., (2018) revealed that individuals with glaucoma displayed lower levels of health-related quality of life compared to those without the disease. Elevated serum and salivary cortisol levels have been associated with a factor for depression in those with POAG (Østergaard Madsen et al., 2021) Furthermore, mindfulness and meditation has been suggested to lower serum cortisol, consequently reducing intraocular pressure and improving overall mental wellbeing (Dada et al., 2018).

An elevated CAR has been associated with poorer wellbeing including burnout, vital exhaustion, impaired executive function and chronic stress (Clow et al., 2010; Bellingrath et al., 2008; Hsaio et al., 2014; Juster et al., 2010; Stalder et al., 2015). An explanation of this relationship returns to the 'Boost Hypothesis' proposed by Adam et al., (2006). The authors established that prior-day feelings (sadness, loneliness, threat, lack of control) were associated with a higher CAR the following day. This is indeed an adaptive explanation of the CAR as a response designed to provide individuals the boost needed to meet demands for the upcoming day.

The most frequently used measure of patient-reported, vision-related functioning in age-related ocular disease studies is the National Eye Institute Visual Functioning Questionnaire (NEI VFQ-25) (Clemons et al., 2003; Jampel et al., 2002; Mangione et al., 2001). Based on qualitative research with patients, the NEI ssVFQ-25 was developed to measure the range of vision-related functioning experienced by persons with a variety of chronic eye diseases (Mangione et al., 2001).

Overall, to date, there are no studies which have specifically observed patterns of PACS in those with POAG. Jean-Louis et al., (2008), explains that POAG may be the principal ocular disease that effects photic input to the circadian time-keeping system as the result of inherent ganglion cell death. Thus, investigation of individuals with POAG provides an opportunity to explore the effects of light

transmission to the circadian system with particular focus on input to PACS and its potential influence on state and trait factors of wellbeing.

5.1.1 Aims

Using the standard measures of CAR research, ensuring adherence to saliva sampling protocol, the current study is the first to monitor patterns of PACS in those diagnosed with primary open angle Glaucoma (POAG). These patterns were subsequently compared to a reference data (RD) group with no visual difficulties. The relationships of PACS in those with POAG were explored in relation to ambient lighting, visual function and other psychosocial state and trait factors. The specific aims and objectives are outlined below (see Table 5.1).

Table 5.1 Specific aims and objectives

Aims	Objectives
Explore patterns of PACS in participants with POAG.	Close monitoring of first morning sample to examine patterns of post- awakening salivary cortisol in participants with POAG and comparing PACS with a RD group with no reported visual difficulties
Explore relationships between PACS and ambient lighting/ self-reported visual function	Exploring associations between PACS and ambient light and self-reported visual function in individuals with POAG
Explore relationships between PACS in psychosocial state/trait factors	Exploring associations between post waking salivary cortisol patterns alongside state (anticipated mood and demands) and trait psychosocial variables (mental wellbeing, perceived stress, and fatigue)

5.1.2 Hypotheses

Noting recent studies which have observed PACS in those with a neurodegenerative disease such as Parkinson's Disease (Skogar et al., 2011; Smyth, Skender, et al., 2019), it was hypothesised that there will be a main effect of sample on post awakening secretion in those with POAG. However, with reference to studies that identified high intraocular pressure in association with elevated cortisol levels (Kasimov &, Aghaeva, et al., 2017; Østergaard Madsen et al., 2021; Schwartz et al,1987; McCarty and Rosner, 1987) it was also hypothesised that there will be elevated composites of PACS in those with POAG in comparison to the RD group. Further, similar to Study I, it is predicted that composites of PACS will be associated with measures of ambient light and self-reported visual function. It is also hypothesised that greater anticipated demand will be associated with elevated PACS and particularly the CAR. With respect to ambient lighting, it is hypothesised that brighter light (greater lux) will be associated with a more elevated PACS. Composites of cortisol secretion were hypothesised to be associated with measures of state variables including, anticipated mood over the two-day study as well as psychosocial trait factors including wellbeing, fatigue and stress. Finally, it was hypothesised that a lower score visual function/quality of life as measured by the NEI-VFQ-25 will be associated with a higher PACS and specifically the CAR.

5.2. Method

5.2.1 Participants

A total of 109 participants (29 male, 80 females) were recruited. 54 participants were recruited from the International Glaucoma Association (IGA)

Male (N=16) and female (N=38) participants with POAG were recruited from the IGA following a call for participants advertised in a monthly newsletter. Ages ranged from 46-84 years (70.3 ±7.3). The majority were married (54 %) and retired (72%).

For comparison of the POAG group, a reference data (RD) group with normal vision was used (see Smyth, Skender, et al., 2019). Cortisol and demographic data were taken from participants recruited from an ongoing study (N=55) in 2017 at the University of Westminster. Data from male (N=13) and female (N=42) participants were used. Ages ranged from 54-89 years (68.5 ±8.5). Similarly, most participants in the RD group were married (67%) and retired (75%)

Following an expression of interest, either by phone call or email, each participant was contacted via an informal phone call, in which the researcher ensured the prospective participant met the inclusion criteria; participants using oral or topical corticosteroid medications and/or suffering from a medical (excluding open angled glaucoma) or psychiatric illness were excluded. Participants received no financial incentive to take part in the study. The University of Westminster ethics committee approved the protocol.

5.2.2 Materials and measures

Participants were sent a study pack, which included full, standardised written instructions, an information sheet, a questionnaire booklet, a saliva sampling kit

containing two Ziploc bags labelled day 1 and 2. Each Ziploc bag contained five colour-coded labelled Salivettes (saliva sampling devices, Sarstedt Ltd., Leicester, England) labelled ‘evening’ and tube 1-4 (awakening – 45 min post awakening). Participants were provided with a record sheet to confirm their awakening and saliva sampling collection times. Also included were electronic devices, which monitored ambient light, participant awakening times (wrist-worn Actiwatch-score device) and the saliva sampling times (MEMS cap), containing cotton swabs required for saliva collection.

5.2.2.1 **Psychosocial measures**

Demographic questions included age, sex, employment and marital status. Participants also reported their smoking/drinking (current, occasional, ex-smoker/drinker, never smoked/drank alcohol) and health status (1=poor - 5= excellent). The questionnaire booklet included measures of subjective wellbeing and quality of life, assessed using the 14-item Warwick-Edinburgh Mental Well Being Scale (Tennant et al., 2007; WEMWBS; higher scores indicated greater mental wellbeing), the 10-item Fatigue Assessment Scale (Tennant et al., 2007; FASS; higher scores indicated greater feelings of fatigue) and the 10-tem Perceived Stress Scale (Cohen et al., 1983; PSS; higher scores indicated greater feelings of perceived stress).

5.2.2.2 **National Eye Institute Visual Function (NEI-VFQ)**

A 25-Item Visual function questionnaire from the national eye institute (Mangione et al., 1998) was used to determine the varying ‘subjective’ range of visual function (see Chapter 2, Section 2.5 for details of scoring). Higher scores indicated better visual function. Cronbach’s alpha was low (0.64) for the NEI-VFQ but improved after removing an item (item 9 from the ‘Distance activities’ subcategory)

5.2.2.3 **Lighting and electronic monitoring**

Light was recorded using the light data logger (LDL) (HOBOware device). Recordings of the LDL were analysed using HOBOware software. Light data recorded on the HOBOware, was measured in 'lux' every 5 minutes from activation of the device. An average of light measured every 5 minutes one hour before sleep was calculated to give a mean evening light score (EL). This was repeated for morning light, where average light measured every 5 minutes for one hour after awakening was calculated to give a mean awakening light score (AL).

The Actiwatch and MEMs devices were used in combination to identify real sampling time from awakening. Electronically-monitored (EM) sampling times were determined by the actigraphy estimates of awakening and MEMs-verified sampling times. Awakening times were independently assessed, then agreed, by at least two members of the supervisory team following examination of actigraphy measures on each study day. The interval between EM waking and collection of saliva sample 1 were calculated as a difference score and indication of sampling delay. A delay of more than 8 minutes was considered 'severe' and formed part of the analyses.

5.2.2.4 **Diary measures**

Self-reported measures were recorded on each study day using diaries (See Appendix 9). Prior to sleep and on awakening participants were asked to rate their anticipated demands for the day on a five-item Likert scale ranging from 1 = not at all busy to 5= very busy. A higher score is reflective of a higher level of anticipated demands for the day.

Anticipated mood was measured as participant's self-reported feelings for the day ahead (Stalder et al., 2009). This was recorded on a five-item Likert scale, ranging from 1= anticipation of a very negative feeling to 5= very positive. A higher score is therefore reflective of anticipations of a more positive and happier feel for the day ahead.

5.2.3 Procedures

Participants had a standardised one-to-one research induction session conducted over the telephone with the researcher (NR). During this session (duration 25-30 min) participants provided verbal informed consent, received full verbal instructions on completing the questionnaires and the saliva sampling protocol. During the induction a number of preliminary research questions were asked based around their health which included understanding how long they suffered with POAG, details of medication and treatment and if they were experiencing any ocular pain (see appendix to be included). Participants were talked through the steps of the saliva sampling process and given every opportunity to ask questions.

Participants were asked to place the LDL in their bedrooms, on a flat surface, away from direct electrical lighting. The LDL was activated from 18:00 for 48 hours on the first study day.

Participants were informed about the need to adhere to the strict sampling regime relative to awakening time and were informed that the electronic devices would be used to verify the accuracy of their saliva sampling. Prior to sleep and to verify awakening times, participants were asked to wear Actiwatch (Cambridge Neurotechnology, Cambridge, UK) or the Actiwatch-2 (Philips Respironics, UK) device. Participants were reminded to prepare for the study day (e.g. place samples next to their bed and wear the Actiwatch-score device to bed) via automated text messages.

Participants were instructed to collect saliva samples immediately on awakening (S1),15 (S2), 30 (S3), and 45 (S4) minutes post awakening. Cotton swabs were stored in a medication event-monitoring (MEM) bottle where participants were instructed to open the device removing the swab for each specified sampling time. They were instructed to awake in their usual way either spontaneously or by an alarm clock. During the saliva collection period participants were asked to refrain from smoking, brushing their teeth, exercising and take nil by mouth except water.

Samples were initially stored in a domestic freezer until they were returned to the laboratory to be stored at - 20ºC until assayed. Participants were asked to complete a record sheet and evening/morning diary, entering their awakening times, their protocol-required saliva sampling times based on their awakening time that day, their actual saliva sampling times and scoring the anticipated demands (1=not at all busy-5=very busy) and mood (1=very negative feelings-5= very positive) for the following day. The study materials and the saliva samples were returned to the researcher at the end of the study.

5.2.4 Cortisol assessment and assay

Salivary cortisol assays were carried out at the Psychophysiology and Stress Research laboratory at the University of Westminster. Saliva samples were thawed and centrifuged for 10-15 minutes at 3,000-3,500 rpm. Cortisol concentrations were determined in duplicates by Enzyme Linked Immune-Sorbent Assays developed by Salimetrics LLC (USA). Intra- and inter-assay variations were below 10% for the analysis of the hormone.

5.2.5 Treatment of data and statistical analysis

Distribution and normality analyses were conducted on the whole sample (POAG and RD group; see Table 6.1 for a quick view of analyses conducted). Subsidiary analyses removing those that delayed their sampling following awakening, were conducted to check results were consistent. A standardised procedure to identify non-adherence to sampling protocol was used (see Salivary Cortisol and Melatonin section 2.3.5)

Awakening cortisol values were positively skewed and square root transformations were used to normalise sample distributions. Composites of post awakening cortisol secretion (PACS) were computed on raw data. This included an estimate of total cortisol secretion over the 45-minute post-awakening period represented as the area under the curve with respect to ground (AUCg) [s1 + s2 + s3 + [(s4 – s1) / 2]. The distribution of AUCg was positively skewed and was

normalised by a square root transformation. The CAR was calculated as the mean increase (MnInc) of subsequent samples (s2-s4) from the first sample (s1) taken at awakening [s2+s3+s4]/3-s1]. The MnInc followed a normal distribution and z-score analysis was used to highlight any potential and probable outliers. Mean MnInc calculated over the two-day study period revealed two extreme z-scores. Subsequent analyses were repeated excluding the two outliers to ensure results were consistent. State measures including evening and morning light data were also all positively skewed. Subsequently, data were normalised using square root transformations. Z- score analyses did not reveal any outliers for prior/same day anticipated demands and mood data. Each factor here also followed a normal distribution and statistical transformations were not required.

For both the POAG group and RD group, an initial two 2(day) x 4(sample) within-subject ANOVA was conducted on post awakening cortisol of participants with POAG and the RD group to highlight the main effects of study day, sampling time and the interaction between factors.

For comparison of PACS of POAG to the RD group, A mixed ANOVA was conducted on post awakening cortisol data (Sample Day x Group) to observe potential main effects and interactions in data of participants with POAG in comparison to the RD group over the two study days.
Analyses of sampling adherence were conducted using chi-squared test to examine statistical differences between the sample group (POAG and reference data).

The remainder of analyses observing psychosocial trait and state variables were conducted for the POAG group only. Descriptive analyses via stem and leaf plots, revealed a small number of potential outliers within the distribution of the psychosocial scores. One participant's score of wellbeing (WEMWBS=26) remained outside of the mean 95% confidence intervals (lower=51 upper=56). To prevent a loss of data, a method of winsorising was used by manually changing the score to the next lowest score consequently evenly distributing the data. Similarly, an outlier was also identified within the distribution of the FASS. One

participant's score of fatigue (FASS=35) remained outside the mean 95% confidence intervals (lower=18 and upper=21). Using the same winsorising method, this score was manually changed to the next highest score, evenly distributing the data. In addition, there were two outliers that were revealed within the distribution of the NEI-VFQ. Two participants visual function scores (NEI-VFQ= 41 and 46) were outside the mean 95% confidence intervals (lower= 64 and upper=70). These scores were again manually changed to the next lowest score, evenly distributing the data.

Paired t-tests were also used to determine differences between lighting measured in the evening and on awakening the following day. Similarly paired t-tests were used to determine differences in anticipated demands and mood measured before sleep and on awakening. In addition, bivariate correlational analyses were used to analyse relationships between PACS, psychosocial and state measures. The first waking sample was used to check adherence and the assumption of the 8-minute interval accuracy in providing post waking samples 2-4. Monitoring the results for consistency, all analyses were conducted on the full sample including subsidiary analyses removing those that delayed the first morning sample.

5.3. Results

5.3.1. Sampling adherence

Similar to study I, electrically monitored awakening times (EM) and saliva sampling times were highly concordant with participant's self-reported timings (90% matched timings). All self-reported (SR) awakening data were assessed with reference to EM awakening. A simple *t*-test revealed there were no significant differences between EM awakening and SR (*day 1: t* = -1.427*, df* = 97*, p* = .157*;* day 2*: t* = -.910*, df* = 95*, p*=.365).

On repeating the below ANOVA analyses with only adherent participants with POAG (N=15) and RD group (N=40), all findings were consistent. With the exception of a significant association identified between the cortisol AUCg and

anticipated demand, findings were also consistent when repeating bivariate correlational analyses with adherent participants with POAG.

Cross tabulation analyses demonstrated that participants with POAG 'delayed' the first morning sample (>8 minutes) more often than the RD group (see Table 5.2). A chi-square test was performed to examine the association between the sample group (POAG and RD group) and adherence (delayer and no-delay). Analyses revealed that participants with POAG delayed more often than the RD group X^2 (1, N = 106) = 21.71, p = <.*001*.

Table 5.2 Total number and (%) of participants in the POAG and reference sample that delayed cortisol sampling on either the first [missing n=5], second [missing n=7] or both study days

Sample (N)	No delay	Delay	Delayed first study day	Delayed second study day	Delayed both study days
POAG (54)	15	37	32 (59)	34 (63)	29 (54)
Reference (55)	40	14	9 (16)	8 (15)	3 (5)

Means and standard deviations of the mean increase in cortisol (MnInc) and total cortisol (AUCg) for participants with POAG are presented in Table 5.3 below. The cortisol data were within the normal range for the age of the POAG sample (Wüst et al., 2000; Stalder et al., 2010). The mean increase in cortisol within the first 45 min post-awakening was 5.3 nmol/l, and the mean peak for cortisol concentrations occurred at 30 min post-awakening. Cortisol data for the RD group

was within the normal range for age and health of the sample (Wüst et al., 2000; Stalder et al., 2010 ; Stalder et al., 2015, 2022; Smyth et al., 2019).

Table 5.3 Descriptive statistics of untransformed at sampling times 0, 15, 30 and 45 minutes following awakening and composite cortisol concentration (MnInc, AUCg, nmol/L) in the POAG group (N=54) and the RD data group (N=55

	Mean (SD±)		Min-Max	Min-Max
	Day 1	Day 2	Day 1	Day 2
POAG Group				
Sampling times (minutes)				
0	9.04(0.68)	10.51(1.05)	11.68-23.46	2.76-38.53
15	13.24(1.14)	13.51(1.19)	2.43-42.90	2.31-40.64
30	16.86(1.23)	16.63(1.16)	4.66-45.02	3.36-40.75
45	15.86(1.29)	14.32(1.08)	0.36-51.23	1.89-34.90
mnInc	6.28(0.97)	4.32(0.87)	-9.58-23.94	-13.50-21.96
AUCg	42.56 (2.95)	42.57(3.04)	12.75-116.94	10.22-107.35
RD group				
0	8.82(0.76)	7.09(0.511)	1.72-29.64	0.87-17.85
15	11.73(1.07)	9.61(0.64)	1.33-38.61	2.74-22.31
30	12.84(0.83)	13.89(0.98)	3.18-36.11	3.83-36.50
45	11.21(0.84)	13.65(1.06)	1.05-32.21	3.25-43.64
mnInc	3.10(0.81)	5.29(0.83)	-11.07-24.03	-7.53-24.60
AUCg	34.60(2.23)	33.86(1.92)	11.89-83.12	10.91-73.24

5.3.2. Patterns of post-awakening salivary cortisol (PACS) in participants with POAG and comparison with a reference data (RD) group

Post awakening cortisol concentrations for the POAG sample ranged between 0.36 and 51.23 nmol/L. Analyses of the POAG sample cortisol data revealed a typical CAR profile, with a significant increase in cortisol over the initial 45 minutes post awakening, with a main effect of sampling time $F_{2.86,\ 104.28}$= 23.13, p <0.001. For illustration purposes untransformed data is presented in Figure 5.1. There

was no main effect of day $F_{1, 50}$ = 0.35, p = 0.56 and analyses revealed that there was no significant interaction between day and sampling time $F_{2.01, 100.59}$ = 0.82, p = 0.44.

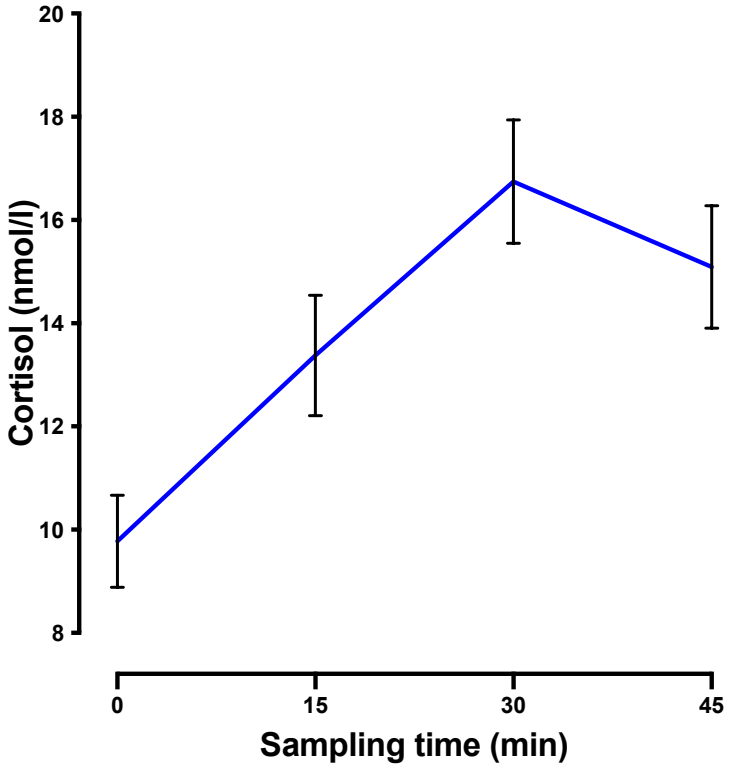

Figure 5.1 Mean (±SEM) cortisol for samples (0,15, 30, 45 min post-awakening) in the POAG group across two study days

Post awakening cortisol samples for the RD group are also outlined in Table 5.3 above. An additional two 2(day) x 4(sample) within-subject ANOVA revealed a typical CAR profile in the RD group, with a significant increase in cortisol over the initial 45 minutes following awakening. Similar to the POAG sample, there was a main effect of sampling time $F_{1.74, 92.33}$= 32.58, p <0.01 and no main effect of day $F_{1, 53}$ = 0.34, p = 0.85.

Comparing PACS between participants with POAG and the RD group, a two-way mixed ANOVA (Sample x Group) revealed a significant main effect of group $F_{1, 106}$ = 6.46, p=0.012. Post awakening cortisol levels for the POAG group are higher at each time point than for the RD group (see Figure 5.2 below). No interaction between sampling and group were identified $F_{1.98, 210.08}$ = 0.187, p=0.828.

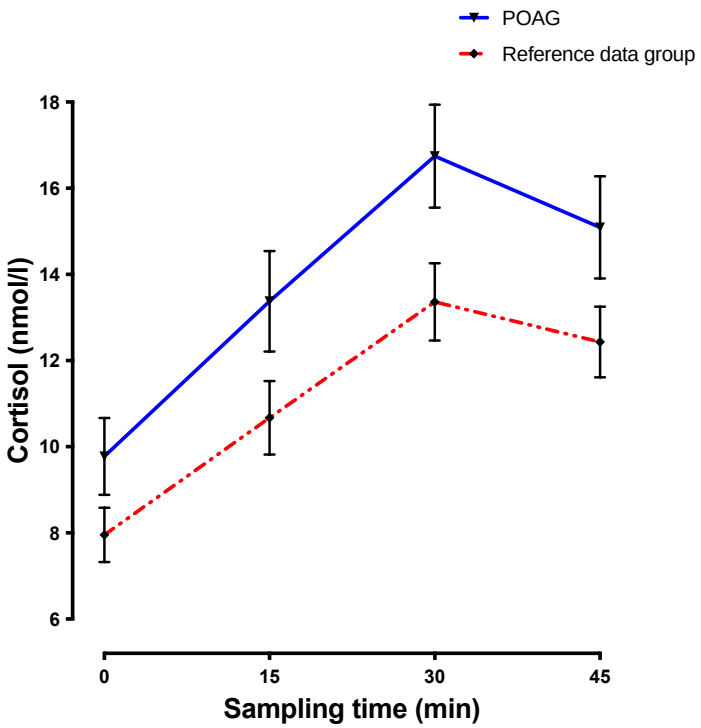

Figure 5.2 Estimated marginal mean of post awakening cortisol of the POAG and RD group at 0, 15, 30 & 45 min following awakening across two study days.

5.3.3. Relationship between salivary cortisol patterns, ambient light and self-reported visual function in participants with POAG

5.3.3.1. Relationship with ambient light

Evening light ranged between 0 and 3671.60 lux and light measured an hour after awakening ranged between 0 and 531.6 lux (see Table 5.4). A paired t-test showed no significant study day differences between evening light t = .978, df = 25, p= .338 and awakening light t= -.268, df= 24, p=. 791. An overall mean evening light score (EL) and awakening light score (AL) was calculated.

Table 5.4 Means, standard deviations and ranges of ambient light measured in the evening (EL) an hour after awakening (AL) (N=44)

	Mean (SD±)		Min	Max
	Day 1	Day 2	Day 1	Day 2
Ambient light (lux)				
Evening light	199.30(555.33)	87.15(160.86)	0.00-3672.60	0.00-704.40
Awakening light	132.13(272.42)	158.32(531.46)	0.00-1504.47	0.00-3669.48

A significant positive relationship between EL and AUCg *r*=.0.320, n=46 *p=.030* was observed, see Table 5.5 below. This suggests that the brighter the light before sleep, participants with POAG were likely to have the higher levels of total post awakening cortisol (see Figure 5.3).

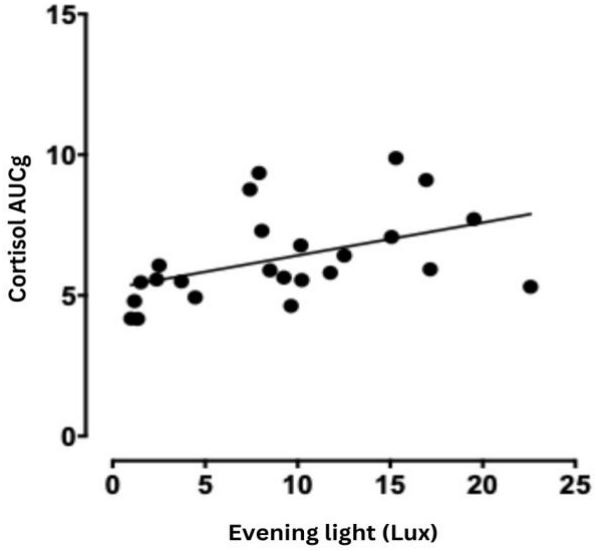

Figure 5.3 Scattergraph for Evening light (Lux) and total cortisol secretion (AUCg)

Table 5.5 Pearson's correlation coefficient for evening cortisol, PACS (MnInc, AUCg), mean ambient light in the evening (EL), mean light an hour after awakening (AL) and NEI-VFQ score

	AUCg	EL	AL	NEI-VFQ
MnInC	.585**	.069	.252	-.297*
AUCg		.320*	268.	-.205
EL			.448*	-.132
AL				-.056

p<.05*, P<.01**

5.3.3.2. Relationship with self-reported visual function

Visual function measured by the NEI-VFQ ranged between a score of 47 to 82. The mean score was 67.07 (±SD= 9.84). Correlational analyses revealed a significant negative relationship between scores of the visual function questionnaire (NEI-VFQ) and the MnInc r=-0.297, n=46, p=0.045. Figure 5.4 illustrates this association; participants with POAG reporting a poorer level of visual function displayed a greater rise in cortisol following awakening.

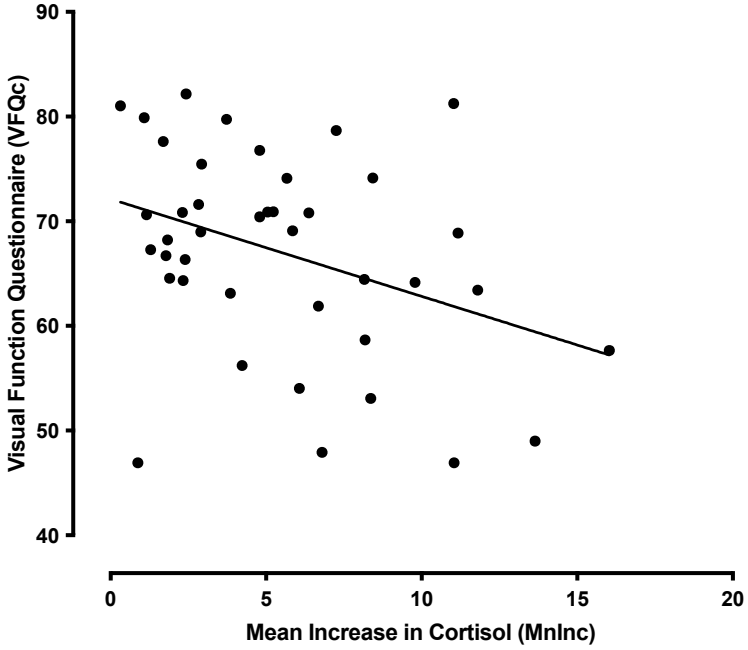

Figure 5.4 Scattergraphs for visual function as measured by the NEI-VFQ and post awakening mean increase in cortisol (MnInc)

5.3.4. Relationship between salivary cortisol patterns and psychosocial trait and state variables in participants with POAG

5.3.4.1. Psychosocial trait measures

Descriptive statistics of the psychosocial trait measures from participants with POAG are outlined on Table 5.6 below.

Table 5.6 Means, standard deviations and ranges of psychosocial trait measures including the Warwick and Edinburgh mental wellbeing scale (WEMWBS), the Perceived stress scale (PASS) and the Fatigue assessment scale (FAS) N=45

	Mean (±SD)	Min-Max
WEMWBS	54.13 (7.56)	41-70
PSS	16.311 (4.39)	9-27
FAS	19.61 (5.38)	10-31

Presented in Table 5.7 are the bivariate correlations of the trait questionnaire measures and evening cortisol and PACS. In general, analyses revealed there

were no significant relationship between scores of trait psychosocial measures and patterns of salivary cortisol.

Table 5.7 Pearson's correlation for PACS and trait psychosocial measures in participants with POAG

	AUCg	WEMWBS	FAS	PSS
MnInc	.585**	-.124	.041	.089
AUCg		-.274	.099	.198
WEMWBS			-.414**	-.633**
FASS				.602**

p<.05*, p<.01**

5.3.4.2. Psychosocial state measures

Table 5.8 below, presents descriptive data of psychosocial state variables for the two study days for participants with POAG. In this study, measures of anticipated mood and demand were analysed using paired t-tests to observe potential day differences.

No differences were observed between anticipated mood reported on the two evenings prior to the study day *t*=-.151*, df*=45*, p*=0.88. This was replicated when observing day differences between anticipated demand reported on the evenings prior to the study day *t*=1.67*, df*=45, *p*=0.102 and on the same day *t*=-1.31*, df*=44*, p*=0.20. Mood and demand reported prior to the study day as well as demand reported on the same study day were combined into one measure. The only remaining diary measure which was different across the two study days was anticipated mood. A higher level of mood was reported on the first day of the study compared to study day 2 *t*=3.50*, df*=44*, p*=0.001. An overall mean was not calculated, and subsequent correlational analyses below use mood reported on the two study days as independent variables.

Table 5.8 Means, standard deviations and ranges of psychosocial state variables with regards to prior-day and study day anticipated mood/demand and lighting.

Diary	Mean (SD±)		Min	Max
	Day 1	Day 2	Day 1	Day 2
Prior study day				
Anticipated mood	4.00(0.89)	4.02(0.83)	1.00-5.00	2.00-5.00
Anticipated demand	3.41 (0.97)	3.13(1.00)	1.00-5.00	1.00-5.00
Study day				
Anticipated mood	3.95(1.03)	3.55(0.94)	1.00-5.00	2.00-5.00
Anticipated demand	3.40(0.83)	3.62(1.00)	2.00-5.00	1.00-5.00

Correlational analyses outlined on Table 5.9, revealed a significant relationship between AUCg and anticipated mood reported prior to each study day and reported on the day of study day 1 and 2 r=-.326, p=0.027; r=-.349, p=0.017; r=-.352, p=0.018. Greater post awakening cortisol levels therefore are suggested to be linked to a lower reporting of mood prior to and on each day of saliva collection in participants with POAG (See Figure 5.5).

Table 5.9 Pearson's correlation for PACS and the psychosocial measures in participants with POAG

	AUCg	Prior day demand	Study day demand	Prior day mood	Day 1 mood	Day 2 mood
MnInC	-	-.030	.157	-.188	-.276	-.295
AUCg		.-.044	.111	-.326*	-.349*	-.352*
Prior day demand			.819**	.285	.321*	.548**
Study day demand				.266*	.299*	.206
Prior day mood					.803**	.589**
Day 1 mood						.549**

p<.05*, p<.01**

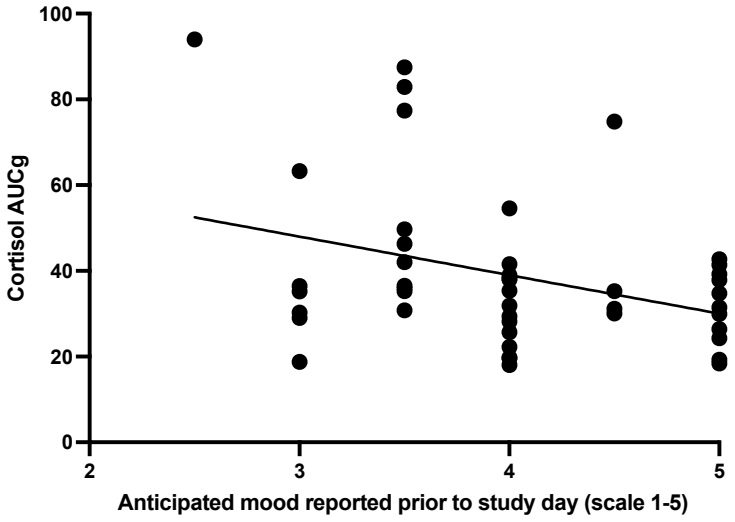

Figure 5.5 Scattergraphs for mean total cortisol secretion (AUCg) and mean anticipated mood report prior to study days

On repeating the above correlational analyses with only adherent participants with POAG (N=15), a significant association was identified between post awakening cortisol AUCg and anticipated demand reported on the same day *r*=.737, *p*=0.015. Therefore, participants with POAG who adhered to the sampling protocol, reporting a high level of demand on the study day were likely to have higher levels of total post awakening cortisol (see Figure 5.6).

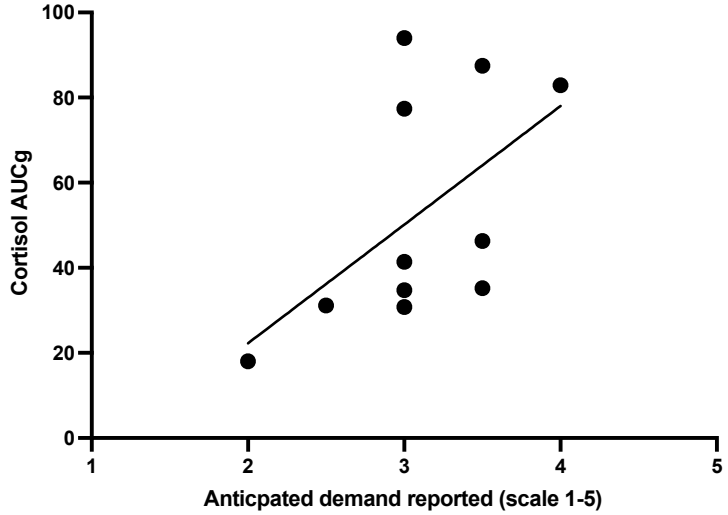

Figure 5.6 Scattergraph for mean total cortisol secretion (AUCg) and mean anticipated demand reported over the study days.

5.4. Discussion

This study for the first time has explored the patterns of PACS in individuals with POAG. As predicted, participants with POAG displayed the usual peak in cortisol following morning awakening over the two-day study period. This indicates that the CAR is preserved in individuals with POAG. Also, in support of the experimental hypothesis, those with POAG appeared to have a greater AUCg in comparison to the RD group with no visual difficulties. There was a positive relationship between ambient evening lighting and AUCg; however, lighting following awakening was not associated with any of the composites of PACS. As hypothesised, visual function was negatively associated with the CAR, suggesting that the lower the self-reported visual function the higher the CAR. This relationship was restricted to the CAR as no statistically significant associations were identified between the NEI-VFQ and cortisol AUCg. Moreover, there were significant relationships identified between anticipated demands over the course of the two-day study and the AUCg but not the CAR in the POAG sample. Thus, the greater the demand experienced by the participant during the study, the greater the overall total cortisol concentration following awakening. In addition, anticipated mood, or rather the feelings felt for the day ahead was negatively correlated with AUCg but not statistically associated with the CAR. Thus, participants with POAG, who reported a greater demand and a low mood over the two study days had the propensity for an elevated total cortisol concentration following awakening. Like study I, and accepting of the null hypothesis, there were no statistically significant correlations between wellbeing, fatigue and stress with PACS in individuals with POAG.

For the first time, this study has illustrated the patterns of PACS in participants with POAG. Observed by the main effect of sampling, participants displayed the usual peak in cortisol following awakening and thus demonstrated that a typical CAR existed. Findings are not too dissimilar to a study conducted in 2019 by Smyth and colleagues. The authors observed the patterns of PACS in participants with Parkinson's Disease and noted the typical peak in cortisol following morning awakening. Whilst there are significant differences in symptoms between

Parkinson's disease and POAG, they share some similarity in how the disease manifests. Both have been described as neurodegenerative, due to selective loss of neuron populations (Ly et al., 2011). The findings of the present study support the very notion, demonstrating that individuals with such diseases are resilient, and thus physiologically unimpacted with respect to their PACS.

Initial thoughts would suggest that the damage to mRGCs due to increased intraocular pressure would reduce post awakening cortisol secretion from the adrenals. However, the usual peak in cortisol following morning awakening is typical of a CAR. Thus, despite the potential impact of loss of mRGCs the CAR is preserved in the POAG group.

The HPA-axis activated response, triggered by psychological stress, pain, immune system activation, awakening and several other physical functions appear to override the secondary non-HPA axis dependent mechanism. Furthermore, visual function as measured by the NEI-VFQ encompassing 'quality of life' and thus a measure of wellbeing, was negatively associated with the CAR, suggesting that the lower the self-reported visual function the higher the CAR. However, no statistically significant associations were identified between the NEI-VFQ and AUCg. These findings support the conclusions of studies which have associated higher levels of cortisol and poorer wellbeing (Clow et al., 2010; Bellingrath et al., 2008; Hsaio et al., 2014; Juster et al., 2010; Stalder et al., 2015).

In comparison between the POAG group and the RD group, a significant greater total cortisol concentration (AUCg) was observed. This finding provides support for studies which have demonstrated that elevated cortisol is associated with higher intraocular pressure in those with POAG in comparison to a healthy control (Kasimov &, Aghaeva, et al., 2017; Østergaard Madsen et al., 2021; Schwartz et al,1987; McCarty and Rosner, 1987).

An unexpected finding was the evident difficulties of participants with POAG adhering to the sampling protocol in comparison to the RD group. Approximately 54% of the POAG sample delayed the sampling protocol on both study days

compared to only 5% of the RD group. Nevertheless, after controlling for this delay, PACS findings were consistent, apart from the total cortisol post awakening level - the AUCg composite which was positively related to anticipated demand reported on the same day. Accepting the experimental hypothesis, adherent participants with POAG who reported a high level of demand on the study day were likely to have greater levels of total post awakening cortisol. Here, findings support the boost hypothesis suggested by Adam et al., (2006) who provide a functional interpretation of the CAR composite of PACS as an adaptive response designed to provide individuals with the "boost" needed to meet anticipated demands for the upcoming day. Furthermore, individuals with POAG who anticipated a lower mood for the next day had an elevated cortisol AUCg. These findings provide some insight into the mindset of the individual during the time of the sampling protocol. Independent to the CAR, cortisol AUCg has been associated with poorer trait wellbeing (Smyth et al., 2015b). The present study's finding consequently informs past literature by Smyth et al., (2015) by investigating a clinical population.

Furthermore, whilst Smyth et al., (2015) did not capture daily variations in state wellbeing, this provides additional support of the AUCg being a discrete measure of neuroendocrine function with distinctive associations with state and trait wellbeing. On the contrary, despite controlling for adherence, there were no statistical relationships between trait psychosocial variables (wellbeing, fatigue, and perceived stress) and PACS in individuals with POAG. These conflicting findings can perhaps also inform past literature as contradictory to Smyth et al., (2015), the state-like factors may be more dominant, impeding trait psychosocial associations within a clinical population.

In acceptance of the null hypothesis, there were however, no associations identified between the CAR and ambient lighting, yet there were positive associations between evening lighting and total post awakening cortisol secretions (AUCg). The CAR has been suggested to be influenced by light inducing effects (Clow et al., 2010; Figueiro &and Rea, 2012; Clow et al., 2010; Thorn et al., 2004a). It should be noted that this is the first study to investigate PACS in individuals with POAG. Several studies have in fact demonstrated that retinal ganglion cells

(RGCs) are the most affected cell type which progressively degenerate over the course of POAG (Dul, Ennis, Radner et al., 2015; Nickells, 1996). Consequently, patients with severe POAG have a recognisable level of damage to the optic nerve and a decreased threshold to light, resulting in blurrier vision and the need for more lighting (Hu et al., 2014; Jean-Louis et al., 2008; Kalaboukhova et al., Fridhamamar & Lindbolm, 2007). Thus, the effects of lighting on the CAR may be disrupted in this current sample population.

Although this study was cross-sectional in design and therefore cannot demonstrate causality, it provides evidence to help unravel the intricate interaction between PACS and POAG. The study was limited to two sampling days and a moderate sample of POAG patients was used. Nevertheless, it was strong enough to demonstrate the usual peak rise in cortisol following morning awakening as well as the effects of self-reported visual function. The comparison between the POAG and RD group however should be treated with caution as some covariates were not controlled for in the RD group. group. These variables included lighting and trait psychosocial fatigue which may have impacted overall PACS. In addition, socioeconomic status, ethnicity, and BMI were not measured in the study and could also potentially confound the overall findings when comparing the two groups.

Future work could examine a POAG sample in comparison to healthy, age-matched controls taking into consideration all covariates related to PACS. This would clarify whether individuals with POAG maintain an elevated PACS in comparison to those without visual deficits. Furthermore, stringent measures of retinal sensitivity could also be utilised in future work to quantify the amount of light being transduced to the HPA-axis in POAG patients.

In conclusion, using the gold standard morning awakening salivary cortisol methodology, this study for the first time showed that participants with POAG displayed the usual peak in cortisol following morning awakening over a two-day study period. Low levels of self-reported visual function in individuals with POAG were the only measure of wellbeing associated with an elevated CAR Individuals

with POAG also displayed a higher total cortisol level following awakening in comparison, to the RD group and this provides support for studies which have suggested an elevation of cortisol in those with high intraocular pressure. Whilst there were no associations between PACS and psychosocial trait factors, psychosocial state variations did reveal that individuals with POAG who anticipated a low mood, and greater demand for the day ahead and in environments with brighter lighting in the evening, had an elevated total post awakening cortisol secretion (AUCg) but this was not reflected in the CAR. The fundamental finding of this study is that despite potential impact of degradation of mRGCs, individuals with POAG have an intact, preserved CAR and important implication for regulated diurnal secretion and maintenance of overall health and wellbeing.

Chapter 6 Study III

PACS Research and the moment of morning awakening: A qualitative exploration

6.1. Introduction

There is compelling evidence that post awakening cortisol secretion (PACS) research studies are pivotal for understanding of both mental and physiological wellbeing. PACS research has informed health literature with respect to a plethora of illnesses, including Seasonal Affective Disorder (SAD) (Thorn et al., 2014), major depression (Refsgaard, Schmedes and Martiny., 2022), Parkinson's Disease (Smyth, Skender, et al., 2019) and coronary artery disease (Weber et al., 2022a).

PACS research measures the CAR from saliva samples that are self-collected. It aims to attract participants during the initial stages of recruitment based on the principle that the study, following instruction, can be conducted from the comfort of one's home and conveniently fitting into one's daily routine. The adherence data findings from study II of this thesis, suggest that participants with POAG experienced difficulties adhering to the sampling protocol in comparison to the RD group with no known visual difficulties. Despite the usual study briefing and intricate guidelines, vulnerable participants struggled with the first morning sampling. Thus, there are two fundamental issues this study seeks to address. Firstly, with successful measures of the CAR taking place in a variety of sample groups, little is known about the overall experiences of being a participant in PACS research. Furthermore, to achieve a successful first morning sample, a crucial element of PACS research is the assumption that participants have fundamentally the same understanding of "morning wakefulness". To date, no research has been

published to explore this important but taken-for-granted element. Therefore, this study aims to provide a qualitative exploration of the experience of being a participant and crucially how participants understand the moment of morning awakening.

Providing the participant with sufficient and accessible information about potential research study promotes a positive attitude towards participation and is pivotal to successful recruitment. (Jones et al., 2015; Lux et al., 2015; Moorcraft et al., 2016). However, successful recruitment has also been associated with minimal participant commitment and demand. A study by Newington & Metcalfe (2014) explored the factors influencing research recruitment within the clinical setting. The authors' thematic analyses revealed three themes which influenced the success of research recruitment. The first was 'infrastructure' – the ability to identify eligible participants within a particular institution. The second was the 'nature of the research', this theme discussed the varying commitments required from individuals e.g., there was difficulty in recruitment for clinical trials in comparison to observational studies. The third theme was recruiter characteristics. In this study, participants were more likely to agree to participate if asked by their medical doctor. In addition, 'altruism' was the main reason that individuals participated in research, maintaining the intention to help future patients as well as to give something back to the hospital and team which cared for them.

Studies investigating participation in clinical trials have reported the element of moral obligation felt by the participant to take part, and a sense of wanting to reciprocate the 'returning the favour' to their care providers (Canvin & Jacoby, 2006; Lowton, 2005; Newington & Metcalfe, 2014; Ulrich et al., 2012). A recent study by Sheridan et al., 2020) used a mixed methodology to investigate the determinants of participation in health research. They identified several facilitators and barriers that influenced participation. The researchers found that 'potential for personal benefit', 'altruism' and 'trust' were all dominant themes in facilitating successful participation. In contrast, researchers are often challenged with low recruitment rate. Here, the authors identified that 'context', 'population', 'design',

‘participant information and ‘social influences; acted as a barrier to research participation.

PACS research methodology relies on participants carrying out the required saliva sampling regime where the most crucial element is to collect the first saliva sample ‘immediately upon the moment of awakening’ (Stalder et al., 2015). Awakening from sleep described by Clow et al., (2010), involves a rapid attainment of consciousness followed by the relatively slow re-establishment of full alertness, some 20-30 minutes later. The period between regaining consciousness (i.e., awakening) and the attainment of full alertness is described as ‘sleep inertia’.

Sleep inertia has been examined by a few studies in relation to the CAR. Supportive of the role of the CAR in the regaining of state arousal, a positive association has been suggested between arousal at 45-minutes post awakening, post awakening cortisol levels (Thorn et al., 2004a) and the dynamic of the CAR (Thorn et al., 2009). The boost hypothesis suggested by Adam et al., (2006) provides a functional interpretation of the CAR as an adaptive response designed to provide individuals with the “boost” needed to meet anticipated demands for the upcoming day. Further, it has been proposed that the role of the CAR provides a recovery from the sleep inertia state as well as the provision of an ‘energetic boost’ in morning awakening and put simply - the restoration of alertness (Clow et al., 2010).

It has been stated that the onus to explain precisely what is meant by ‘the ‘moment of awakening’ to the participant is on the researcher during the initial face-to-face briefing. The definition in the first consensus paper published in 2015, focuses on the ‘regaining of consciousness’. Stalder et al., (2015, p.43) explains:

“*When you are awake, i.e. you are conscious: you know who and where you are; you are in a state that clearly is different from when you were sleeping even though you may still feel tired….”*

Although PACS research has ecological validity when they are conducted outside of laboratory settings, such that four saliva samples are self-collected from awakening and every 15 minutes for 45 minutes -studies of this nature, lack researcher oversight. Participant non-adherence to requested saliva sampling regimes can lead to inaccurate CAR estimates. For example, simply delaying the collection of the first sample following awakening (S1) by more than 15 minutes has resulted in false-high estimates of S1 and false low estimates of the CAR (Desantis et al., 2015; Dockray et al., 2008; Okun et al., 2010). Recent research studies by Smyth et al., (2013, 2015) carefully controlled awakening and sample times in healthy participants, sampling at 5-minute intervals. Findings revealed that cortisol levels remained relatively unchanged over the first 5-10 minutes following awakening ('latent period'), with a significant increase first being detectable in the 15-minute sample. One implication of this research is that accurate CAR measures can only be determined from data with strict adherence to commencement of saliva sampling following awakening Smyth et al., (2013, 2015). Therefore, there is an understanding that in order to achieve an accurate measure of the CAR, a participant is required to accurately and consciously be aware of the moment of their awakening. However, this can pose some complications for those who find it problematic in defining that point of wakefulness. Smyth et al., (2016) demonstrated that if participants delayed up to 15 minutes, they could still be included in the data analysis provided electronic estimates were available. Therefore, the authors highlighted the importance of electronic monitoring of awakening and collection of the awakening sample (S1) in order to obtain known sampling timings in the post awakening period.

Of the several measures taken within this intensive PACS research protocol, researchers can objectively monitor morning awakening via an electronic monitoring device such as the Actiwatch (Phillips, Cambridge). Sampling times have been determined by the use of a Medication Event Monitoring System (MEMS) which contain the cotton swabs required for saliva collection. Opening of each MEMS cap device records the date and time. Interestingly, the differences in self-reported wake time, wake time as measured by the Actiwatch and the recording of the first morning opening of the MEMS cap can inform researchers

about the duration of participant sleep inertia (Stalder et al., 2015). This reduced state of cognitive and motor performance (Tassi et al., 2006), may increase the difficulty of adhering to the saliva sampling protocol, preventing the precise determination of the moment when one is fully awake (Clow et al., 2010; Smyth et al., 2013).

The use of electronic monitoring can therefore identify the timing of unconsciousness during sleep via muscle movement measured by the Actiwatch. However, of the objective measures established to ensure critical adherence to the timing of the first morning saliva sample, the most crucial aspect for a researcher, is to assume that a participant is aware of the moment they are awake from a night's sleep. The Actiwatch does not give any insight into the establishment of full alertness. Therefore, filling the gap in the existing literature, this research will focus on exploring the subjective experience of the moment of morning awakening, a process which is highly subjective and occurs outside of the researchers' direct oversight.

Although Actiwatch and MEMs Cap allow for objectivity, they add additional procedures on top of an intense sampling protocol, which may be perceived as potentially onerous and burdensome. There also remain unanswered questions surrounding personal experiences of taking part in PACS research studies. This study will adopt a participant perspective lens and aim to 'give voice' to the participants by exploring the motivations for participation, the experiences of taking part in PACS research and ultimately understanding some of the challenges they face.

There are two fundamental issues this study seeks to address. Firstly, with such insight into sampling data, little is known about the motivations and overall experiences of being a participant and establishing a point of wakefulness for the first morning sample in PACS research. Secondly, with no literature published to date, this study for the first time will qualitatively explore participants' experience of the moment of morning awakening – a crucial element of PACS research.

6.2. Method

6.2.1 Participants

Participants were sampled from two populations: Participants with POAG (N=11; males=2, females=9) were recruited from the International Glaucoma Association (IGA) newsletter. All participants were retired and previously took part in study II (See Chapter 6) of this thesis. Ages ranged between 62-82 years old. A second group of participants were all members of the academic community (MAC, N=9; males= 4, females=5) in the Social Sciences department at the UOW, they had no visual impairment. Ages ranged between 48-55 years old. These participants previously took part in a study conducted by Smyth et al, (2021). All participants self-reported that they were in good health.

6.2.2 Procedure

Face to face semi-structured interviews were conducted by a member of academic staff in the Social Sciences at the UOW - Dr Sanjay Joban, (SJ). SJ followed up on the data collected for the study described in Smyth et al., (2021), within a week of the final PACS collection. All participants collected saliva using cotton swabs and salivettes. SJ addressed research questions which investigated the experiences of academic staff (within the Psychology department) taking part in PACS research studies and exploring the subjective meaning of morning awakening. Face to face interviews took place in either the qualitative research laboratory, health laboratory or a research cubicle within the Psychology suite of the University of Westminster. In this instance, interviews occurred on the final day of cortisol sampling.

Following the recruitment of participants with POAG taking part in study II, during the telephone screening, participants were asked if they would be interested in taking part in an extended study investigating the aims described above. Semi-structured interviews using the interview topic guide were conducted over

telephone (see Table 6.1 below) by the researcher (author of this thesis) and were completed alongside the quantitative research and analysis. The process of qualitative data generation, therefore, began while the researcher continued to collect quantitative data from other participants. Thus, on this occasion, interviews were conducted within a week of the last cortisol sample taken for study II. Telephone interviews have been considered less inhibiting for participants as the interview occurs within an environment familiar to participants (Ward et al., 2015).

Table 6.1 Interview topic guide

Topic (prompts, if necessary)
• **What were your reasons for taking part in this study?** • **What were your initial thoughts?** • **What were your general feelings about participation? (e.g., providing saliva samples upon waking up?)** • **How did you feel the night before, when you were aware that you needed to provide saliva samples the following morning?** • **How did your routine/experience compare to your usual routine?** • **Did you do anything differently?** • **What was the experience like for you to collect the saliva samples over the two mornings?** • **How long was it between waking up and collecting the saliva samples?** • **What was it like collecting samples as soon as you woke?** • **How did you find collecting samples every 15 minutes for 45 minutes after the first sample?** • **How did you fit the morning saliva sampling in with your morning/daily routine?** • **How did you find collecting the samples as soon as you woke?** • **Tell me about your thoughts of the other aspects of the study e.g., wearing the watch, completing the diary etc.** • **How did you determine this 'moment of awakening' as requested by the team?** • **What does being awake mean to you? (What do you understand by the moment of waking up?)** • **At what point, after gaining consciousness, do you normally feel fully alert?** • **How would you describe your awaking process? What do you go through as you are waking up?** • **How do you feel in the morning when you wake up?** • **How long does it take you from waking up to getting out of bed and going about your daily business*?***

6.2.3 Transcription and analysis

For the interviews conducted in 2016, recordings were transcribed and coded using thematic analysis by the author of this thesis. Coding and themes were checked by at least 2 other researchers from the team (NS & LT).

Five interrelated phases are recommended by (Braun & Clarke, 2014, 2021) and allowed for the analysis of qualitative data produced by this study. The phases included familiarisation, generating initial codes, searching for themes, reviewing themes, defining themes and interpretation (further detail described in Chapter 4, Section 4.52).

6.2.4 Interpretation

Once overarching and subthemes were identified, all data was interpreted in relation to overall participant experience but more specifically to initial research questions. Braun & Clarke, (2014, 2021) explain that each theme should have a detailed and logical explanation. Here, interpretation of the sub themes and overarching themes is an opportunity of further assessment to establish recurrent explanations for attitude and behaviours of resultant experiences.

6.3. Findings

In accordance with the research questions, the analysis identified nine subthemes; these have been grouped into three overarching themes. These themes encompass the various stages of participation, including initial motivations about taking part in the study, the experiences of going through the process of participation, and finally their understanding of the moment of awakening – a fundamental aspect of PACS research. These are shown in Figure 6.1 below:

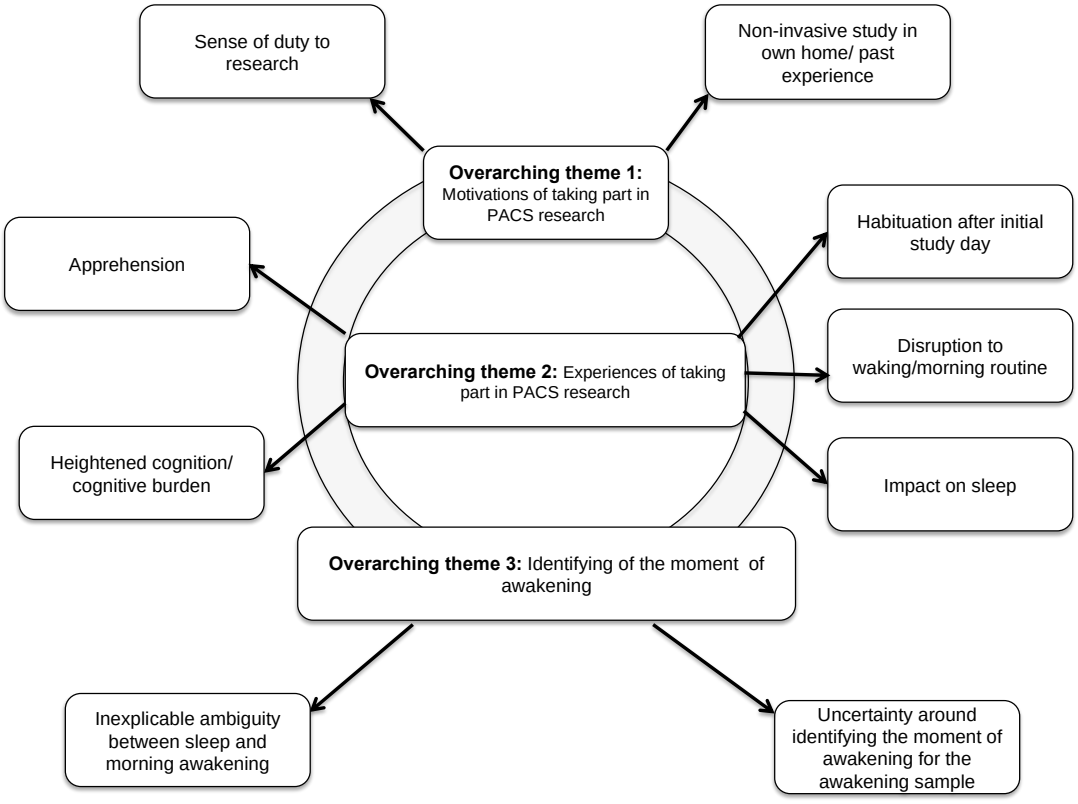

Figure 6.1 Diagrammatic illustration of overall thematic analysis for the motivations and experiences of taking part in a PACS research study and identifying the moment of awakening

6.3.1. Overarching theme 1: motivation of taking part

The theme ‘motivation of taking part’ captures participants initial thoughts about their interest or otherwise in participating in the PACS research study. The theme can be divided into two subthemes, which encompasses 'a sense of duty’ and that the study was ‘non-invasive and conducted from one’s own home’. Those with prior knowledge of the study were also encouraged by the ease of saliva sampling which is categorised under the second subtheme.

6.3.1.1. A sense of duty to research

This theme encapsulates the sense of duty participants felt towards taking part in research in general and specifically this study. An active feeling for doing the study was expressed by both samples. Members of the academic community at the University voiced a motivating awareness of ethical responsibility to participate in research in order to gain insight into the participants experiences, given that they also recruit participants for studies. For example, P18 (Male, Member of Academic Community), an experienced researcher has conducted several studies in the past explained:

“*So I think that’s [it’s] almost like an essential [reference to being an academic]; which is one of the reasons why I am quite happy to take part in research as a participant, because if we’re doing the research ourselves, it’s important to know what the research participant feels like. And that [taking part] was quite useful for that, because you realise... you know... sometimes the time and the effort that participants have to put into it [research studies] “ (*P18, M, MAC)

This excerpt is illustrative of the members of academic community sample being motivated to experience research from the standpoint of the participant and the potential burden of participation. They were also motivated by the awareness of the difficulties of research design and the importance of successful study

recruitment. P15 (F, MAC) is also supportive with the recruitment procedure and comments:

"*I guess because I sympathise with people trying to recruit participants; so that's the first thing, is that I wanted to be able to be helpful".*

Participants with primary open angle glaucoma (POAG) also expressed a sense of duty in research participation linked to altruism. Participants were motivated to be involved in research participation without any self-interest. For example,

"*I feel a sort of* '*duty* '*but [it] comes off a bit strong, a duty to do our bit. If [I] can be of any help, even if it helps you. I have taken part in medical trials, and I feel it is worth doing!" (*P08**,** Female, POAG)

Additionally, participants with POAG, voiced a sense duty linked to promoting awareness about their condition as well as benefiting others. This was highlighted by P03 (M, G) who stated they wanted to "*help out and get that knowledge out there"*. Building on the knowledge of POAG literature was important to other participants. One who regularly participates in research studies, explained:

"*Since I was diagnosed with glaucoma, I'm happy to do anything that I can to shed any light that may help with it all. I also do arthritis research as well, if there's anything I can do at all [to help]…. I also like to help anyone else that would benefit"* (P11. F, G)

6.3.1.2. A non-invasive study conducted in one's own home and prior experience of the ease of sampling

Participants were motivated to take part in this study because PACS research can be done in the domestic setting of one's own home. Conducting the study from the comfort of one's own home was mentioned often during interviews, particularly with participants with POAG and appears to be an important motivator for taking part in PACS research. The ease of accessibility of PACS research is an advantage for the POAG group. P05 (F, POAG) discusses their eagerness to take part in past research but have felt the burden of the distance they had to travel which often prevented participation:

"*Well, I had glaucoma for quite a few years now and it has got worse. I did apply once before to the glaucoma magazine [to take part in a study]. They wanted me to go to London three times, I think. There was no way I could do that. And so, when this came up in the glaucoma magazine, I thought oh that's interesting and that's something I could do"*

Another participant (POAG) expresses a sense of comfort *"...I didn't have to leave the home to do it. I could do it all in my own home"* (P01, F, POAG)

On recruitment, the study appeared non-invasive to participants, indicating that they saw saliva sampling as something non-invasive. P07 (M, G) summarises 'convenience 'being a subtheme of motivation in one sentence:

"*Oh well, I mean it all seemed very straightforward, non-invasive, not a problem".*

Others from the academic community, were very vocal with the ease they felt with the sampling protocol. Through prior participation, one participant was aware of the guidelines which required the participant to not doze or fall asleep between saliva sampling. Due to the nature of their own sleeping and waking habits they

found the protocol easy to fit into their waking routine as they explained that they usually stay awake once awake. P18 (M, MAC) states:

"*Once I kind of wake up, I usually [stay] up.... I might listen to the radio – just lie and listen to the radio or something like that – for half an hour… so that... so in that sense... doing the samples was... was relatively easy"*

Another member of the academic community - also through prior participation, was familiar with the study guidelines and felt confident about the ease of sampling. P12, (F, MAC) explains:

".. I've done it before; it felt familiar; I don't have any problems with taking saliva samples first thing in the morning… it doesn't worry me…it felt... it felt easy, yeah; it did feel quite easy."

6.3.2. Overarching theme 2: Experiences of taking part in PACS research

The theme 'experiences of taking part in PACS research', encapsulates the burdens of PACS research experience. Whilst there were some comments about the ease of the sampling protocol as exemplified above, what emerged from the interviews was that participants found the nature of the study arduous in terms of their apprehension, cognitive burden and disruptions to routine. 'Apprehension' forms the first subtheme. Both POAG and members of the academic community highlight their 'anxieties', 'concerns 'and 'worries when discussing their experiences about participation.

Despite apprehensions, both samples were keen to achieve the best outcome for the study. Consequently, there was a tendency for participants to feel more alert than usual during the course of the study leading to the development to a second subtheme of heightened cognition. Members of the academic community vocalised a 'disruption to routine more frequently than the POAG group, which forms the third subtheme. All of the members of academic community were of

working age and differences in daily commitments are apparent between the two populations. The penultimate subtheme addressed the study's 'impact on sleep' with both samples discussing their experiences. Finally, there is an expression of 'habituation during the study period' which is expressed by participants' account of the ease of saliva sampling, improvements in sleep quality, wake time and mood.

6.3.2.1. Apprehension

Apprehension was prevalent in both samples and there were often points in conversation where this feeling was highlighted. Participants expressed 'anxiety', 'worry' and 'concern' when asked to describe their experiences before and during the study protocol. Most of this apprehension stemmed from the participant concern about their poor sleep pattern impacting on the saliva sampling and *"making sure that I was awake on time"* (P13, F, MAC) and that their poor sleep pattern may be detrimental to the strict study protocol. For example, P01 (F, POAG) explained:

"..I was a bit anxious because I had spoken to you before about a very erratic sleep pattern - not regular. Some days I do wake up really early. I was never sure. I'm never sure if I can get back to sleep again. That was my concern really …."

In the protocol participants were advised to return to sleep if wake time is premature. There was an expectation that this was possible, which for some participants - this may have not been. The combination of the uncertainty of premature awakening, timing of the first waking sample and returning to sleep created anxiety. Participants were also apprehensive about multiple nocturnal awakening and the implications for the study protocol. These apprehensions surrounding sleep and awakening occurred from a sense of wanting to get the study completed correctly. This is illustrated by P02, (F, G) when consciously considering the protocol and the enduring nature of their worry throughout the study:

"Yeah, you know, [I] tried to visualise in my mind exactly what I had to do and what could go wrong here. And I think my biggest worry from the beginning to the end of it was the fact that I sleep so badly." (P02, F, POAG)

Some participants had previously taken part in PACS research studies and were aware of the protocol. Nevertheless, they appeared reluctant to admit that certain apprehensions remained. They reiterated the challenges previously experienced which aided preparation for all components of the study:

"Yeah, I was a little bit nervous about it, because – well, not really nervous – but.. I was aware that when I did it before, I did find it quite challenging…It is a real commitment; it's not something where you're just filling in a questionnaire and sending it off; it did require me to be mentally prepared, and to commit myself to it. (P15, F, MAC)

6.3.2.2. Heightened cognition/cognitive burden

Participants made efforts to internalise the study protocol which seemed to be somewhat of a cognitive burden that required heightened cognition. They explained that participation required them to be focused and "*on the ball*" due to the protocol perceived as being elaborate and challenging "not just filling out a questionnaire" (P15, F, MAC). Both samples of participants experienced being more alert than usual, again with the aim of getting the study right and achieving the best outcome for the study.

"[I] think I was more conscious than usual, I wanted to get it [the study protocol] right…" (P12, F, MAC).

Prior to the study day, there was a sense of 'mental preparation' that was required which included a continuous internal dialogue about the saliva sampling protocol:

“I think just being mentally prepared and... you know... reminding yourself that that’s what you’re going to be doing when you wake up [with reference to the saliva sampling protocol]” (P15, F, MAC).

Some participants further elaborated on their internal dialogue, by explaining that prior to sleeping the night before the morning sampling, there was concern about remembering what they were being asked to do and that they were conscious about overlooking aspects of the sampling protocol:

“I thought that was... yeah, it actually was more... and I say, a concern, rather than anxiety, about forgetting or not putting things [electronic devices] right, and... sort of... ‘[I have] got to remember this’ and sort of like going to bed and thinking "I hope I remember properly when I wake up” and all that kind of thing, to [be able to] actually do the stuff [sampling]”. (P18, M, MAC)

6.3.2.3. Disruption to waking/morning routine

Throughout the study days, participants were required to include the study protocol into their daily, morning routine. Both samples of participants discussed their delayed routine during the morning. There were however, differences in the type of delay being expressed. For example, some participants of the POAG group were vocal about the delay in administering their eye drops. The researcher kindly requested participants to carry out the saliva sampling prior to their usual morning administration of POAG medication (eye drops) as it causes dry mouth. This appeared to also change the way in which the participants woke, disrupting their usual morning routine. P03 (M, POAG) explains:

“I changed my routine. I switched off the alarm clock and I didn't take my eye drops too. Then I'm taking the swabs”

With the prioritisation of the sampling protocol, the delay in taking the POAG medication appeared to also delay the commencement of the day. Following this, there were disruptions in usual sleep routines, such that participants voiced that

family members were eager to prevent interferences to the study and so alternate arrangements were made for sleeping.

"*I had to take the drops before I did the [saliva sampling] test. So that made me [begin my day] a bit later and my husband moved into another room…. we sort of came to an arrangement between us because he gets up sometimes early morning, to go to the bathroom and he said he didn't want to disturb me….*" (P05, F, POAG)

Members of the academic community further expand on the experiences of the delay in routine. Several voiced disruptions to morning routine, by explaining that the study delayed important elements of their routine by 45 minutes. P14 (F,MAC) explains:

"It did influence my routine; because, for me, I wake up in the morning and the first thing I do is brush my teeth; that's my routine… And plug in the kettle and have a cup of tea; that's the start of my day. And so that was disrupted because I could do neither for 45 minutes"

The explanations from the members of the academic community establishes the importance of routines such that it organises the day to facilitate productivity. For example, P18 (M, MAC) enters a discussion about enjoying physical activity in the form of jogging soon after waking in the morning. By taking part in the study therefore, they are clearly restricted from carrying out their usual routine jog and they comment on the delay before it can resume, they explain:

"…you couldn't do any kind of physical exercise and stuff like that; and I often sort of do go jogging first thing in the morning when I get up, and that kind of... disrupts the routine then; sort of... because nearly an hour goes by..." They also attempt to not let themselves get too engrossed in the disadvantages of routine disruption caused by the study, perhaps in an attempt to not cause offense or seem troublesome as they ended their statement by saying:

"*…but I mean, it's not a major thing; it was only a minor thing".*

Routines automate basic elements of daily life, conserving energy to dedicate towards safely achieving tasks during the day. The disruption of a routine therefore was also described as an 'intrusion'. P16 (M, MAC) explains:

"…… you could argue that having a standardised routine of waking up is safer. You know [laughs wryly] if you're going to start doing weird things, and thinking about stuff, and 'Oh no; I forgot to feed the cat, because I'm not following the routine'… A break to a previously established routine will intrude upon your standard patterns of waking up"

Both samples, go so far to explain amusingly, that everything had appeared to stop for the prevention of errors to the saliva sampling protocol. P12 (F, MAC) put everything on hold to complete the sampling protocol and this indeed disrupted their morning routine:

"45 minutes I felt, for me, my life was on hold! I was a little bit 'clock watching 'and not doing very much else, really".

Waking routines appeared to be so instilled within the self, that simply a break in one element of the routine, prevented the completion of elements that may have followed straight after. For example, P14 (F, MAC) explains:

"Funnily enough, now that I think about it, I didn't even get dressed until the 45 minutes were up!"

This was similar for participants in the POAG sample, one in particular explained:

"I stayed in bed for the first one [sample] and then got up then went downstairs and I don't think I got washed or dressed" (P03, F, POAG)

6.3.2.4. Impact on sleep

On reflection, participants from both samples, were not deterred by the impact the study had on their sleep pattern. They continued with the sampling process and attempted to adhere to the protocol for the duration of the study. Yet, there were consistencies in the accounts participants gave about the impact on sleep and the disturbances experienced by taking part in the study. There was a tendency for participants to have a shortened duration of sleep, as they were waking earlier in the morning. The apprehension about first morning sample in combination with premature waking appeared to have a direct impact on some participant's sleep. P06 (F, MAC) explains that in usual circumstances following premature waking, she would return to sleep:

"So normally my alarm would go off at about 7am; I might wake up before it, but this [time]...I was definitely waking up, and... I guess, under normal circumstances, I would wake up and go back to sleep; but I couldn't; I didn't want to risk it [missing the first morning sample]". This resulted in the participant waking 2 hours earlier on every study day.

Other members of the academic community were intrigued by the notion that they woke an hour earlier during every study day. There also appears to be some 'intrusion' experienced, such that they seemed to feel quite literally captivated by wakefulness and is described clearly as a phenomenon beyond their control - P17 (M, MAC) states:

"…it's interesting that on all 4 occasions I actually ended up waking up before the alarm sounded; so, I think that's quite an interesting observation.... I actually got up a whole hour before I had allowed myself to wake up".

For some participants in the POAG sample, early wake times were usual and normalised by participants. Following early wakening they would habitually return to sleep, however because the study required them to commence sampling as

soon as morning wake occurred, there was some confusion about when to do take the first waking sample. one participant notes the difficulty:

"I realised that I wake at 5am to go to the lavatory and then go back to bed, sometimes go back to sleep…. waking up [during the study] is when I think 'well I should be getting up [to start the saliva sampling]'…it was tricky" (P07, F, POAG).

There were differences experienced between the two samples in relation to the mechanism of waking which potentially led to early waking times. For example, members of the academic community were actively employed and so wake times were often governed by the demands of the day ahead. Morning wake time was therefore intended to be prompted by an alarm clock. However, the study had an impact on the duration of sleep as members of the academic community all appeared to wake before the alarm sounded.

P18 (M, MAC) explains *"I always woke before the alarm"* and P19 (F, MAC) states*" I found myself waking up [an hour] earlier [than the alarm]*

In contrast participants with POAG appeared to rely on a natural waking process instead of an alarm clock. The POAG group were retired and there were no consistent mentions of immediate demands for the day following wake during the study period. Despite this, there were report of sleep disruptions, with a justification of why they were waking: *"I probably woke couple of times before [the time intended to wake] but I think I was going to get up [anyway]"* (P05, F, POAG). Others with POAG reported disrupted sleep during the study period, with difficulties establishing middle night and morning waking. P03 (M, POAG) explains:

"Yes. I seem[ed] to have a disturbed sleep pattern because…I woke up in the middle of the night… but well when I was waking up in the early morning, it was still quite dark… I felt like I was going to get up….it was earlier [than my usual wake time]

6.3.2.5. Habituation after initial study day

Several comparisons between study days were made and these included differences in sleep quality, wake time and mood. These discussions suggest a clear easing of demands of the study protocol following the initial study day. Some participants explain that morning awakening was later in comparison to the first study morning. Participant P06 (F, POAG) and P14 (F, MAC) explain:
"I think one morning I woke later than I had the day before…." (P06, F, POAG)

"…. because we only had four days; and the first two of those, it [morning wake] was markedly earlier..." (P14, F, MAC)

There were also clear differences with the quality of sleep, such that the second study day was preceded by a markedly better night's sleep:

"The first night [prior to first study day] - no I didn't sleep very well but the second night was much better" (P09, F, POAG)

Alongside sleep quality and duration, there was a sentiment of an improvement in mood. Participants began to feel more positive following the initial study day which they felt subsequently eased the saliva sampling protocol- as P04 (F, POAG) explains:

"Yeah, and you know, I felt more positive in terms of [my] mood on the second day as well, so that made the sampling easier" (P04, F, POAG).

The ease of saliva sampling was a recurrent expression as an initial motivator for those who had taken part in PACS research studies before (see overarching theme 1 and subtheme 1.1). This ease of saliva sampling arises upon discussion of participants most recent experience of the PACS study. There was an understanding of the saliva sampling protocol following the initial study day which contributed towards a sense of confidence regarding the saliva protocol the following day. P10 (F, POAG) felt more organised and states:

"I suppose the second morning was easier than the first, because I knew what I was doing.... I got the things ready…" (P10, F, POAG)

6.3.3. Overarching theme 3: Identifying the moment of morning awakening

The final overarching theme captures participants 'identifying the moment of morning awakening'. The identification of being conscious following sleep has been reflected as a general behaviour that has never been thought about before but is a necessary element of the sampling protocol and PACS research P20 (F, MAC) state:

"*Well I…I wouldn't have contemplated… I wouldn't…the conversation we're having now is not a conversation I've really had with myself before; I've never thought ' how do I wake up?' "*

The development of a first subtheme highlighted both the samples' account of 'the inexplicable ambiguity between sleep and wake'. Here, participants attempted to capture the essence of moving from a state of unconscious to fully alert. Finally, both samples of participants reflected on their 'uncertainty around identifying the moment of awakening' which forms the second subthemes.

6.3.3.1. The inexplicable ambiguity between sleep and morning awakening

The question about participants description of wakefulness resulted in much thoughtful reflections. This was characterised by lengthy pauses and expressions of the general difficulty of pinpointing the precise nature of awakening *"Oh dear, you're asking very difficult questions…"* expressed P08 (F, POAG). Some participants highlighted the complexity of the question and stated: *"It's a mystery,*

*isn't it?" (*P11, F, POAG). The formation of the first subtheme 'the inexplicable nature of waking from sleep' captures the curious and careful accounts of morning wakefulness from both samples.

Morning awakening, can be described as the gaining of an explicit awareness and having a sense of consciousness. Yet conversations trended towards a more confused and uncertain account. Participants often required an internal dialogue to clarify their thoughts during their account. One particular participant ponders on the indistinguishable differences between a complete resting state, deep sleep and feeling awake by explaining:

"And you kind of wonder to yourself 'Have I actually been to sleep, or have I, you know, have I just been sort of laying here in a deep state of rest, but not quite asleep'; It just felt like that, really". (P19, F, MAC)

This hazy zone between wakefulness and sleep continues amongst participants who highlight that there was never a clear point of consciousness from sleep, particularly during the PACS study period.

*"I've never really had – on any of the 4 occasions, in this study – a clear point at which I could say I was asleep and then awake". (*P19, F, MAC)

Similarly, despite waking considerably earlier than usual, P14 (F, MAC) commented:

"I wasn't entirely clear-cut, because some mornings you... you kind of surface slowly, don't you? It's a bit 'foggy'…It's a progressive event, rather than 'asleep – awake'".

The uncertainty and confusion about that precise awakening moment persists. The process is often merged
with a state of dreaming. Here participants observe their awakening process as a gradual transition from the amalgamated sound of the radio within their dreams:

“I sort of hear the radio in the background and sometimes it merges with the dream because I dream about something similar. ‘There's something strange there…probably this is the radio’… it is similar to a transitional period. Eventually you do wake up and you realise what you were dreaming about…. then you realise your [I’m] conscious”. (P03, M, POAG)

The internal dialogue of questioning wakefulness was repeated amongst participants. Participants described ambiguity and doubt surrounding sleep/wakefulness and deciphering between shallow sleep and wakefulness. P17 (M, MAC) states:

“*I remember on one of the mornings I wasn’t sure whether I’d been awake or not; or whether I was just coming into wakefulness from a... you know... shallow sleep”*

6.3.3.2. Uncertainty around identifying the moment of awakening for the awakening sample

The most pivotal element of all PACS research studies is to collect the first morning sample immediately upon awakening. A consistent finding in both samples were the recurrent uncertainty participants expressed when asked whether their first morning sample was taken on awakening due to the difficulty in identifying the moment of awakening. There was a sense of internal dispute about wake times and subsequent first morning sampling time. For example, P03 (M, POAG) centres their wake and first morning saliva sample as an act of probability by referring to it as *“a difficult call’*, they explain:

… It was still quite dark when I felt like I was going to get up. So there was a little bit of a strange moment on whether I was going to get up or not…. it was a bit of a difficult...call...”

In line with the study impacting on sleep, participants were experiencing difficulties with staying asleep and recognising wakefulness. Participants also required confirmation of their experiences and stated that once wake had been identified there was an ease in the sampling procedure, for example P04 (F, POAG) states:

"I was awake a lot but and it was also 'you know' , when you sleep fitfully, it can be very difficult to recognize when you're actually awake…[sampling was ok] once I realised I was awake" (P04, F, POAG)

For some participants awakening was identified as a behavioural state. There were also the internal confirmations of wakefulness defined by the participants physical movement and internal thought in which wakefulness was a recognised state. It was the moment that the participant consciously recognised the movement their arm reaching out to hold the salivette. P14 (F. MAC) explains:

"I say 'Yes, I'm awake; check the time, grab a salivette; I've moved"

Others from the POAG sample also explain with a tone seeking reassurance from the interviewer that they recognise the moment of waking in the form of physical activity. P04 (F, POAG) states:

"It's the start of activity [physical movement] isn't it? The start of you know, just realising that you [I] need to get up off the bed"

There is a sense of overlap between physical movement and movement in the form of cognitive effort or consciousness. This has been depicted by internal dialogues from the participant. For example, P15 (F, MAC) questioned her waking "*I was kind of going 'Right, hang on a minute; what am I going to do; shall I go with this?"*. There was also the sense of becoming aware of one's environment "*I suppose, it's difficult"* explains P10 (F, POAG), they continue *"its consciousness, something is moving and you're becoming aware of things. I think before I open eyes, I'm aware that I'm conscious"*. Another participant who purposely does not set an alarm due to the dislike of being startled awake, corroborates by providing

a detailed examination of morning awakening. They describe the comforting familiar noises of the arrival of the dustmen on a Tuesday morning and explain:

"…*if I'm thinking about this now, when I say that the dustmen are [there]... I'm woken by the noise, it's familiar because I know they come every Tuesday. So there isn't... any surprise to the noise that they make; so it's almost as though it's... it's a... it's [waking] is a gradual thing, that's sort of seeping in; it's almost a familiarity – almost a comfort – that they're there... that I'm not being surprised, and being awoken, which is generally what an alarm will do"* (P20, M, SM).

6.4. Discussion

The aim of this study was to understand both the experiences of participants with POAG and members of the academic community, participating in a PACS research study and to explore the subjective understanding of the moment of morning awakening. A thematic analysis of the interview transcript led to the development of three overarching themes: (i) motivations for taking part (ii) experiences of taking part in a PACS research study and (iii) identifying the moment of morning awakening.

The sense of duty to research and the notion that the study was non-invasive, conducted in one's home as well as the past experiences of the ease of saliva sampling formed the motivations and reasons for study participation. It was evident that the individuals who were putting themselves forward for research participation, maintained a conscientious commitment to research. Members of the academic community were able to empathise with the researcher's effort required to recruit participants and therefore felt a duty to place themselves in the shoes of the participant. Participants with POAG also maintained a sense of duty to research and regularly participated in research trials in which they were committed to helping anyone else that would benefit. Altruistic behaviour has been previously documented to "lie at the heart of research on human subjects" (p873, de Angelis et al., 2007) and this is consistent with previous literature (Jones et al., 2015; Newington & Metcalfe, 2014).

PACS research practice encompasses a self-reporting methodology, fully reliant on the participant to firstly understand the general guidelines proposed by the researcher during the initial meeting. In accordance with Moorcraft et al., (2016) researchers aimed to promote and maintain a positive attitude with the participant from the outset, providing full information, guidelines, practice, and the opportunity to ask questions. The initial meeting (both face-to-face and over telephone), is where individuals are ensured that participation is done at their convenience and within the comfort of one's home. The 'non-invasive study conducted in one's own home and prior knowledge of ease of sampling' therefore formed the final subtheme of one's motivation to take part in this study. Individuals commented on the benefit of avoiding the commute to the university and others observed the sampling protocol being both straightforward and non-invasive. Members of the academic community who had prior experience of taking part in PACS research also were motivated by the common knowledge that saliva sampling was easy.

Whilst motivations were positive and encouraging, the second overarching theme, observing the experiences of being a participant, pointed to a number of challenges where several disruptions were felt. The subthemes encompassed 'apprehension, 'heightened cognition', 'disruption to routine', 'impact on sleep', however the final subtheme of this overarching theme addresses experiences of eventual 'habituation over the study period'.

The feelings of apprehension and worry emerged, when asking participants to provide insight of their experiences prior to and during the study. Participants felt it essential to do their very best and several reiterated their trouble with sleep and its potential negative impact on the study. Others detailed the challenges they expected to face due to prior experiences of participating on previous PACS research studies. Some simply expressed the need to be "mentally prepared". This intent to prepare, taps into the feelings of a heightened cognition/ cognitive burden, the second subtheme identified as part of the overarching theme of the experiences of taking part in PACS research.

It is without doubt, that participants were more alert during the study period. They provide indication that they were more conscious than usual, with psychological demands placed on ensuring that they 'got the study right', demonstrating an ongoing commitment to the research. The study therefore promoted rumination and the demand of a prospective memory. The mental preparation appeared to reflect the burdens of the sampling protocol and gave rise to the third subtheme; disruption to morning routine.

Upon asking participants to describe their morning routine during the study and as to whether it differed from their usual morning routine, most participants agreed that there was a form of disruption. For some, it was the simpler tasks, for example, the delay of cleaning one's teeth or drinking a cup of coffee in the morning. For others, they experienced a complete halt to the morning routine by focusing on the sampling and then continuing the day. Despite sampling occurring either once a week for four consecutive weeks or, in the case of participants with POAG, for two consecutive days, the impact in both morning routine and sleep appeared to be consistent over the duration of the study.

The experiences of actual study completion highlighted the clarity of the researcher's instructions as well as the participants' conscientious commitment to complete the protocol. The adherence to the saliva sampling protocol is clarified during the face-to-face and telephone induction. Therefore, participants were aware of the importance of timing and summarised that they wanted to be the good participant, vocalising how organised they had to be and detailed the methods taken the night before to ensure all went smoothly on the morning of saliva sample collection. However, it is apparent that such meticulous and potentially burdensome preparation led to the fourth subtheme which was an underlying 'impact on sleep'.

Many participants reported the disruption of sleep based on the experience of overall poor quality in comparison to their usual sleep pattern with the increasing incidence of early waking. There were consistencies, particularly in the accounts of participants with POAG and almost all of whom spoke about premature waking

and sleep disruptions. Through engaging with the conversations surrounding motivation, both the POAG sample and members of the academic community, were consistent in expressing an attitude of conscientious participation which is reflective of the final subtheme.

The final subtheme of the second overarching theme ‘experiences of taking part in PACS research’, highlights the benefit of conducting the study over a number of days. There is an expression of ‘habituation during the study period’ which is expressed by participant’s account of the ease of saliva sampling, improvements in sleep quality, wake time and mood.

The moment of awakening is a crucial element of PACS research. The participant is required to confidently understand the moment they are conscious following a night’s sleep and to commence saliva sampling at that timepoint. Upon asking participants to expand on this concept, two distinct subthemes were generated. There were (i) the inexplicable ambiguity between sleep and morning wakefulness and (ii) the uncertainty around identifying the moment for the awakening sample.

The experiences of the moment of awakening can be understood by observing the difficulty participants had in reflecting and cognitively evaluating the behaviour. There were many moments of contemplation upon being asked the question of defining this moment. It is very important to note therefore, that simply the contemplation and internal assessment of describing what it means to wake and gain consciousness, is a demanding task alone. However, once participants began to elaborate, the conversation along with internal dialogues - grew. Participants commented on the difficulty in identifying the actual point of wakefulness, with recurrent conversations describing the inability to confidently distinguish the state of wakefulness, particularly during the study period. Participants also attempted to describe the haziness involved between the sleep and wake state, which prompted discussions of lucid dreaming. This inexplicable ambiguity between the sleep and wake state therefore prompted the development of the final subtheme. There was a sense of uncertainty around identifying the moment of awakening for the awakening sample.

There were clear doubts about the first morning sample. Several participants shared the thought processes that occurred during that time. These included a series of internal affirmations which attempted to establish the waking process. In some instances, it was evident that there was a certain delay before the first morning saliva sample was collected. However, this may have varied depending on the disruption and quality of sleep.

The emphasis on the importance of taking the first saliva sample on the moment of awakening or “strict adherence of sampling protocol” is recommended during the initial face-to-face, telephone or video meeting (Stalder et al., 2016, p.422, 2016). There is some indication from the current study, that a participant may be subconsciously anxious about the demands of the study which may in turn affect their usual sleep pattern. Whilst PACS researchers promote their research on the basis that it is to be conducted from the comfort of one’s home and to fit in with one’s routine, they do not usually discuss the potential for sleep disruption during the study. This is perhaps crucial when promoting the study to those who are particularly vulnerable. To improve the strategy of ensuring participants are informed and prepared for all outcomes of the study, a conversation during the initial face-to-face meeting regarding sleep habits is recommended. It may even be a pre-requisite, to ascertain whether a) adherence to sampling protocol can be maintained and b) to ensure the wellbeing of participants are prioritised. Despite researchers observing that mild sleep disturbances have little effect on the CAR (Elder et al., 2014), the potential to exacerbate sleep quality may therefore potentially be minimised.

In accordance with consensus guidelines (Stalder et al., 2015, 2022), it is encouraging that participants are correctly following the instructions for accurate CAR measures. Nevertheless, it is also important to emphasise the effect this had on the participants with respect to the ‘impact on sleep’; a subtheme identified as part of the experiences of participating in PACS research.

The adherence to the saliva sampling protocol has been described to be a fundamental aspect of PACR as outlined by authors such as Smyth et al., (2013, 2015 & 2016) and (Stalder et al., 2015, 2022). It appears that the study itself may form the anticipated demand/ challenge. There is clear heightened rumination and development of a prospective memory as a result of the study protocol. It is recommended that study days be repeated for at least 2-3 days to eventually habituate the participant's responses

Like many qualitative studies, there are some limitations. Conducting face-to-face semi-structured interviews is always beneficial to ensure the researcher captures non-verbal cues on rapport. 90% of participants with POAG were interviewed over telephone and this potentially led to the loss of that non-verbal data. This may explain why the interview conversation was shorter as a more in-depth conversation may have been prohibited. To improve, future studies should limit telephone semi-structured interviews and attempt to ensure all interviews are either conducted face-to-face or via video conferencing. Since the pandemic, there is a normalisation of video conferencing resources (e.g. Skype, Zoom, Teams etc). Participants may feel comfortable with this method due to the advantages of remote access. Where video conferencing is not possible, efforts should be made for the researcher to travel to a place of convenience to conduct a face-to-face semi-structured interview with the participant.

In addition, as with all semi-structured interviews, there is a reliance on the participants to recall experiences without biases. As a doctoral researcher conducting PACS research, participants may have been polite and guarded in response to questions about experiences being posed by myself as to not offend the value of the work. On reflection, there is a difference in the interview transcripts between those conducted by NR and the participants being interviewed by SJ. In the latter case, members of the academic community took part in the PACS research study under the guidance of NS and LT, thus interviews were independent to the study in question insofar as these interviews were conducted by a researcher who was not involved with the original PACS study involving this cohort.

In conclusion the aim of this study was to understand both the experiences of volunteers participating in a PACS research study and to explore the subjective meaning of the moment of morning awakening. This study for the first time reveals the development of three overarching themes: (i) motivations for taking part (ii) experiences of taking part in a PACS research study and (iii) identifying the moment of morning awakening. This study attempts to inform and improve the methodology of future PACS research studies. From the experiences of the participants, it is evident that there is no definitive points of consciousness and wake time is very subjective. It is therefore proposed that PACS researchers consider discussing this phenomenon with each participant during every study induction. In addition, researchers should also consider prioritising the length of the study period to maximise the importance of sampling protocol adherence and to allow habituation. Finally, particularly relevant to participants who are vulnerable, researchers should attempt to fully understand the potential impact the study may have on individual sleep quality before recruitment.

Chapter 7 General Discussion

Saliva tissue sampling developed to assess post awakening cortisol secretion (PACS) has greatly informed literature within the psychoneuroendocrinological discipline. The HPA-Axis is activated in response to both internal and external triggers. However, an additional pathway has been associated with basal, non-stress activity and highlights the critical role of cortisol as a master hormone that is responsible for regulating physiological function around the 24-hour light-dark cycle. Via melanopsin containing retinal ganglion cells (mRGCs), upon awakening the hypothalamic suprachiasmatic nucleus (SCN) enhances cortisol secretion at wake, in response to light. The same projection of stimuli to the SCN also suppresses melatonin production, inducing sleep. The current work of this thesis firstly explored PACS in relation to post awakening melatonin and secondly investigated PACS in participants with POAG – where mRGCs are reported to be compromised. Moreover, methodological implications of the PACS research study become apparent during study II which informs the rationale for study III. Here, an exploratory investigation of individual experiences in participation in PACS alongside identifying morning wakefulness is conducted. Accordingly, this Chapter discusses and integrates the results of all three studies.

7.1. The retinohypothalamic tract and hormones of the SCN

The presence of intrinsically photosensitive melanopsin containing retinal ganglion cells (mRGCs) in the retinohypothalamic tract (RHT), project light information to the SCN. Here, it either activates the paraventricular nucleus (PVN) and HPA-axis to release cortisol or it takes a non- HPA-axis trajectory, sending signals to the splanchnic nerve innervating the adrenal glands, increasing glucocorticoid release. The latter route has been suggested to be involved in facilitating light induced effects on PACS (see review by Clow et al., 2010) and has particular importance

with respect to the fine tuning of circadian influences (Buijs et al., 2003b; Ulrich-Lai et al., 2006).

When photic information is sent to the SCN it also stimulates the PVN, suppressing the release of melatonin from the pineal gland. During times of darkness the SCN secretes glutamate which stimulate the PVN, synthesising melatonin from the pineal gland. No study to date has observed the patterns of post awakening melatonin concentrations from the moment of morning awakening. This has also been the first time in which an investigation has been conducted to observe the relationship between PACS and post awakening melatonin secretion (PAMS).

Past literature has established that mRGCs in primary open-angle glaucoma (POAG) sufferers are often compromised (Gao et al., 2022; Jean-Louis et al., 2008; la Morgia et al., 2011b) and this has been associated with circadian dysfunction. Numerous studies have observed plasma diurnal cortisol and melatonin in POAG sufferers, demonstrating irregularities in both circadian hormones (Østergaard Madsen et al., 2021). Subsequently, this thesis also initiates an exploration of observing PACs with particular interest in those with POAG and comparing this data to a healthy reference data group.

7.1.1. Measurement of post awakening salivary cortisol and melatonin

In study I, using a strict adherence to protocol method, saliva samples were self-collected from healthy participants at 0-, 15-, 30- and 45-minutes post awakening. Saliva collection required a passive drool method and participants successfully followed the guidelines on waking and taking their first morning sample (with 90% adherence).

Morning lighting is a fundamental undisputed factor which can influence the waking process (Benarroch, 2011a; Leproult et al., 2001). With sufficient intensity, light projects through the retina activating the SCN and in turn inhibiting melatonin secretion. This study for the first time attempts to understand whether the influence of ambient morning lighting at awakening determines a decline in melatonin and

whether this can be related to the PACS. Analyses revealed a usual peak in cortisol following awakening, however, no statistically significant decline in post awakening salivary melatonin was observed, refuting the initial hypotheses suggesting that melatonin concentrations would significantly fall after awakening.

In healthy participants, concentrations between PACS and PAMS were not associated. This may be attributed to the difference in pathways responsible for each hormone. For example, the secondary, non-HPA-axis pathway detailed above, has been documented to be accountable for the influence of morning light on the CAR (Clow et al., 2010). Innervation of the splanchnic nerve to the adrenals therefore acts independently to the decline in melatonin. It would appear therefore, a significant decline in melatonin cannot be captured within the usual CAR monitoring times of 45-minutes following awakening.

The decrease in melatonin concentrations occurs after approximately 4am during the second half of sleep. It is from this time that there are noticeable changes occurring in melatonin secretions. Increasing environmental lighting due to the Earth's 24-hour light/dark cycle, from 4am to wake time, would justify an inhibition of melatonin concentrations. However, when controlling for ambient lighting, there were no relationships identified with either PAMS nor PACS. This study is the first to document melatonin secretions at the point of wake and 45 minutes after. Furthermore, it is concluded that underlying changes in melatonin secretions are unlikely to be associated with effects linked to the CAR. Power analyses however revealed a large sample size would be necessary to capture any potential associations between PACS and PAMS.

7.2.1 Measurement of PACS in participants with POAG

Due to the build-up in fluid and intraocular pressure, retinal ganglion cells (RGCs) including – non-image forming mRGCs present in the retina and the RHT has been noted to be damaged in those with POAG (Gao et al., 2022; Jean-Louis et al., 2008; la Morgia et al., 2011b). These specialised cells known for projecting light stimuli to the SCN, are assumed to prevent the pathways from the HPA-axis from

promoting the release of cortisol. Yet, high intraocular pressure has been associated with elevated cortisol levels control (Kasimov & Aghaeva, 2017; Østergaard Madsen et al., 2021; Schwartz et al., 1987). Study II, therefore attempted to dispel some of the contradictions in current literature. In addition, the non-associative relationship between PACS and PAMS justified the halt in a costly investigation in melatonin in those with POAG and the focus of the investigation remained with PACS in individuals with POAG.

Continuing the method of using a strict adherence to protocol, saliva samples were self-collected from POAG participants at 0-, 15-, 30- and 45-minutes post awakening. Participants were required to collect saliva samples using the traditional, gold standard method of chewing on a cotton swab as opposed to the passive drool method used in Study I.

Individuals with POAG displayed the usual peak in cortisol following awakening however they appeared to have an elevated CAR in comparison to the RD group. Furthermore, self-reported visual function as measured by the national eye institute visual function questionnaire (NEI-VFQ) was negatively associated with the CAR. This study for the first time, demonstrated that poorer self-reported visual function was related to an elevated CAR in individuals with POAG.

Implications from Study II are twofold. Firstly, it has been established that a higher CAR is associated with those with POAG, refuting the mRGC-RHT-SCN-splanchnic-adrenal innervation hypothesis. Thus, despite the potential impact to mRGCs due to increased intraocular pressure, the CAR is preserved in those with POAG. This suggests the HPA-axis activated response, triggered by psychological stress, pain, immune system activation, awakening and several other physical functions may override the secondary non-HPA axis dependent mechanism.

Secondly, this theory offers support to a recent study published in the International Review of Neurobiology. Smyth, Skender, et al., (2019) identified that the CAR levels in those with Parkinson's Disease, another type of neurodegenerative disease, were in accordance with levels of healthy controls. Consequently, an

alternative hypothesis is at work. The boost hypothesis suggested by Adam et al., (2006) who provide a functional interpretation of the CAR as an adaptive response designed to provide individuals with the "boost" needed to meet anticipated demands for the upcoming day.

When observing state covariates in relation to PACS, adherent participants with POAG reporting a high level of demand during the study days were likely to have greater levels of total post awakening cortisol concentrations. An additional finding supporting this notion was that the POAG group struggled with adhering to the sampling protocol with 54% delaying the protocol compared to only 5% of the RD group. Despite achieving patterns of PACS in POAG sufferers, a clear demand was in place. Thus, in conclusion the combination of the demands of the disease and the demands placed on the participant taking part in PACS research study elevates the CAR in those with POAG, a neurodegenerative disease. These deductions prompted the research questions for the third and final study of the thesis.

7.2. Experiences of participation in PACS research studies

Following on from Study II, it was evident that participants with POAG struggled with adhering to the saliva sampling protocol. Whilst PACS were measurable, this finding prompted the research questions proposed for the final study of this thesis, with two fundamental issues arising. Firstly, with successful measures of the CAR taking place in a variety of sample groups, little is known about the true motivations and overall experiences of being a participant in PACS research.

Semi-structured interviews were conducted following participation of healthy individuals (academic staff members at the UOW) in a study conducted by Smyth et al., (2021). Interviews were replicated with participants with POAG from study II. Both sets of data were merged and thematically analysed using the five interrelated phases including familiarisation, generating initial codes, searching for themes, reviewing themes, defining themes and interpretation suggested by Braun & Clarke, (2014, 2021).

Three overarching themes and nine subthemes were generated during the final analyses. Overarching themes included (i) motivations for taking part in a PACS research study (ii) experiences of taking part in a PACS research study and (iii) identifying the moment of morning awakening (discussed further in Section 7.2 below).

The motivations for taking part in PACS studies included a ‘sense of duty to research’. To continue the research study to completion, it is important that one demonstrates conscientious commitment. This may have been influenced by the next subtheme which is usually highlighted to participants during the recruitment stage. PACS researchers promote the methodological convenience of the study, explaining that it is participant-led and conducted from the comfort of one’s home. Consequently, participants agreed that this ‘’non-invasive study conducted in one’s own home’ as well as the past knowledge of the ‘ease of saliva sampling’ promoted their motivation for taking part in PACS research which supported previous literature surrounding the notion of convenience by Newington & Metcalfe, (2014) A convenient study methodology would also promote commitment to the research and subsequent motivation to participate.

The second overarching theme were the ‘experiences’ of taking part in PACS research which were notably a little more adverse. Subthemes included ‘apprehension, a ‘heightened cognition/cognitive burden’, ‘disruption to morning/wake routine’, ‘impact on sleep’ and ‘habituation after initial study day’. Participants felt apprehensive during the study because of regular sleep disturbances, and others detailed the need to feel mentally prepared for the days ahead. This finding is quite relevant to the concluding findings in Study II. Participants with POAG struggled to adhere to the sampling protocol, emphasising the demands placed on the participant during the study period. Moreover, participants of PACS research explain heightened cognition and cognitive burden, which formed the second subtheme of this overarching theme. An increased consciousness ensured participants carefully followed all guidelines and

processes of the study. Participants also discussed a disruption to routine and an impact on sleep which formed the third and fourth subtheme.

The impact on sleep is an important finding, with many participants reporting the disruption of sleep during the study. Interestingly, all participants with POAG reported an initial problem with sleep and emphasised the impact the study had on their sleep pattern. Several spoke about early morning and middle night waking with issues returning to sleep. PACS research relies on the participant to accurately wake and stay awake to commence the saliva sampling process. These findings suggest that the POAG group may have had prior issues with sleep before commencing the study[1] and a worthy question remains as to whether this can be controlled at the recruitment stage. Encouragingly, there was a final subtheme which encompassed a sense of habituation after the initial study day. Here, participants discussed the continued ease of saliva sampling, sleep quality, wake time and mood following the first study day.

To achieve a successful first morning sample, a crucial element of PACS research is the reliance on participants to fundamentally understand their point of morning wakefulness. Therefore, with no research published to date, this study finally aimed to provide a qualitative exploration of identifying the moment of morning awakening.

The final questions of the semi-structured interview asked “What do you understand by the moment of waking up?” Two distinct subthemes were generated. There was a sense of difficulty to coherently communicate one’s understanding of their own personal waking process. It led to the development of the first subtheme which was the ‘inexplicable ambiguity between sleep and wake’. It was often mentioned this was the first time they had considered such a question. Several participants were asked to recall the wake process for the current day to

[1] On reflection, during telephone recruitment of individuals with POAG, many mentioned problems with sleep, however it was a fleeting comment as participants usually explained that sleep disturbances were not regular.

prompt conversation. Participants began to express the difficulty in identifying the actual point of wakefulness, with recurrent conversations describing the inability to confidently distinguish the state of wakefulness, particularly during the study period. Participants continued to explain their transient sleep inertia as a period of fogginess and uncertainty. This inexplicable ambiguity between the sleep and wake state therefore prompted the development of the final subtheme.

The findings of studies I and II extends knowledge to current literature exploring the RHT-mRGC-SCN pathway (Buijis et al., 2003; Clow et al., 2010; Scheer & Bujis, 1999; Thorn et al., 2004; Ulrich-Lai et al., 2006). Study I is the first to document melatonin secretions at the point of wake and 45 minutes after and non-association between PACS and PAMS. Further exploring the RHT pathway, Study II is also the first to document PACS – specifically the CAR in participants with POAG extending support to the typical CARS demonstrated in other neurodegenerative diseases (Hoile et al., 2019; L. Shi et al., 2018; Smyth, Skender, et al., 2019). An elevated CAR in those with POAG compared to a healthy reference data group is suggestive of the primary HPA-axis route in PACS activity. Findings from Study II is also supportive of the 'Boost Hypothesis' described by Adam et al., (2006). Additional research questions arise from Study II, specifically around the methodology of PACS research. The delays experienced by participants with POAG in the saliva sampling protocol is an example of the demands placed on these individuals during the study is further addressed in Study III. The final study generates a series of overarching themes that include: (i) motivations for taking part (ii) experiences of taking part in a PACS research study and (iii) identifying the moment of morning awakening. This study attempts to inform and improve the methodology of future PACS research studies. Table 7.1 provides a summary of the strengths and novelty of the programme of research.

Table 7.1 A summary of the strengths and novelty of the programme of research

Measurement of post awakening cortisol and melatonin patterns	- The first study to measure post awakening melatonin in saliva samples alongside PACS - Using strict adherence to protocol, PACS and PAMS were measured in saliva samples at 0-, 15-, 30- and 45-min post-awakening providing optimal assessment of PACS (and the CAR) on each study day - Controlled for majority of trait and state covariates known to influence the CAR - First study to show non-associative patterns of PACS and PAMS
Measurement of PACS in individuals with Glaucoma	The first study to explore patterns of PACS in individuals with POAG. - The first study to compare PACS in those with POAG and a healthy reference data group - Using strict adherence to protocol, PACS were measured in saliva samples at 0-, 15-, 30- and 45-min post-awakening providing optimal assessment of PACS (and the CAR) on each study day
Expands research on PACS and neurogenerative diseases	- First study to show usual post awakening cortisol peak in POAG group - First study to show an elevated CAR in POAG group compared to healthy reference data group. - The first study to show association between visual function and the CAR in the Glaucoma group. - Adherence data highlights demand placed on Glaucoma group participating in PACS research
Qualitative Methodology	- The first study to explore the experiences of taking part in PACS research and understanding of the crucial element of PACS research – the moment of morning awakening - The first study to generate overarching themes and subthemes in relation to PACS research participation

7.3. Limitations

7.3.1. Study I

Salivary Melatonin Enzyme Linked Immuno-Sorbent Assay (ELISA) developed by Salimetrics LLC (USA) were an important resource for investigating post awakening melatonin in relation to post awakening cortisol secretion. However, assays were expensive and time-consuming. The incubation time of 3 hours (in comparison to 1-hour with cortisol ELISAs) limited the number of participants in the study. Additionally, post-hoc power analysis suggested that the sample size required for observation of the expected relationship between PACS and PAMS was large. This may have limited the generalisability of the findings. Moreover, several covariates including individual posture and eye colour should have also been controlled. Finally, future research should account for other covariates such as socioeconomic status and Body Mass Index (BMI) known to have differing effects on PACS

7.3.2. Study II

Comparing the POAG group and the RD group was challenging due to the presence of several covariates that were not controlled for. Matching the control group with gender and age would strengthen the study. Additionally, like study I, repetition of this study would consider all covariates, including socioeconomic status and BMI increasing generalisability of all findings,

7.3.3. Study III

Telephone interviews were a convenient and cost-effective way to collect data from participants. However, this method potentially led to the loss of non-verbal data, such as facial expressions and body language. This in turn, could limit the accuracy of the findings. Additionally, there may be potential researcher biases during telephone interviews, as the author of this study NR directly recruited for the PACS study and participants may have felt they couldn’t be fully open with

the researcher. To mitigate these limitations, additional measures such as video conferencing or in-person interviews should be considered.

7.4. Future directions

Using a mixed methodology, the focus of this thesis has been to accurately measure salivary hormones of circadian function in relation to the light-dark cycle. In consideration of the saliva sampling protocol, this thesis also explores the experiences of participants that have taken part in PACS, giving particular attention to defining the moment of morning awakening. Underlying changes in post awakening melatonin secretions are unlikely to be associated with effects linked to the CAR, therefore future research on morning/awakening patterns of cortisol do not need to observe salivary melatonin. However, it is possible that melatonin supplementation may have a differing effect on PACS, although evidence is very inconsistent (Lopresti, Smith & Drummond., 2021). The causal pathways between salivary hormones and the light-dark cycle cannot be determined from day-to-day or cross-sectional studies. It is therefore crucial to understand the pathways mediating these associations. The investigation of the RHT-mRGC-SCN pathway therefore, merits further exploration.

To fully quantify the amount of light potentially being transduced to the HPA-axis, stringent measures of retinal sensitivity should be utilised in future work in both a POAG group and healthy controls. Using the gold standard Humphreys Visual Field Test provides a non-invasive technique which can determine the threshold of one's retinal sensitivity which is expressed in numeric form as decibel of light attenuation. Incorporating this measure in future PACS research would therefore accurately ascertain the function of mRGCs in morning cortisol production.

The 24-hour light-dark cycle regulates cortisol which is known to impact on mental health. In this thesis, low levels of self-reported visual function, a measure of quality of life; has been associated with a greater CAR. Through the use of a randomised trial future research could attempt to test the hypothesis, that dawn simulation will improve mental health and subsequent quality of life in individuals with POAG.

Finally, Study III gives rise to several methodological recommendations. Commencing with participant recruitment stage, it is important to understand the sleeping habits of individuals prior to research participation. Study II and III highlight the demands of the study placed on participants who are particularly vulnerable. Therefore, researchers should account for sleep patterns, controlling for the potential for individuals to frequently experience sleep disturbances.

There is clear heightened rumination and development of a prospective memory as a result of the PACS research studies. A recommendation of a minimum repeated study days (3 days), therefore, are necessary to habituate to these initial participant responses. The findings in this thesis also suggests an improvement of the definition surrounding the moment of morning wakefulness outlined in the consensus paper by Stalder et al., (2015). The recommended definition is:

"Morning awakening is subjective, in which a temporary moment of uncertainty occurs. Though you may feel sleepy, when fully awake things are familiar, and full consciousness is evident"

It is during the initial induction stages that PACS researchers should promote open discussions with each participant regarding their personal definition of morning awakening. Reiterating the morning awakening process in conversation reinforces this biological process that is often taken for granted. It also allows participants insights into the process of PACS research and the importance of adhering to the saliva sampling protocol. Furthermore, researchers should attempt to fully understand the potential impact the study may have on individual sleep quality before recruitment of participants with greater emphasis and care with those who may be particularly vulnerable.

Finally, inclusive of healthy participants, to further aid and support participants who are vulnerable with illness, it is recommended that PACS research studies consider the development of a smartphone application to ease participation. The use of smartphones has recently been applied to capture timed-stamped photographs as

an objective method of awakening verification (Zhu et al., 2019). Used as a supplementary resource where possible, a smartphone application may promote accessibility to all, the application would enhance participation by removing some of the burden of PACS study methodology. This would encompass the elimination of hardcopies of questionnaires, guidelines and saliva sampling records. The mobile phone application could be used to practice timings of saliva sampling, through the use of in-application alerts. Researchers could also pre-set, agreed timings for morning awakening, these functions may aid in omitting some of the apprehensions observed in this thesis prior to study participation.

Overall, this innovative piece of work offers promise to the understanding of the connections between the 24-hour light dark-cycle and its effect on salivary circadian hormones. It also establishes the requirement to recognise methodological implications when recruiting vulnerable individuals with a neurodegenerative disease such as POAG and inform best practice methodology for PACS research.

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9 Appendices

Appendix 1

UNIVERSITY OF
FORWARD
THINKING
WESTMINSTER

Natasha Madhewoo
Psychology - SSHL
154A West Hendon Broadway
London
NW9 7AA

23 May 2013

Dear Natasha,

Application Number: 12/13/30A_Psych
Natasha Madhewoo: School of Social Sciences, Humanities & Languages
Mode: SSHL MPhil/PhD Research

Project title: The Cortisol Awakening Response (CAR): Hormones of the Sleep-Wake Cycle

I am writing to inform you that your application was considered by the SSHL Research Ethics Committee (REC) by correspondence in May 13. The proposal was **approved**.

If your protocol changes significantly in the meantime, please contact me immediately, in case of further ethical requirements.

Yours sincerely

Debs Harris
School Administrator, School of Social Sciences, Humanities and Languages

cc Louise Sylvester, (Chair) SSHL Research Ethics Committee
Dr Jeremy Colwill, Deanof SSHL
Mike Fisher, Research Degrees Manager

SSHL REC Secretary; School Office, Room 314, 309 Regent Street, London, W1B 2UW

UNIVERSITY OF
FORWARD
THINKING
WESTMINSTER

Natasha Madhewoo
154A West Hendon Broadway
London
NW9 7AA

18 October 2013

Dear Natasha,

Application Number: 13/14/04_PSY
Natasha Madhewoo: Department of Psychology, Faculty of Science and Technology
Mode: Psychology PhD Research

Project title: The Cortisol Awakening Response (CAR): Vision and the Sleep-Wake Cycle

I am writing to inform you that your application was considered by the Psychology Research Ethics Committee by correspondence in October 13. The proposal was **approved**.

If your protocol changes significantly in the meantime, please contact me immediately, in case of further ethical requirements.

Yours sincerely

Debs Harris
Psychology Administrator

cc Laura Boubert, Chair of Psychology Research Ethics Committee
John Colwell, Chair of Faculty Research Ethics Committee

I am advised by the Committee to remind you of the following points:

1. Your responsibility to notify the Psychology Research Ethics Committee immediately of any information received by you, or of which you become aware, which would cast doubt upon, or alter, any information contained in the original application, or a later amendment, submitted to the Psychology Research Ethics Committee and/or which would raise questions about the safety and/or continued conduct of the research.
2. The need to comply with the Data Protection Act 1998
3. The need to comply, throughout the conduct of the study, with good research practice standards
4. The need to refer proposed amendments to the protocol to the Psychology Research Ethics Committee for further review and to obtain Psychology Research Ethics Committee approval thereto prior to implementation (except only in cases of emergency when the welfare of the subject is paramount).
5. The requirement to furnish the Psychology Research Ethics Committee with details of the conclusion and outcome of the project and to inform the Psychology Research Ethics Committee should the research be discontinued. The Committee would prefer a concise summary of the conclusion and outcome of the project, which would fit no more than one side of A4 paper, please.
6. The desirability of including full details of the consent form in an appendix to your research, and of addressing specifically ethical issues in your methodological discussion.

UNIVERSITY OF
FORWARD
THINKING
WESTMINSTER

Project title: The Sleep and Wake Transition: A mixed methods approach

Application ID: ETH1819-0331

Date: 08 Nov 2018

Dear Natasha

I am writing to inform you that your significant amendments to protocol was considered by the Psychology Ethics Committee.

The proposal was approved.

Yours,

Coral Dando

Psychology Ethics Committee

I am advised by the Committee to remind you of the following points:

Your responsibility to notify the Research Ethics Committee immediately of any information received by you, or of which you become aware, which would cast doubt upon, or alter, any information contained in the original application, or a later amendment, submitted to the Research Ethics Committee and/or which would raise questions about the safety and/or continued conduct of the research.

The need to comply with the Data Protection Act 2018 and General Data Protection Regulation (GDPR) 2018.

The need to comply, throughout the conduct of the study, with good research practice standards.

The need to refer proposed amendments to the protocol to the Research Ethics Committee for further review and to obtain Research Ethics Committee approval thereto prior to implementation (except only in cases of emergency when the welfare of the subject is paramount).

The desirability of including full details of the consent form in an appendix to your research, and of addressing specifically ethical issues in your methodological discussion.

The requirement to furnish the Research Ethics Committee with details of the conclusion and outcome of the project, and to inform the Research Ethics Committee should the research be discontinued. The Committee would prefer a concise summary of the conclusion and outcome of the project, which would fit no more than one side of A4 paper, please.

Appendix 2

Information sheet and consent form for Study I

The Hormones of the Sleep-Wake Cycle: Participant information sheet.

Study Overview

You are invited to take part in 'The hormones of the sleep-wake cycle' study being conducted by the Psychophysiology and Stress Research Group at the University of Westminster. This sheet contains important information about the study being supervised by Professor Angela Clow.

When awaking from sleep in the morning, our body releases cortisol which has a marked 24-hour rhythm. There is evidence from a number of studies that this process is associated with mood, general well being and light. On the contrary, melatonin is released in the evening, initiating sleep. Levels of melatonin lower towards the end of the sleep cycle.

The aim of the research is to investigate possible interactions between awakening cortisol, melatonin and other personal variables such as wellbeing, feelings of fatigue and stress.

The study will involve:

1. Completing four questionnaires, taking approximately 15 minutes.
2. On two consecutive, typical weekdays you will also be asked to complete an evening diary prior to sleep and a morning diary on awakening.
3. Wearing a special watch measuring your activity during sleep.
4. Collecting saliva samples on the 2 study evenings before sleep and mornings for 45 minutes after awakening.

If you agree to participate you will be given an equipment pack to take away with you. This includes all of the necessary equipment for saliva sampling and sleep/wake assessment. You will be expected to return this equipment pack upon completion.

Consent Form

Please read the information given below and sign the consent form. This form will be stored separately from any data you provide so that your responses remain anonymous

Please note:

- Participation is entirely voluntary and will not affect your treatment in anyway.
- You have the right to withdraw at any time without giving reason.
- Your responses will be confidential. No individuals will be identifiable from any collated data, written report of the research, or any publications arising from it.
- You have the right to ask for your data to be withdrawn as long as this is practical, and for personal information including saliva samples to be destroyed.
- You do not have to answer particular questions on questionnaires or in interviews if you do not wish to.
- All personal data will be kept in a locked cupboard on University premises. Saliva samples will be stored in a freezer within a locked laboratory on University premises.
- Please notify us if any adverse symptoms arise during or after the research.
- If you wish you can receive information on the results of the research.

The researcher can be contacted at any time during the study by email or by telephone.

Email: Natasha.ramachandran@my.westminster.ac.uk Tel: 02079115000 Ext. 69029

Please print name _____

Signature_____ Date _____

Researcher

Natasha Ramachandran

Signature_____ Date_____

Appendix 3

Information sheet and consent form for Study II (Phase I and II)

UNIVERSITY OF WESTMINSTER

Participant information sheet:
Vision and the sleep-wake transition

important to the accurate measurement of the hormone. So, we can monitor the times at which you sampled relative to awakening we will require you to wear an actiwatch to bed - this will provide an objective time of your awakening. The cotton swabs used for saliva collection will be stored in a track cap, each opening of the cap will provide a date and time - these times will be used as a proxy of your sampling time.
Diaries. You will also be asked to complete a brief diary in the evening before bed (prior to each study day), and in the morning on all of the study days. This will be completed at home (approx. 2 min to complete) and assesses mood and anticipations of the day ahead and your sleep that night. You will also report you awakening and sampling times.

Phase 2: Following completion of first phase, in which participants collect saliva samplings in their domestic setting on 2 consecutive days, participants will be asked to take part in a 30-minute semi-structured interview. Interviews will be audio recorded.

Who can take part in the study? Male or female healthy adults aged >40 years, not suffering from any serious medical other than primary open angle glaucoma, or psychiatric illness.

What if I change my mind during the study? You have the right to withdraw from the study at any time, including withdrawing your data without explanation.

What happens to the information? Your data and personal details will be treated confidentially, and will only be seen by the researcher (Natasha Ramachandran) and her research team. This information will not be shared with anyone else. Should the results of this research be published, your data will be anonymized prior to being added to a larger data set, and you will be completely unidentifiable within that data set. All information you provide will be stored in such a manner so that no specific details will be linked to individuals. Your saliva will only be tested for the hormone cortisol. Although we will not be able to give feedback on individual data, we will provide all participants with a summary of the overall findings.

Please do not hesitate to contact one of the members of the team if you have any questions or problems: Natasha Ramachandran: Natasha.ramachandran@my.westminster.ac.uk or Dr. Nina Smyth: n.smyth@westminster.ac.uk

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Informed Consent Form:
Vision and the sleep-wake transition: Phase 1

		Please circle
1.	Have you read the information sheet about this study?	YES / NO
2.	Have you had the opportunity to ask questions, and received satisfactory answers to your questions?	YES / NO
3.	Have you received enough information about the study?	YES / NO
5.	Do you agree to your saliva being tested for the hormone cortisol only?	YES / NO
6.	Do you understand that you are free to withdraw from the study at any time, without giving a reason for withdrawal?	YES / NO
7.	Do you understand that you do not have to answer all questions asked, and can decline to answer questions as you see fit.	YES / NO
8.	Do you understand that you will be anonymised, and all identifying features will be removed so that your contribution will not be identifiable when reporting this research	YES / NO
9.	Your data will be securely stored, and destroyed in accordance with the Data Protection Act, 1998.	YES / NO
10.	Do you understand that your identity, contact details and the information that you provide will be treated confidentially and in accordance with the University of Westminster ethical guidelines and British Psychological Society code of human research ethics.	YES / NO
11.	Do you understand that the duty of confidentiality is **not absolute** and in exceptional circumstances this may be overridden by more compelling duties such as to protect individuals from harm	YES / NO
12.	Do you understand that the data from this study may be used for future research, and may undergo secondary analysis. Future research may be related or	YES / NO

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Informed Consent Form:
Vision and the sleep-wake transition: Phase 2

		Please circle
1.	Have you read the information sheet about this study?	YES / NO
2.	Have you had the opportunity to ask questions, and received satisfactory answers to your questions?	YES / NO
3.	Have you received enough information about the study?	YES / NO
4.	Do you understand that you are free to withdraw from the study at any time, without giving a reason for withdrawal?	YES / NO
5.	Do you understand that you do not have to answer all questions asked, and can decline to answer questions as you see fit.	YES / NO
6.	Do you understand that your interview will be digitally audio/video recorded for later transcription and analysis.	YES / NO
7.	Do you understand that you will be anonymised, and all identifying features will be removed so that your contribution will not be identifiable when reporting this research	YES / NO
8.	Your data will be securely stored, and destroyed in accordance with the Data Protection Act, 1998.	YES / NO
9.	Do you understand that your identity, contact details and the information that you provide will be treated confidentially and in accordance with the University of Westminster ethical guidelines and British Psychological Society code of human research ethics.	YES / NO
10.	Do you understand that the duty of confidentiality is **not absolute** and in exceptional circumstances this may be overridden by more compelling duties such as to protect individuals from harm	YES / NO
11.	Do you understand that the data from this study may be used for future research, and may undergo secondary analysis. Future research may be related or unrelated to the goals of this study	YES / NO
12.	Do you agree with the publication of the results of this study in appropriate outlets?	YES / NO
13.	Do you agree to take part in the study?	YES / NO
14.	Do you agree to be contacted about future research?	YES / NO

The Psychophysiology and Stress Research Group
University of Westminster
https://www.westminster.ac.uk/psychophysiology-and-stress-research

Appendix 4

Research participation scheme approval at the University of Westminster

- Please complete and submit to Alison Eardley via Email at: a.eardley@westminster.ac.uk
- Please EMAIL the completed form (with scanned signatures) as a word document.
- The RPS scheme is now run via the online system: http:westminster-RPS.sona-systems.com
- You will need to sign your study up on this system. We are NOT using the paper scheme.
- Students have to do 3 credits (which is equivalent to three hours participation time). So, if your study is 15 minutes, then you will award 0.25 credits.
- **Please DO NOT be overly generous in your hours allocation. The time allocated should honestly reflect the duration of participation.**
- You need to allocate timeslots so that students can sign up. If you are using an online questionnaire you can define a time period during which time students can sign up to the study.
- Once your study has been added to this system correctly, request approval, and I will check the application.
- Once the study has been approved online, I will allocate an RPS code, which I will add into your study. I will also email back the completed RPS form (with my signature and the RPS code).
- You are responsible for assigning credits to students, once they have completed the study. Please do so promptly. If a student fails to attend, register that on the system and they will be penalised by having to do an additional 0.5 credits (30 minutes participation time).
- Because research participation is a requirement to pass 1PSY408, you must keep your own record of the names and ID numbers of your participants, along with the amount of time contributed (rounded up to nearest 15 mins.). These should be kept separately from the data for the study.

Title of Research:

Hormones of the Sleep Wake Cycle

This should be ethically neutral, and descriptive, but should not be so specific that it reveals details you do not wish students to be aware of (e.g., your research hypothesis). Your research will be referred to by this title in communications with students (e.g., adverts, lists of current studies).

Brief description of research and procedures to be used:

The aim of the research is to investigate possible interactions between awakening cortisol, melatonin and other personal variables such as wellbeing, feelings of fatigue and stress.

The study will involve:

1. Completing four questionnaires, taking approximately 15 minutes.
2. On two consecutive, typical weekdays participants will also be asked to complete an evening diary prior to sleep and a morning diary on awakening.
3. Wearing a special watch measuring activity during sleep.
4. Collecting saliva samples on the 2 study evenings before sleep and mornings for 45 minutes after awakening.

Approximate number of participant hours requested:

_2__ session(s) per participant of __1__hours per session = __2__total hours per participant,

x ___30_____ participants = __60_____ participant hours

Example: 2 sessions per participant of .25 hours each = .5 hours per participant,

x 50 participants = 25 participant hours (round up session duration to nearest .25 hour)

When do you intend to start data collection (approx):

_October 2012

Researchers on this study who will be collecting data: *(only those listed are authorised to sign student participation records)*

NAME POSITION Uni Username SIGNATURE

Natasha Madhewoo PhD student w12039175

Briefly describe *how* you intend to recruit participants:

In groups of six (or less), participants will be provided with full information, instructions and demonstration of saliva collection for the study. Participants will be given the opportunity to either consent or refuse participation during the allocated timeslot.

Signature of staff member responsible for the study:

I confirm that this study is staff or postgraduate research that conforms to the BPS Ethical Guidelines for Research with Human Participants. I confirm that University/Departmental ethical approval has been granted. I undertake that researchers collecting the data will debrief participants adequately on the purpose and methods of the study, in accordance with the educational purpose of the Participation Scheme, and that a record will be kept (separately from the data) of participants names, ID numbers, and the time credit awarded to each student. I take responsibility for crediting students' participation by the published deadline in the event of the unavailability of the researchers named above.

NAME: Natasha Madhewoo University username: w12039175

SIGNATURE  DATE 24th Sep 2013

APPROVED STUDY CODE DATE

Appendix 5

Call for participants study II

VISION AND THE SLEEP-WAKE TRANSITION STUDY

The Psychophysiology and Stress Research Group specialise in understanding the burst of cortisol following morning awakening. This is known as the Cortisol Awakening Response and is crucial for daily functioning and healthy living.

We are recruiting for an exciting research study…
We need healthy adults, diagnosed with primary open angle glaucoma, aged 40-90 years to collect saliva samples at home to measure your cortisol awakening response and to complete some questionnaires about yourself, your experiences and your visual function.

Eligibility to participate: Not taking psychoactive or steroid medication or suffering from any illness (excluding primary angle glaucoma), not pregnant or breast feeding. Get in touch if you're unsure about your eligibility to participate.

If you are interested in taking part in this research or have any questions, please contact:

Natasha Ramachandran
Tel: 07903012648
Email:
Natasha.ramachandran@my.westminster.ac.uk

Project Supervisor: Dr. Nina Smyth
Tel: 0207 911 5000 ext. 64425
Email: n.smyth@westminster.ac.uk

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Appendix 6

Study Guidelines Study I

Hormones of the Sleep-Wake Cycle

STUDY SAMPLING GUIDELINES

Project ID_____ Date___/___/___

If you have any questions,
Please contact Natasha Madhewoo on 0207 911 50000 Ext. 69029
07903012648 Natasha.madhewoo@my.westminster.ac.uk

1. Study Overview:

1.1. What is involved?

During the study you will be asked to collect ten saliva samples over two consecutive weekdays in total. The first sample should be collected on the evening prior to the study, before sleep. This will be followed by four samples on each morning. The time of collection will be based on the time you wake up.

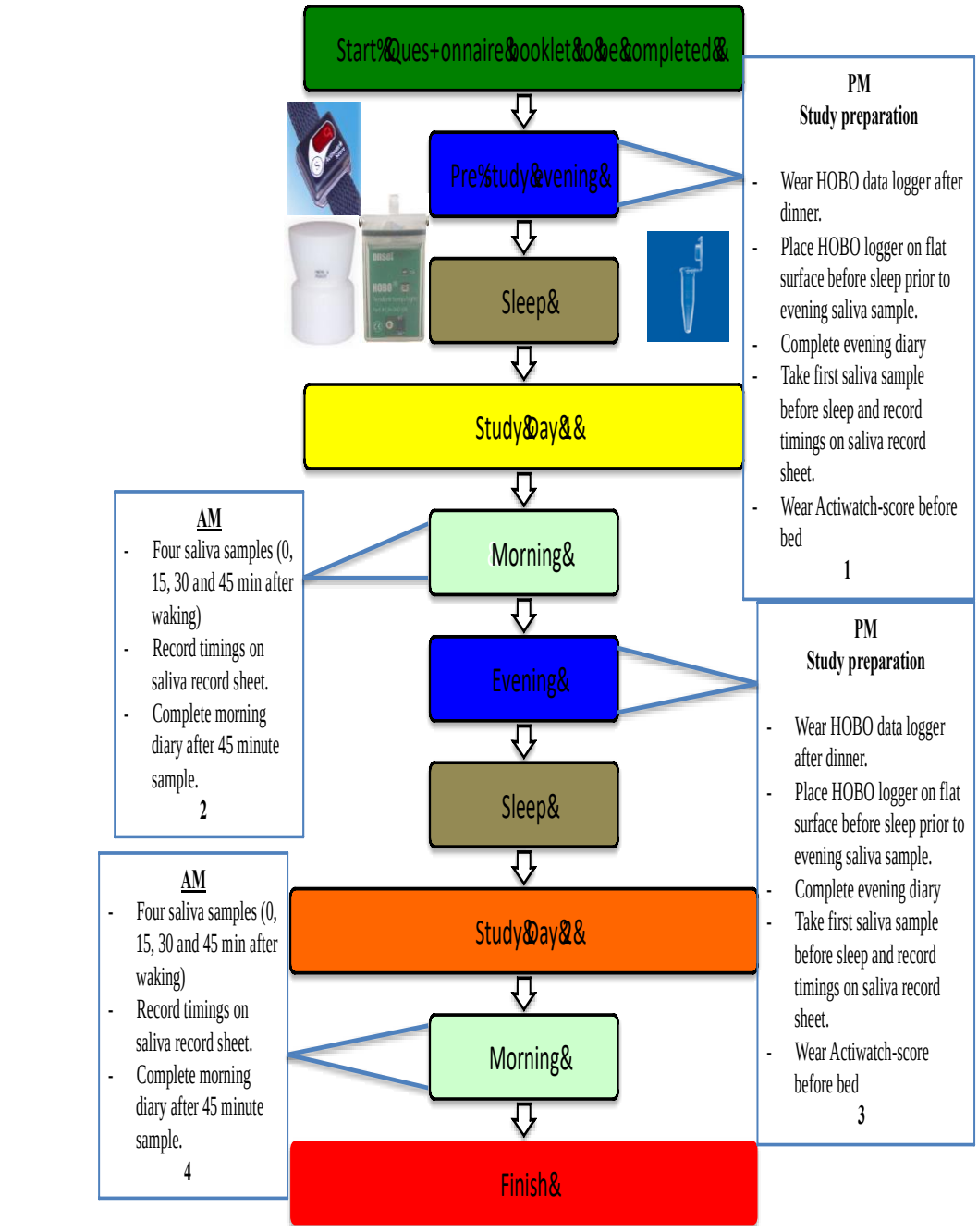
You will be asked to complete four questionnaires, which can be completed at any point over the two days.

On the nights prior to morning saliva collection, you will be asked to complete an evening diary. You will also be asked to complete a morning diary following the fourth saliva sample on each study day. An SMS message will be sent to your mobile phone, reminding you to prepare for the study and diary completion.

You will be provided with a testing pack containing the following:

1. An Actiwatch – This should be worn on your non-dominant arm before sleep.
2. A light detector, which is to be worn during saliva sampling.
3. Medication Event Monitoring bottle caps (MEMsCap) containing 10 straws.
4. Two cortisol and melatonin packs labelled for each study. These packs will contain eppendorf tubes.

2. Study Protocol



2.1 Saliva sampling instructions:

i. *Saliva Sampling Instructions:*

1. Do not eat or drink, smoke, brush your teeth or exercise for 30 minutes before you collect each of the saliva samples. You can drink water only.

2. Open the MEMSCap bottle (see figure 1) and take one straw. **Please close the MEMsCap as soon as the straw has been taken.** Place the straw inside the Eppendorf tube (See figure 2).

3. Imagine eating your favourite food and allow saliva to pool in the mouth. With your head tilting forward, drool down the straw and collect saliva until the Eppendorf tube is full to the 100ml mark.

4. Once the Eppendorf tube is full, dispose of the straw and tightly seal the tube, placing it in the plastic bag provided for that sampling day.

Figure1. Medication event monitoring system caps (MEMSCap)

Figure 2. Eppendorf tube

ii. *Cortisol & Melatonin Packs*

You have 2 cortisol packs, one for each study day, it is very important that you put your cortisol tubes in the appropriate bags. The packs are labelled and colour coded:

Day 1 = Yellow
Day 2 = Orange

It is very important that you place the straw in the appropriate Eppendorf tube. Each tube is clearly labelled and is colour coded:

Tube 1  (Pre-study evening) = no label

Tube 2  (Study day morning, immediately upon awakening) = Green

Tube 3  (15 mins post waking) = Blue

Tube 4  (30 mins post waking) = Orange

Tube 5  (45 mins post waking) = Red

i. *MEMSCaps Bottles (see figure 1.)*

A total of 10 straws (total for the study days) are stored in the MEMSCaps bottle. Only open this bottle when you are collecting your saliva samples.

DO NOT put it back in this bottle.

These bottles record the times the bottle was opened, this will be used to check that you collected the saliva sample at the appropriate time.

2.2 Saliva cortisol & melatonin sampling times:

Prior to the first study day, the first sample will be collected in the evening before sleep. During each of the study days you will collect a total of five saliva samples.

The first sample will be collected prior to sleep before the first study day, this will be labelled evening sample E.

The morning sample should be collected as soon as you wake, please do this **STRAIGHT** away, even if you are a little sleepy. Please do not dose back to sleep because you will need to complete your samples 15, 30 and 45 minutes after you wake up.

Getting accurate timings of the saliva samples is fundamental to the researcher. If for any reason you miss the timing of a sample, please indicate this in the record sheet provided.

This table gives you an overview of each of the saliva sampling times (left side) and gives you examples of calculating your sampling times based on your waking times (right side).

Sampling times			Examples of calculating your sampling times based on your waking times
Sample No.	**Sample Time**	**Instructions**	**e.g. weekday sampling times**
Sample 1	Prior to sleep	This first sample should be collected before sleep	10.30pm
Sample 2	Waking	This first sample should be collected **as soon as you wake up** and **before** you **get out of bed.** Even if you are still half asleep you should still take it.	6.00am
Sample 3	Waking + 15 minutes	This second sample should be collected 15 minutes after you have woken up.	6.15am
Sample 4	Waking + 30 minutes	Take this sample 30 minutes after your awakening sample.	6.30am
Sample 5	Waking + 45 minutes	Take this sample 45 minutes after your awakening sample.	6.45am

2.3 Using the Actiwatch-Score:

- Wear this watch whilst in bed (the watch records your activity which will indicate you sleep and waking times).
- Do not wear the watch in the shower.

2.4 Using the HOBO light intensity data logger

- The HOBO light intensity logger measures **ambient** light. The logger should be placed upright and vertical (see above) on your bedside table or on any flat surface.
- Do not place the logger directly under any lamp or other sources of light.
- Keep logger away from any strong magnets (as this may trigger a false start).

2.5 Diaries and Questionnaires

i. *Morning Diary:*

The morning diary asks you to record the time you went to sleep the night before, awakening time, your sleep quality, anticipations of the day ahead and your current stress levels.

You need to complete this on the mornings of the two study days (after the fourth saliva sample). You will be reminded by an SMS message to complete this.

ii. *Evening Diary:*

The evening diary asks you to report your anticipations of the following day. You need to complete the evening diary prior to sleep.

iii. *Questionnaires*

The questionnaire booklet can be completed at any point during the two study days.

iv. *Saliva Sampling Time Record Sheet*

Please record all timings of the saliva sampling in the record sheet provided, noting any discrepancies.

2.6 Saliva samples and Storage.

- Once saliva samples have been collected for each day, please place the full Eppendorf tubes in the zip-sealed pack provided, labelled (for the relevant day). Please store the pack in your home freezer.
- At the end of the study you should have two packs containing five full Eppendorf tubes (ten in total), both should be stored in your home freezer as soon as possible. Upon returning the samples to the researcher, place these packs in the master testing pack along with the Actiwatch-score, MEMScap HOBO light intensity logger, questionnaire booklet, evening and morning diaries.

3. Returning study materials and equipment

All materials and equipment can be returned directly to the researcher at:

Natasha Madhewoo
Department of Psychology
University of Westminster
309 Regents St Campus
London W1B 2UW

Tel: 0207 911 5000 Ext 69029
Email: Natasha.madhewoo@my.westminster.ac.uk

Alternatively, arrangements can be made for materials to be collected by the researcher.

Thank you for taking the time to read the sampling guidelines. It is very important that you understand the instructions and are able to clearly follow the sampling instructions.

The content of this guidebook has be covered thoroughly in the initial introductory meeting however, if you have any questions regarding the procedure of this research project, or need to clarify something, this guidebook will serve as a useful reminder of all necessary information to complete this study.

However, should you have any additional questions not covered by the content of this guidebook, please do not hesitate to contact Natasha Madhewoo on any of the following numbers:

020 7911 5000 ext. 69029

Or by email:

natasha.madhewoo@my.westminster.ac.uk

Thank you very much for participating in this study!

Appendix 7

Guidelines Study II

VISION AND THE SLEEP-WAKE TRANSITION STUDY

Participant ID: ……..

Study Dates: ……

If you have questions at any time, please contact the team:

Lead Researcher:

Natasha Ramachandran : natasha.ramachandran@my.westminster.ac.uk or 07903012648

Project Supervisor

Dr Nina Smyth: smythn@westminster.ac.uk or 020 7911 5000 ext. 64425

Participant ID: ……..

Study Overview

Thank you very much for agreeing to participate in The Vision and The Sleep Wake Transition study. The study aims to investigate patterns of cortisol secretion after you wake up in the morning. Cortisol is a hormone that is essential for healthy functioning and we are interested in examining this in those who are diagnosed with primary open-angled glaucoma in relation to vision as well as how you feel.

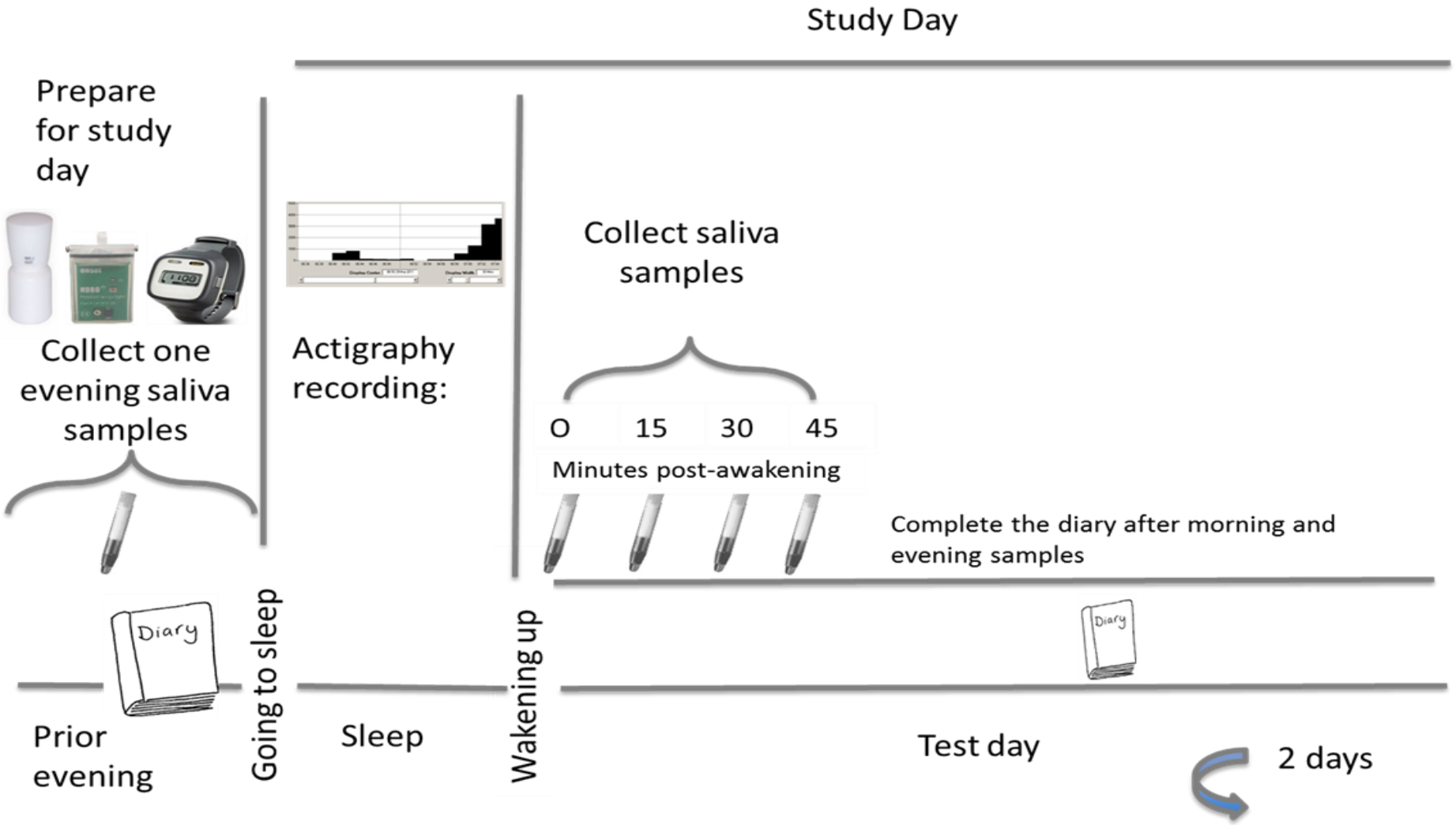
This booklet is intended to serve as a guide, describing the research protocol. It should provide answers to many of your questions, if you are unsure of something please do not hesitate to contact the Natasha, the lead researcher.

What is involved in the study?

Over two days, in your home environment, you will be asked to collect a saliva samples to measure the hormone cortisol. You will be asked to collect a sample before sleep and the following morning. We need you to collect the first sample **as soon as you wake up** and again 15, 30 and 45 minutes later. You can get up at your usual time, but we do ask you to try and wake up before 11am. Your sampling timings for each day will depend on the time you wake that day (and wake your wake time can differ on each of the study days).

You will be provided with a watch to wear to bed the nights prior to each study day. This watch provides an objective measure of your sleep and waking times. You will also be provided with a light logger, which should be placed by your bedside before sleep. The light logger measures the ambient light of your surroundings. You will be given two saliva sampling kits; each kit will include 5 tubes (labelled for each sampling day and time). To prepare for your study days you should ensure that you are wearing the watch when you go to bed and place the saliva sampling pack next to your bed to ensure that you collect the first sample immediately on awakening.

You will also be asked to complete an evening and morning diary: the evening diary will ask you questions about the day ahead and the morning diary will ask you to record your wake time, sampling times and questions about your upcoming day and your sleep. The evening prior to each study day, you will be sent a text message to your mobile phone reminding you to prepare for the upcoming study day. Over the 2-days, you will complete questions about your vision and wellbeing.


Study Day
Prepare for study day
Collect one evening saliva samples
Actigraphy recording:
Collect saliva samples
0
15
30
45
Minutes post-awakening
Complete the diary after morning and evening samples
Diary
Diary
Prior evening
Going to sleep
Sleep
Wakening up
Test day
2 days

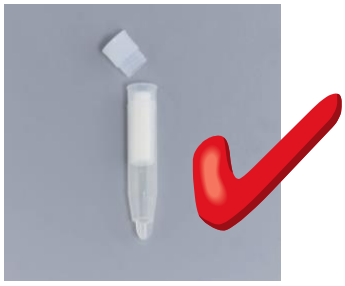
Saliva sampling instructions:

- Please do not eat or drink, smoke, brush your teeth or exercise during the sampling period. You can drink water only.
- Take one cotton swab from the track cap and place the cotton swab in your mouth.
- Gently chew on the swab until it is soaked with your saliva, this will usually take about 2 minutes.
- Once the swab is soaked, place it back in the appropriate salivette tube, trying not to use your hands. Put the cap on securely, and place the tube in the plastic bag provided for that sampling day.
- Store the salivette tube in a cold place or in a refrigerator.

1.1.8.

Placing the cotton swab in the salivette tube:

DO: Place in the smaller inner tube



Please DO NOT: remove the smaller, inner tube and place the swab in the larger inner tube.

Track Cap bottle

The cotton swabs used for sampling will be stored in a electronic bottle. Each time the bottle is opened the date and time is recorded, and this is used to verify your saliva sampling times. A total of 10 cotton swabs will be stored in the bottle. **Please only open this bottle when you are collecting your saliva samples.**

For the bottle to register as an event, please make sure you leave the cap off for **at least 3 seconds.** Once sampling is complete, please put the swab in the correctly labelled tube. It is very important that you use the correct tube. If you use the wrong tube please re-label it.

Saliva sampling times

On each of the study days you will collect four saliva samples, the times depend on your waking time. The first sample will be collected as soon as you wake, please do this **STRAIGHT** away, even if you are a little sleepy. Please do not doze back to sleep because you will need to collect samples again 15, 30, and 45 minutes later.

Guidance on determining your wake time

*How do I **determine when I am awake** and **when to collect the sample**?*

What

*Being awake is defined as when you are **conscious**: you know who and where you are; you are in a state that is clearly different from when you were sleeping even though you may still feel tired'*

*Being awake is defined as when you are **conscious**: you know who and where you are; you are in a state that is clearly different from when you were sleeping even though you may still feel tired'*

*Being awake is defined as when you are **conscious**: you know who and where you are; you are in a state that is clearly different from when you were sleeping even though you may still feel tired'*

*Being awake is defined as when you are **conscious**: you know who and where you are; you are in a state that is clearly different from when you were sleeping even though you may still feel tired'*

*happens if I **wake during the middle of the night**, should I start collecting the samples?*

Can I doze during the morning sampling period?

*If you wake during the middle of the night and plan to go back to sleep, do not begin sampling; please only **begin when you are awake for the final time**, when you plan to get up for the day.*

*If you wake during the middle of the night and plan to go back to sleep, do not begin sampling; please only **begin when you are awake for the final time**, when you plan to get up for the day.*

*If you wake during the middle of the night and plan to go back to sleep, do not begin sampling; please only **begin when you are awake for the final time**, when you plan to get up for the day.*

*If you wake during the middle of the night and plan to go back to sleep, do not begin sampling; please only **begin when you are awake for the final time**, when you plan to get up for the day.*

*When collecting the morning saliva samples, please **do not fall back to sleep or 'doze'** after your initial awakening. You can stay in bed or get out of bed but please stay awake (even if you are not fully alert) during and after the saliva sampling period.*

*When collecting the morning saliva samples, please **do not fall back to sleep or 'doze'** after your initial awakening. You can stay in bed or get out of bed but please stay awake (even if you are not fully alert) during and after the saliva sampling period.*

*When collecting the morning saliva samples, please **do not fall back to sleep or 'doze'** after your initial awakening. You can stay in bed or get out of bed but please stay awake (even if you are not fully alert) during and after the saliva sampling period.*

*When collecting the morning saliva samples, please **do not fall back to sleep or 'doze'** after your initial awakening. You can stay in bed or get out of bed but please stay awake (even if you are not fully alert) during and after the saliva sampling period.*

Calculating your saliva sampling times

The Table below gives you an overview of each of the saliva sampling times (left side) and examples of calculating your sampling times based on your waking times (right side). The Table on the next page gives you an overview of each of the saliva sampling times (left side) and examples of calculating your sampling times based on your waking times (right side).

Sample No.	**Sample Time**	**Instructions**	**Examples of calculating your sampling times based on your waking times**	**Your practice**
Sample 1	Waking	This first sample should be collected **as soon as you wake up** and **before** you **get out of bed.** Even if you are still half asleep you should still take it.	6.00	
Sample 2	Waking plus 15 minutes	This second sample should be collected 15 minutes after you have woken up.	6.15	
Sample 3	Waking plus 30 minutes	Take this sample 30 minutes after your awakening sample.	6.30	
Sample 4	Waking plus 45 minutes	Take this sample 45 minutes after your awakening sample.	6.45	

Using the electronic devices

The Actiwatch

Please wear this watch whilst in bed (the watch records your activity which will indicate you sleep and waking times).

Please do not wear the watch in the shower.

The Light Logger

The light logger measures **ambient** light. The meter should be placed upright and vertical on your bedside Table or on any flat surface.

Do not place the meter directly under any lamp or other sources of light.

1.1.9. Keep meter away from any strong magnets (as this may trigger a false start).

Morning Diary

The morning diary asks you to report your calculated and actual saliva sampling times for the morning. As well, some questions about your sleep and anticipations of the day ahead.
You need to complete this on the mornings of the study days (after your sampling in the morning).

	Type of questions
Study day 1 & 2	• Report salivary sampling times • Your night's sleep • Expectations of the day

Evening Diary

The evening diary asks you to report your anticipations for the following day. You will receive a text message reminding you to complete the evening diary.
You need to complete the evening diary at the following times:

	Type of questions
Preparation for study day 1	Anticipations of the following day
Study day 1	Anticipations of the following day
Study day 2	No diary

Returning the study materials

At the end of the study you will need to return your saliva samples, the study equipment and your diaries.

These need to be returned to a member of the research team:

Natasha Ramachandran: natasha.ramachandran@my.westminster.ac.uk or 0790 31 24 68
Or
Dr Nina Smyth: smythn@westminster.ac.uk or 020 7911 5000 ext. 69033

The researcher can arrange a convenient time for either drop off or collection of the study pack. It is very important that these are returned straight after the study so the equipment can be used for other participants. Or you can return by post (stamp addressed envelope will be included), please send to the following address:

University of Westminster, 115 New Cavendish St, Room 6.104, London, W1W 6UW

If you are interested in the results of the study, please let the team know and we will send you a summary following data collection and analysis.

Thank you for your participation
The Vision and Sleep-Wake Transition Study Team

The Psychophysiology and Stress Research Group
University of Westminster
https://www.westminster.ac.uk/psychophysiology-and-stress-research

Appendix 8

Self-report diary used by participants in Study I

Hormones of the Sleep-Wake Cycle

STUDY SAMPLING

EVENING AND MORNING DIARIES

Project ID_____ Date___/___/___

If you have any questions,

Please contact Natasha Madhewoo on

0207 911 50000 Ext. 6907

Natasha.madhewoo@my.westminster.ac.uk

Pre Study day 1 _____/_____/_____ Time of saliva sample___:___(pm)

EVENING DIARY:

Please complete this the evening before your first study day

1. **How busy do you expect tomorrow to be?** 1 = not at all busy – 5 = very busy ☐
2. **How do you feel about tomorrow?** 1 = very negative - 5 = very positive ☐

Study Day 1 _____/_____/_____

MORNING DIARY:

Please complete after your **45 minutes** post-awakening sample

1. **What time did you wake up? (24hr)** _____:_____

2. **What was the approximate time you went to sleep last night? (24hr)** _____:_____

3. **How did you wake this morning?** (Please tick one)
   a. Alarm clock / radio __
   b. Somebody I asked to wake me __
   c. Noises __
   d. Just woke __

1.1.10.

4. **Compared to usual, how was your quality of sleep last night?**
   a. Much better than usual __
   b. A bit better than usual__
   c. Same as usual__
   d. A bit worse than usual__
   e. Much worse than usual __

1.1.11.

5. **How busy do you expect the day ahead to be?** 1 = not at all busy – 5 = very busy ☐

7. **How do you feel about the day ahead?** 1 = very negative - 5 = very positive ☐

Time of saliva sample_____:_____(pm)

EVENING DIARY:

Please complete after your **12 hours** post-awakening sample, prior to sleep.

1. **How busy do you expect tomorrow to be?** 1 = not at all busy – 5 = very busy ☐
2. **How do you feel about tomorrow?** 1 = very negative - 5 = very positive ☐

Study Day 2: _____/_____/_____

MORNING DIARY:

Please complete after your **45 minutes** post-awakening sample

1. **What time did you wake up? (24hr)** _____:_____

2. **What was the approximate time you went to sleep last night? (24hr)** _____:_____

3. **How did you wake this morning?** (please tick one)

   a. Alarm clock / radio__
   b. Somebody I asked to wake me __
   c. Noises__
   d. Just woke__

1.1.12.

4. **Compared to usual, how was your quality of sleep last night?**

   a. Much better than usual __
   b. A bit better than usual__
   c. Same as usual__
   d. A bit worse than usual __
   e. Much worse than usual__

1.1.13.

5. **How busy do you expect the day ahead to be?** 1 = not at all busy – 5 = very busy ☐

7. **How do you feel about the day ahead?** 1 = very negative - 5 = very positive ☐

Appendix 9

Questionnaire booklet used by participants in Study I

Hormones of the Sleep-Wake Cycle

QUESTIONNAIRE BOOKLET

Project ID_____ Date___/___/___

If you have any questions,

Please contact Natasha Madhewoo on

0207 911 50000 Ext. 6907

Natasha.madhewoo@my.westminster.ac.uk

1. a) What is your date of birth ___/___/___
2. Are you male_____ or female_____? (Please ✓)

3. Please read the following **statements** and tick ✓**one response** which best describes you.

Statements	None of the time	Rarely	Some of the time	Often	All of the time
I've been optimistic about the future	[ ]	[ ]	[ ]	[ ]	[ ]
I've been feeling useful	[ ]	[ ]	[ ]	[ ]	[ ]
I've been feeling relaxed	[ ]	[ ]	[ ]	[ ]	[ ]
I've been feeling interested in other people	[ ]	[ ]	[ ]	[ ]	[ ]
I've had energy to spare	[ ]	[ ]	[ ]	[ ]	[ ]
I've been dealing with problems well	[ ]	[ ]	[ ]	[ ]	[ ]
I've been thinking clearly	[ ]	[ ]	[ ]	[ ]	[ ]
I've been feeling good about myself	[ ]	[ ]	[ ]	[ ]	[ ]
I've been feeling close to other people	[ ]	[ ]	[ ]	[ ]	[ ]
I've been feeling confident	[ ]	[ ]	[ ]	[ ]	[ ]
I've been able to make my own mind about things	[ ]	[ ]	[ ]	[ ]	[ ]

I've been feeling loved	[ ]	[ ]	[ ]	[ ]	[ ]
I've been interested in new things	[ ]	[ ]	[ ]	[ ]	[ ]
I've been feeling cheerful	[ ]	[ ]	[ ]	[ ]	[ ]

Please Turn Over.....

4. The following 10 statements refer to how you usually feel. For each statement please tick ✓**one box** out of five answers, varying from *never* to *always*, 1= ***never***, 2 = ***sometimes***; 3 = ***regularly***; 4= ***often***; and 5 = ***always***.

1.1.14.

Statements	1	2	3	4	5
I am bothered by fatigue	[ ]	[ ]	[ ]	[ ]	[ ]
I get tired very quickly	[ ]	[ ]	[ ]	[ ]	[ ]
I don't do much during the day	[ ]	[ ]	[ ]	[ ]	[ ]
I have enough energy for everyday life	[ ]	[ ]	[ ]	[ ]	[ ]
Physically, I feel exhausted	[ ]	[ ]	[ ]	[ ]	[ ]
I have problems starting things	[ ]	[ ]	[ ]	[ ]	[ ]
I have problems thinking clearly	[ ]	[ ]	[ ]	[ ]	[ ]
I feel no desire to do anything	[ ]	[ ]	[ ]	[ ]	[ ]
Mentally, I feel exhausted	[ ]	[ ]	[ ]	[ ]	[ ]
When I am doing something, I can concentrate quite well	[ ]	[ ]	[ ]	[ ]	[ ]

5. Please indicate by numbering either 0-4, how often you *felt* or *thought* a certain way.

0 = Never 1 = Almost Never 2 = Sometimes 3= Fairly often 4= Very often

In the last month, how often have you been upset because of something that happened unexpectedly? _____

In the last month, how often have you felt that you were unable to control the important things in your life? _____

In the last month how often have you felt nervous and "stressed"? _____

In the last month, how often have you thought about your ability to handle your personal problems? _____

In the last month, how often have you felt that things were going your way? _____

In the last month, how often have you found that you could not cope with all the things that you had to do? _____

In the last month, how often have you been able to control irritations in your life? _____

In the last month, how often have you felt that you were on top of things? _____

In the last month, how often have you been angered because of things that were outside of your control? _____

In the last month, how often have you felt difficulties were piling up so high, that you could not overcome them? _____

6. For the following question, **tick** ✓ boxes for all applicable months (displayed in the columns). This may be s single month, a cluster of months or no particular month. 1.1.15.

At what time of year do you…..

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	No particular month
Feel best	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Tend to gain most weight	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Socialise most	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Sleep most	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Eat most	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Lose most weight	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Socialise least	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Feel worst	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Eat least	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Sleep least	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

To what degree do the following change with the seasons?

(Tick ✓**one box** only for each)

	No change	Slight change	Moderate change	Marked change	Extremely marked change
Sleep length	[ ]	[ ]	[ ]	[ ]	[ ]
Social activity	[ ]	[ ]	[ ]	[ ]	[ ]
Mood (overall feeling of wellbeing	[ ]	[ ]	[ ]	[ ]	[ ]
Weight	[ ]	[ ]	[ ]	[ ]	[ ]
Appetite	[ ]	[ ]	[ ]	[ ]	[ ]
Energy Level	[ ]	[ ]	[ ]	[ ]	[ ]

Appendix 10

Self-report diary used by participants in Study II

VISION AND THE SLEEP-WAKE TRANSITION STUDY

Evening and Morning Diary

The Psychophysiology and Stress Research Group

University of Westminster

https://www.westminster.ac.uk/psychophysiology-and-stress-research

Participant ID: ……..

Study Dates: ……

If you have questions at any time, please contact the team:

Lead Researcher:

Natasha Ramachandran : natasha.ramachandran@my.westminster.ac.uk or 07903012648

Project Supervisor

Dr Nina Smyth: smythn@westminster.ac.uk or 020 7911 5000 ext. 69033

Pre Study day 1 _____/_____/_____ Time of saliva sample___:___(pm)

EVENING DIARY:

Please complete this the evening before your first study day

a. On a scale of 1-5 how busy do you expect the day ahead to be?	1 (not at all busy) to 5 (very busy)	☐
b. On a scale of 1-5 how do you feel about the day ahead?	1 (very negative) to 5 (very positive)	☐

Please indicate any comments below:

……

……

……

……

……

……

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University of Westminster
https://www.westminster.ac.uk/psychophysiology-and-stress-research

Study Day 1 _____/_____/_____

MORNING DIARY:

Please complete after your **45 minutes** post-awakening sample

a. Please indicate where you placed the light logger prior to sleep	……
b. Please indicate what time you went to bed last night? (24hr)	……
c. Please indicate what time you went to sleep last night? (24hr)	……
d. Please indicate what time you woke up? (24hr)	……

.1.16.

e. Please calculate your collection times and state the actual times that you collected your saliva samples.

.1.17. Please note, your honesty is very important to us in analysing the data. Therefore, please state your actual collection times for each sample, even if it is different to the designated time, and answer the questions as accurately as possible.

Tube	Collection time (post-awakening)	Calculated collection time (24hr)	Actual collection time (24hr)	Any problems with sampling? (i.e. brushed teeth, ate, smoked etc.)
1	0 min			
2	15 min			

3	30 min			
4	45 min			

f. Did you wear the Actiwatch to bed?	○ Yes ○ No
g. On a scale of 1-5 how easy was it for you to determine the exact moment you woke up?	1 (very difficult) to 5 (very easy) ☐
h. How did you wake this morning? please tick from the list →	○ Alarm clock / radio ○ Somebody I asked to wake me ○ Noises ○ Just woke
i. Compared to usual, how was your quality of sleep last night? please tick from the list →	○ Much better than usual ○ A bit better than usual ○ Same as usual ○ A bit worse than usual ○ Much worse than usual
j. On a scale of 1-5 how busy do you expect the day ahead to be?	1 (not at all busy) to 5 (very busy) ☐
k. On a scale of 1-5 how do you feel about the day ahead?	1 (very negative) to 5 (very positive) ☐

Pre study day 2 _____/_____/_____ Time of saliva sample___:___(pm)

EVENING DIARY:

Please complete this the evening before your second study day

a. On a scale of 1-5 how busy do you expect the day ahead to be?	1 (not at all busy) to 5 (very busy)	☐
b. On a scale of 1-5 how do you feel about the day ahead?	1 (very negative) to 5 (very positive)	☐

Please indicate any comments below:

……

……

……

……

……

……

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Study Day 2 _____/_____/_____

MORNING DIARY:

Please complete after your **45 minutes** post-awakening sample

l. Please indicate what time you went to bed last night? (24hr)	……..
m. Please indicate what time you went to sleep last night? (24hr)	……..
n. Please indicate what time you woke up? (24hr)	……..

.1.18.

o. Please calculate your collection times and state the actual times that you collected your saliva samples.

.1.19. Please note, your honesty is very important to us in analysing the data. Therefore, please state your actual collection times for each sample, even if it is different to the designated time, and answer the questions as accurately as possible.

Tube	Collection time (post-awakening)	Calculated collection time (24hr)	Actual collection time (24hr)	Any problems with sampling? (i.e. brushed teeth, ate, smoked etc.)
1	0 min			
2	15 min			
3	30 min			
4	45 min			

p. Did you wear the Actiwatch to bed?	○ Yes ○ No
q. On a scale of 1-5 how easy was it for you to determine the exact moment you woke up?	1 (very difficult) to 5 (very easy) ☐
r. How did you wake this morning? please tick from the list →	○ Alarm clock / radio ○ Somebody I asked to wake me ○ Noises ○ Just woke
s. Compared to usual, how was your quality of sleep last night? please tick from the list →	○ Much better than usual ○ A bit better than usual ○ Same as usual ○ A bit worse than usual ○ Much worse than usual
t. On a scale of 1-5 how busy do you expect the day ahead to be?	1 (not at all busy) to 5 (very busy) ☐
u. On a scale of 1-5 how do you feel about the day ahead?	1 (very negative) to 5 (very positive) ☐

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Appendix 11

Questionnaire booklet used by participants in Study II

VISION AND THE SLEEP-WAKE TRANSITION STUDY

Questionnaire booklet

The Psychophysiology and Stress Research Group

University of Westminster

https://www.westminster.ac.uk/psychophysiology-and-stress-research

Questionnaire booklet

Participant ID: ……..

Study Dates: ……

If you have questions at any time, please contact the team:

Lead Researcher:

Natasha Ramachandran : natasha.ramachandran@my.westminster.ac.uk or 07903012648

Project Supervisor

Dr Nina Smyth: smythn@westminster.ac.uk or 020 7911 5000 ext. 64425

Participant ID: ……..

Study Dates: ……

The Psychophysiology and Stress Research Group

University of Westminster

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Please answer a few general questions about yourself.

1.1.20.

1.1.21. a) What is your date of birth ___/___/___

1.1.22. b) What is your gender? _____

How would you describe your marital status? Please ✓one.

- ☐ Married
- ☐ Cohabiting
- ☐ Divorced
- ☐ Widowed
- ☐ With a partner but not cohabiting
- ☐ Single

Employment status?

- ☐ In full-time employment
- ☐ In part-time employment
- ☐ Unemployed
- ☐ Student
- ☐ Home-maker
- ☐ Retired
- ☐ Other……

The following questions are about your general health and vision.

1. Other than your glaucoma how would you rate your general health?

☐ Excellent ☐ Very good

☐ Good ☐ Fair ☐ Poor

2. At the present time, would you say your eyesight using both eyes (with glasses or contact lenses, if you wear them) is:
   1.1.23.

☐ Excellent ☐ Very good

☐ Good ☐ Fair ☐ Poor

3. How much time do you spend worrying about your eyesight?
   1.1.24.

☐ None of the time ☐ A little time

☐ Some of the time ☐ Most of the time ☐ All of the time

4. How much pain or discomfort have you had in and around your eyes (for example, burning, itching, or aching)?
   1.1.25.

☐ None ☐ Mild

☐ Moderate ☐ Severe ☐ Very severe

A. The next questions are about how much **difficulty**, if any, you have doing certain activities (with glasses or contact lenses, if you wear them).

1.1.26.

1.1.27. Please tick ✓**one response** which best describes you

1.1.28. 1=No difficulty, 2, Little difficulty, 3= Moderate difficulty, 4= Extreme difficulty, 5= stopped activity (due to eyesight), N/A= not applicable

1.1.29.

Statement	1	2	3	4	5	N/A
5.Reading ordinary print newspapers						
6.Working or hobbies which require close attention (e.g. sewing, cooking)						
7.Finding something on a crowded shelf						
8. Reading street signs						
9.Going down staircases, steps or curbs in dim light or at night						
10.Noticing objects to the side of your vision whilst walking						
11.Seeing how people react to things you say						
12.Picking out and matching clothes						

13.Visiting people at their home, at parties or restaurants						
14. Taking part in social activities (e.g. going to the cinema, sporting events etc.)						

15.Do you currently drive?

☐ Yes (skip to Q 15c.)

☐ No

15a. If **no**: please go to question 15c

☐ Never drove (Skip to Part C, Q17.)

☐ Gave up driving

15b. If you **gave up driving. w**as this due to:

☐ Eyesight

☐ Other reasons ☐ Both eyesight and other reasons

15c. If you are **currently driving**, do you experience any difficulty in driving during the day in familiar places?

☐ No difficulty at all ☐ Moderate difficulty

☐ Extreme difficulty ☐ Stopped due to eyesight

B. The next questions are about how much **difficulty**, if any, you have driving in certain conditions (with glasses or contact lenses, if you wear them). Please tick ✓**one response** which best describes you

16. How much difficulty do you have driving at night?

☐ No difficulty at all ☐ Moderate difficulty

☐ Extreme difficulty ☐ Stopped due to eyesight ☐ N/A

16a. How difficult is it for you to drive in hard conditions such as bad weather, traffic etc.?

☐ No difficulty at all ☐ Moderate difficulty

☐ Extreme difficulty ☐ Stopped due to eyesight ☐ N/A

The following statements are about how your vision affects the things you do. Please indicate your response by numbering each statement 1-5.

1 = All of the time, 2= Most of the time, 3 = Sometimes, 4= A little time, 5= None of the time

Statement	1	2	3	4	5
17. I accomplish less than I would because of my vision					
18. I am limited in how long I can work or do other activities because of my vision					
19. Pain and/or discomfort in or around my eyes (burning, itching) keep me from doing things I would like to do					

The following 6 statements refer to how you usually feel about your eyesight. For each statement please tick ✓**one box** out of five answers, varying from *never* to *always*,

1.1.30.

1.1.31. 1= never, 2 = sometimes; 3 = regularly; 4= often; and 5 = always.

Statement	1	2	3	4	5
20. I stay home most of the night					
21. I feel frustrated					
22. I have much less control over what I want to do					
23. I have to rely a lot on other people					
24. I worry about doing things that will embarrass me or others					

The following 14 statements refer to your mental wellbeing. For each statement please tick ✓**one response**, which best describes you.

Statement	None of the time	Rarely	Sometime	Often	All the time
I've been optimistic about the future					
I've been feeling useful					
I've been feeling relaxed					
I've been feeling interested in other people					
I've had energy to spare					
I've been dealing with problems well					
I've been thinking clearly					
I've been feeling good about myself					
I've been feeling close to other people					
I've been feeling confident					
I've been able to make my own mind about things					

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I've been feeling loved					
I've been interested in new things					
I've been feeling cheerful					

The following 10 statements refers to feelings of fatigue. For each statement please tick ✓**one box** out of five answers, varying from never to always,

1= never, 2 = sometimes; 3 = regularly; 4= often; and 5 = always.

Statement	1	2	3	4	5
I am bothered by fatigue					
I get tired very quickly					
I don't do much during the day					
I have enough energy for everyday life					
Physically, I feel exhausted					
I have problems starting things					
I have problems thinking clearly					
I feel no desire to do anything					
Mentally, I feel exhausted					
When I am doing something, I can concentrate quite well					

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The following 10 statements refers general feelings of stress **in the last month**. For each statement please tick ✓**one box** out of five answers, varying from never to always,

1= never, 2 = sometimes; 3 = regularly; 4= often; and 5 = always

In the last month….

Statement	1	2	3	4	5
How often have you been upset because of something that happened unexpectedly?					
How often have you felt that you were unable to control the important things in your life?					
How often have you felt nervous and "stressed"?					
How often have you thought about your ability to handle your personal problems?					
How often have you felt that things were going your way					
How often have you found that you could not cope with all the things that you had to do					
How often have you been able to control irritations in your life?					
How often have you felt that you were on top of things?					
How often have you been angered because of things that were outside of your control?					

How often have you felt difficulties were piling up so high that you could not overcome them?					

For the following questions, **tick** ✓ boxes for all applicable months (displayed in the columns). This may be a single month, a cluster of months or no particular month.

At what time of year do you

	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Feel best												
Tend to gain most weight												
Socialise most												
Sleep most												
Eat most												
Lose most weight												

Socialise least												
Feel worst												
Eat least												
Sleep least												

To what degree do the following change with the seasons?

Please tick ✓**one box** only for each:

	None	**Slight**	**Moderate**	**Marked**	**Extreme**
Sleep length					
Social activity					
Mood					
Weight					
Appetite					
Energy level					

If you experience changes with the seasons, do you feel that these are a problem for you?

☐ Yes ☐ No

If yes, is this problem

☐ Mild ☐ Moderate

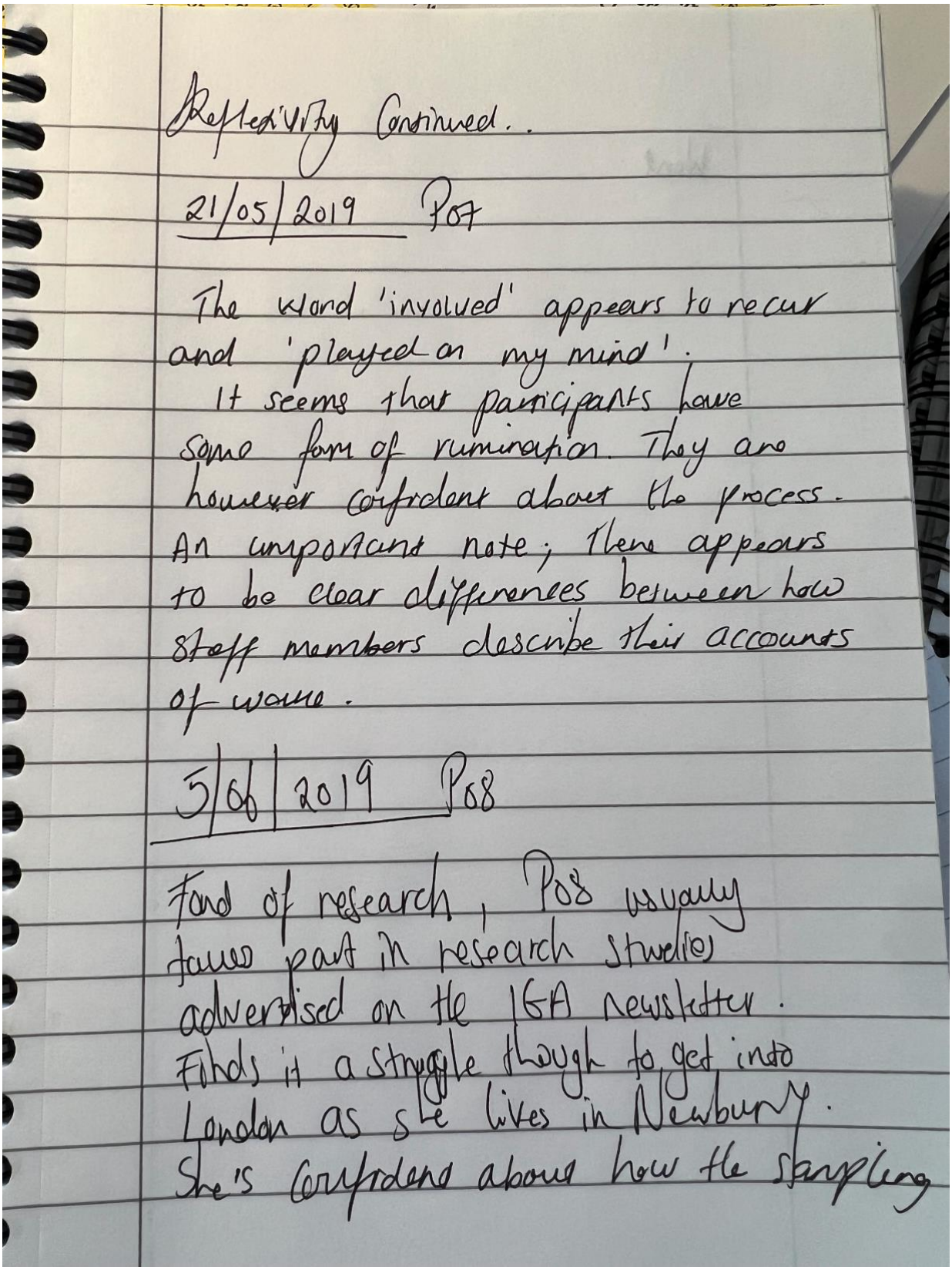
☐ Marked ☐ Severe ☐ Disabling

That was the end of the questionnaire,

Thank you for completing it.

Appendix 12

Excerpt of reflexive diary



Reflexivity Continued..

21/05/2019 P07

The word 'involved' appears to recur and 'played on my mind'.
It seems that participants have some form of rumination. They are however confident about the process.
An important note; there appears to be clear differences between how staff members describe their accounts of work.

5/06/2019 P08

Fond of research, P08 usually takes part in research studies advertised on the IGA newsletter.
Finds it a struggle though to get into London as she lives in Newbury.
She's confident about how the sampling

Appendix 13

Transcript SJ and MAC

P04_MF.mp3

Key:

S – Sanjay (Interviewer)

M – M (Participant)

Start of audio at 00.00

S
Ok. Interview with M, Thursday 2nd June. Great. All right, M; so thank you for taking part – I really appreciate this. M, what I'd like to do to start off with is to ask you some questions about your initial thoughts about taking part in this study. So, what were your reasons for wanting to take part in this study?

M
My main reasons, if I'm honest, were to help out Angela, Nina and Lisa.

S
Right

M
Because that's the study that they were doing; I think it's important to take part in studies that are being done.

S
Yeah; by your friends and colleagues?

M
Yeah, exactly; and also, you know, they've taken part in studies for me, as well.

S
Right, right; so there was a mutual... sort of... helping out.

M
Yeah.

S

Yeah. So if they had not taken part in your studies...

M
Oh, I would still take part in theirs! *[Laughs]*

S
You'd still take part *[laughs]* ok! All right. So what were your initial thoughts, you know, when you *did* decide to take part in the study, knowing that it involved... you know... giving saliva samples in the mornings, over 4 weeks; what were your *initial* thoughts, before it actually started?

M
If I'm honest, initial thoughts are 'Oh!' *[Laughs wryly]*

S
[Laughs]

M
'Oh! I've got to do this study!' Partly because I've done cortisol before...

S
Right

M
And I know that sometimes it's a bit... taking the samples; it's not *difficult*, but it's just... I'd say... slightly a nuisance; it's something that you have to do that's different from the normal. And I find that sometimes, I don't always sleep very well...

S
Right

M
...and so I find it... that I find it hard to sometimes find which... what my awakening time is.

S
Right.

M
...because I might wake up at 5 o'clock in the morning...

S

Right

M
...but then I'll go back to sleep again, maybe at 7, when I'm meant to be getting up.

S
Right. Right.

M
So it was just... if I... if I'm *doing* the cortisol, I know that I'll have to stay awake from 5 o'clock until the next 45 minutes or so.

S
Right, until you've given all your samples?

M
Until I've given my samples.

S
So there was an awareness – based on your *previous* participation in this kind of research – about what was going to be involved; and the fact that it might be a bit of a nuisance...

M
Yeah

S
...from your regular routine, and so on?

M
Yes.

S
Well we'll... we'll... we'll...

M
But... sorry...

S
No-no, no...

M

But I was also very *happy* to take part.

S
Right, right. Yeah.

M
It's just... the...

S
It's just the recognition that you would have to go through all the... the annoying bits? *[Laughs heartily]*

M
Yes! *[Laughs heartily]*

S
Ok. Well we'll certainly come back to some of these issues that you've touched upon, and hopefully explore them in a bit more detail in a minute.

M
Yeah.

S
But talking about... you know... your experiences now, when the study began; you know, talking about your participation, with the different aspects; what was the experience like, for you, of giving the saliva samples in the morning?

M
It was fine, actually; I don't find the... some people talk about dry mouth, and that sort of stuff; I don't have that problem.

S
Right.

M
And what I did was; I set my alarm at 15 minutes... so I naturally woke up, and I woke up very early on those mornings...

S
Right

M

But I set my alarm at 15-minute intervals, to make sure that I complied with the procedure. But I didn't go back to sleep in between; I stayed awake.j

S
Right.

M
But it was just to make sure that I kept on time.

S
Right.

M
But yeah, it was fine; I didn't find it a nuisance or anything. And actually, maybe before the start of the study, and I suppose for the *first* time, you're slightly apprehensive because you don't want to mess it up; because you know that it's important to do the samples at the right time.

S
Right, right. So there was some concern, maybe some anxiety, about making sure that you gave the first sample...

M
Yeah, and that I complied with everything, and adhered to the protocol properly.

S
Right, yeah. So as far as the actual routine was concerned, about taking the cotton wool out and putting it in your mouth, and leaving it your mouth, and all that; that seemed generally fine?

M
Oh, that was fine.

S
It was straightforward?

M
Straightforward; and actually, I found it was very well organised.

S
Right.

M
And I think having the MEMS cap, to put the samples in and take the lid off and on, was good; and also having the reminder the night before, and filling out the questionnaire kind of prepared me for the thing.

S
Right, so it was useful to have the text message the night before?

M
It was useful to have that; and also to have the diary to fill in, and to... you know... the reminders ‘put stuff by your bed’...

S
Right, yeah.

M
And so it was easy, if I’m honest.

S
Yeah. And so did you find that it sort of *prepared* you, then; those reminders and filling in the night-before diary?

M
Yes, yeah.

S
Right.

M
It kept it in my mind that I needed to do that in the morning.

S
Right. And then was...

M
And putting the watch on, as well.

S
Right. Right. And then was that straightforward; did that create any problems or hassles for you, having to wear the watch at night?

M
No. Actually, it was all very easy.
S
Right.

M
The... the only thing that I would say *might* have been slightly problematic was the sleeping.

S
Right.

M
But I actually have disturbed sleep quite a lot anyway; so it's hard to tell whether... it was apprehension to do with the study, and making sure that I was awake on time...

S
Ok

M
...that woke me up; or whether I just naturally woke up, because... yeah.

S
What might be... maybe worth exploring that in a bit more detail, when you say that there was an apprehension about sleeping; and then you're saying that maybe you *didn't* have... sort of... un-interrupted sleep; maybe the sleep was somewhat disturbed; is that what happened?

M
What I'm saying is; it could *possibly* have happened, but it may *not* particularly have happened.

S
Right.

M
It...

S
What makes you think that it *might* have happened?

M
Well... hmm, that's an interesting question. Because... sometimes, if I'm awake at 5 – which I am – I might lie around for half an hour, but then I'll go back to sleep; whereas I *couldn't* go back to sleep in this case, because I was doing the sampling.

S
Right

M
So there was a consciousness of 'I'm awake – but I must *stay* awake', so it might – I'm not saying necessarily that it woke me up any *earlier* than I might necessarily have woken up – but it could have done, because I have little... yeah, it's hard to *[laughs]*

S
[Laughs]

M
I'm not explaining this well, because sometimes I have great, un-interrupted sleep...

S
Right

M
...and then I go through little periods where my sleep is interrupted.

S
Right

M
And I didn't start the study for a little while, because I *was* going through a period of interrupted sleep.

S
Right

M
And then my sleeping was a bit better, and then I started the study.

S

I see. So you've had... sort of... interrupted sleep previously, and based on that awareness, you waited until you felt you were ready, whereby your sleep would not be *unduly* interrupted...

M
Yeah

S
...so that you could take part in the study?

M
Yes.

S
Right. Right. Yeah. And you were saying earlier that you're still not quite sure whether that might have possibly continued – that sense of interrupted...

M
I think certainly, probably the *first* time; the first time I did it. I might have been a bit interrupted.

S
Right.

M
It's hard to tell, with the other times.

S
Sure, yeah. And if it *might* have been interrupted the first time around; what were the signs of that interruption; what happened, do you think, that might have led you to believe that perhaps it was... it might have been interrupted?

M
Well I think... I didn't go to bed till 2.30, the night before I did it.

S
Right. And was that quite later than usual?

M
That was later than I normally did; but that was because we were doing stuff, so nothing to *do* with the study.

S
Right.

M
So...and then I think I took my sample at 5, or 6, but I was also awake around 3 or 4, I think, in the middle of the night.

S
Right.

M
And when I woke up in the middle of the night, I was thinking ‘Oh!’ about my sampling *[laughs]*

S
[Laughs]

M
And then thinking ‘Oh no; no, no, it’s not’ so that’s why I think maybe it was...

S
Right. So... so in that... so... for the first night, you were up till 2 anyway; you went to sleep around 2 o’clock; you ended up waking up at 5 o’clock; so in that 3-hour period, you woke up at least once or twice more?

M
Yes.

S
And you remember thinking about needing to take the saliva samples...

M
Yes

S
When you kept waking up?

M
Yes.

S

Yeah. Yeah; so it's possible that the *thought* of it may have been playing on your mind.

M
The *thought* of it may have been, a little bit *[laughs]*

S
Yeah *[laughs]*

M
But I did have *other* nights where I had interrupted sleep.

S
Right; for other reasons, nothing to do with this?

M
Yeah. Mm.

S
Yeah

M
But I think possibly the first night, it *was*; the other nights, possibly.

S
Right, yeah. And are you able to recall whether the other 3 nights were, if you like, qualitatively different from your first night?

M
Erm... if... if I'm *really* honest, I can't; I just remember feeling... I think maybe because I went to bed so late.

S
For the first night? Right.

M
Yeah.

S
Right.

M
That... that...

S
That it was particularly noticeable?

M
Yes.

S
Yeah. Yeah. But maybe not so for the other 3 nights?

M
For the other nights. Mm-hm.

S
Yeah. Yeah. Overall then, M, talking about those 4 nights, overall; would you say those 4 nights' sleep were *generally* ok, or were they... would you say they were a bit more disturbed?

M
I think they were *generally* ok.

S
Right; apart from that first night?

M
Yeah. Generally ok.

S
Ok. Yeah.

M
Maybe a slight disturbance, which I can't tell.

S
Yeah, for sure?

M
Yeah

S
Yeah.

M
If that's related to the cortisol taking.

S
Sure. All right. I think it's really *interesting*, reflecting on the *possibility* of thinking about needing to give samples in the morning, and how that might affect one's sleep.

M
Mm.

S
And just talking about... you know... waking up in the morning, and giving those... you know... *taking* those samples, over the course of 45 minutes; to what extent would you say that... got in the way of your regular routine?

M
Because I was awake so early, I... it didn't get in my regular... in the way, at all.

S
Right.

M
If I had been woken up at alarm-time – at 7.15 or 7.30 – *then* it would have got in the way.

S
Right.

M
But as it was, I just made sure that I stayed awake, as opposed to going back to sleep! *[Laughs]*

S
Right. Right! *[Laughs]*

M
For that 45 minutes; and then I allowed myself a little snooze, because I like to snooze in the morning. I wake up early...

S
Right

M
...and I have a little snooze; and then I might wake up again, and have another little snooze *[laughs]*

S
Right, right. So it sounds like, by virtue of waking up *earlier* than you might have anticipated, you not only *managed* to take the 4 saliva samples, but you also had a bit of time to spare to maybe have another forty winks, or so.

M
Yes; so it didn't interrupt me having a coffee, brushing my teeth, having a shower, anything.

S
Right, so you were able to do everything.

M
It was... I was able to do everything.

S
Yeah. And the fact that you... you know... that you mentioned that you were waking up possibly earlier than you might have anticipated – or earlier than you might have wanted to – during those 4 mornings; did it have any particular impact later on in the day?

M
No, because... no, I don't think so. Probably on the first day, because I went to bed so late, had interrupted sleep, I was probably a bit tired and dozy that day; but other than that, no; because I'm used to interrupted sleep.

S
Right, right.

M
So...

S

Yeah

M
...I didn't notice any particular difference.

S
Right. So it didn't feel like... you know... by the afternoon or early evening you were feeling unduly shattered or exhausted or anything?

M
No, no, no, no. No.

S
Yeah

M
No; no impact really, at all.

S
Ok.

M
Because that would often be the case that I might wake up at 5; or I might often even wake up at 4; spend a couple of hours awake, have a little sleep. I'm used to that.

S
Right. And talking about waking up at that time, you know; if it was slightly *earlier* than you had anticipated; did you think about going back to sleep straight away, or did you just decide that 'I'm just going to now stay up and then take the saliva samples'?

M
Well I certainly didn't *get* up, any of those mornings. I think... because often I lie in bed and do the crossword anyway, so...

S
Right

M

...and sometimes I snooze after that – sometimes I don't – so it wouldn't have interrupted *that* routine at all. So I might have dozed off a bit; I might have done the crossword; but I certainly didn't get up! *[Laughs]*

S
Right, so you're happy to just lie in bed...

M
Yeah; relax in bed, yeah. *[Laughs]*

S
Yeah. Yeah. *[Laughs]* sounds... sounds... sounds good! *[Laughs]*

M
[Laughs]

S
I like the idea of doing the crossword in the morning; I suppose it must get your brain going, as well, in a relaxed way?

M
Well, I sometimes do it at 3 o'clock in the morning, and...

S
Right!

M
Yeah! *[Laughs]*

S
So... and you mentioned earlier about... following the various steps, as part of the procedure; you said that was well organised?

M
Yes.

S
So it was all clearly laid out?

M
It was clearly laid out – absolutely no issues with doing it, at all.

S
There were no ambiguities in the instructions?

M
No, no. And I suppose at the start of the study, I was thinking ‘Oh, it’s a bit of a nuisance having to do this; because I’m going to have to be reliable!’ but actually doing the study, I didn’t find a nuisance at all.

S
Right, so it was straightforward, just going through the routine.

M
It was straightforward to go through the routine.

S
Yeah. Yeah. And putting the cotton wool in the mouth, and those kinds of things, didn’t feel...

M
No.

S
...unpleasant, or anything?

M
No, not unpleasant at all.

S
Right. Right. Ok. So we’re just coming to the next set of questions, M, which are really exploring your *general* understanding of the meaning of ‘wakefulness’. Did you find it hard to determine at what point you were awake?

M
I certainly did on the final morning.

S
Right.

M
There was a little period where I was between... where I was conscious of thinking ‘I need to do saliva samples’ and when was I actually awake?

S
Right. And that was during your last morning, did you say?

M
Yes; I definitely noticed it most on the last morning.

S
That you were not quite clear...

M
Yeah

S
Erm...

M
Whether I had been awake for a minute or two, or not.

S
Right. Yeah.

M
On the other mornings, I think that I knew that I was awake.

S
Right. So you ... so you felt... generally, that the first 3 mornings, you had a sense that you were awake...

M
Yeah

S
...and there wasn't any ambiguity; with the 4th morning, you weren't *quite* sure how long you *may* have been awake...

M
Or... yeah; I couldn't tell whether I had been quite... consciously awake *[laughs]* or not, for a minute...

S
[Laughs] Right.

M

I think it was only a minute or two; but yeah, there was a little ambiguity on the last morning.

S
Ok.

M
And actually, I think that was the night I slept best, as well, out of them.

S
Right, right. So the other 3 nights were possibly not *un*-interrupted – that there was some interruption – but when you woke up in the morning...

M
I was awake.

S
...you were able to tell quite categorically that 'I'm awake'.

M
I *think* that is true! *[Laughs]*

S
Right *[laughs]* yeah; so perhaps there's an *element* of uncertainty with those as well?

M
There could be. My... my recollection is that I didn't feel too much ambiguity over those; but I *was* definitely aware of a little ambiguity on the 4th one.

S
Right. Yeah. There's an interesting paradox here, isn't there?

M
Mm.

S
That there is... a... a sense that... you were conscious, but you're also aware that you're not *quite* sure at what point you gained that consciousness. And I'm just wondering – relating this back to taking the saliva samples – I'm just wondering; at what *point* do you feel certain that you *took* the saliva samples *at the moment of gaining consciousness* – or awareness, from having been asleep?

M
Hmm. Well, I would say that it was within the first 30 seconds to minute...

S
Right

M
...of feeling 'I'm awake'
S
Right.

M
Because it... yeah. Is that the first thing that comes into my mind, when I wake up? Hmm. Difficult to say. I *think* it was! *[Laughs]*

S
Right *[laughs]* yeah.

M
Yeah. It is... I think... I think there was a clear distinction.

S
Between having been asleep, and the point – the moment – of gaining wakefulness; of consciousness of awakeness?

M
Yes.

S
Right. Yeah. So 30 seconds is *fairly*, fairly sort of rapid transition.

M
Mm-hm.

S
Yeah. And so you feel...

M
And I think maybe, because I was doing the study, I was more... more... mindful of being 'Oh, when I wake up, I need to *do* something' whereas a lot of the time there might be a little 'drifting' into wakefulness.

S
Right.

M
But I don’t feel that I drifted into wakefulness...

S
Right

M
...on those mornings.

S
Right. So the... so the *point* of wakefulness – or the *awareness* of the point of wakefulness – came quite soon?

M
Yes.

S
You feel?

M
I feel! *[laughs]*

S
Yeah. *[laughs]*

M
I may be wrong! But I think...

S
Well, it’s... it’s... it’s really... it’s...

M
Subjectively, that’s how I felt.

S
Yeah, yeah; it is quite difficult, isn’t it, I think, to *tell*...

M
Yeah

S
You know... which is... which is obviously one of the points about this *aspect* of the study...

M
Yeah

S
...trying to explore people's *awareness* of when do they *gain* consciousness, you know?

M
Mm-hm.

S
And it's not always...

M
No, it's not.

S
...clear-cut.

M
Yeah; definitely on the 4th morning, it wasn't.

S
Right, yeah. So the first 3, you reckon maybe within 30 seconds to a minute; but the 4th morning, could have been possibly longer?

M
Yeah. Yeah. It could have been a minute or two; I don't think it was much more. Well, I... no, I don't think it... I would say that I'm sure it wasn't, but... I was just... I remember thinking to myself 'Oh, have I been awake for a minute, or two, or...' there was a little... confusion about it.

S
And what do you think that's about; why do you think it might have been different during that 4th morning? What was different about the 4th morning, compared to the other 3 mornings?

M

Well, I'd come back from a holiday *[laughs]* but... so I'd travelled that afternoon.

S
Right.

M
I was quite relaxed, and I did have a night... I think I had a good night's sleep...

S
Right.

M
Erm... *[Pause from 23.52 to 24.00]* I don't... I don't know, really.

S
Ok. So you were much more relaxed the night before, of the 4th morning?

M
Yeah; I... I was fairly relaxed all the other evenings as well; but I think maybe I was more relaxed.

S
Ok. So there was something slightly different, possibly, leading up to the 4th night; compared to maybe the other 3.

M
And maybe it's getting used to the fact that you know that you're not going to miss the samples, or...

S
Right, yeah. And was it a relief to know that you were coming to the end of the 4th day?

M
I wouldn't say a *massive relief* *[laughs]*

S
[Laughs]

M
But I was pleased, yes.

S
Right, yeah. Yeah. So it wasn't terribly traumatic or anything, but...

M
No; it's just something that you have to remember to do.

S
Yeah. Ok. Yeah. Right. Well M, I think we've gone through all the questions I wanted to ask you. Is there anything else *you* wanted to say, that you feel is interesting; things that we may not have covered, based on your experiences?

M
Erm... *[pause from 25.15 to 25.19]* I suppose I... it *has* made me think about my own cortisol profile, and thinking about my own, unhealthy lifestyle practices! *[Laughs]*

S
Right.

M
And I'm interested in seeing what... so I suppose for the start, as well as thinking 'Oh, it's a bit of a nuisance' I'm also *interested* to see what the outcome of the study is; and I'll be interested in the variations of myself over the days.

S
Right.

M
And I think not just related to the cortisol part of it; certainly for the first day there's a slight apprehension about doing the attention switching task.

S
Right. Right. That was during the *first* day, did you say?

M
During the first day, yeah; thinking that I might be an idiot! *[Laughs heartily]* Not... you know... not be able to do it very well!

S
Right

M
And what else? And *this* isn't related to the cortisol either; but the *shock* of not being able to stand on my leg with my eyes closed, for very long!

S
Right, right. Yeah. So did you find that you had to put your foot down after a little while?

M
[Laughs] After a couple of seconds!

S
[Laughs]

M
It was a shock! Because like this, you're fine; then you close the eyes, and...

S
Right, yeah. And so was it... was it a concern, or was it just an unusual experience; something that you might have taken for granted that it wouldn't affect you?

M
I... I took it for granted that it... I would be *fine*...

S
Right

M
Because I've never particularly *done* it, I just thought that I'd close my eyes and stand on my leg. *[Laughs]*

S
Right! *[Laughs]*

M
And then found that I *didn't!*

S
Right *[laughs]* yeah.

M
[Laughs] and it was a bit disconcerting!

S
Yeah. What do you think it might *indicate?*

M
Erm... perhaps a lack of balance? *[Laughs]*

S
[Laughs]

M
Beyond that, I don't... I don't know that it signifies anything terribly... I don't know if I'm that different to other people; because having said to other people, a lot of other people, they couldn't do it either! *[Laughs]*

S
Yeah.

M
I suppose there might have been a little concern of 'Why am I falling over?' at first, but...

S
And was it the same across the 4 days?

M
Yes.

S
Right.

M
Yes.

S
Yeah.

M
I could last about... well I *think* it was about 7 seconds! *[Laughs]*

S
Well fortunately, you don't have to...

M
[Laughs] Do that very often! *[Laughs]*

S
...do that; that's right, exactly! *[Laughs]* So you're ok! *[Laughs]* There was just one final thing that occurred to me, M, that I meant to ask earlier, which was that; do you feel you're *behaviour* changed in any way – in any particular way – across those 4 days of taking the saliva samples?

M
Erm...

S
At *any* stage, you know?

M
Well maybe what I had been doing, because it was a... I did it on a Monday morning, which may have not been the best day to do it, because I might do quite varied things at the weekend that involve me going to bed at half-two in the morning.

S
Right.

M
Whereas midweek, I might have gone to bed at 11 o'clock; so there was a variation, I think, in times that I went to bed and things that I did at the weekend. But I don't think there was any real behaviour change; that would be normal for me, anyway, across those.

S
Ok. So there were no major changes you can think of, either the night before, or in the morning, or later on in the day?

M
No. No.

S
Ok. All right. Yes, well that's... that's all I wanted to ask.

M
Ok. Thank you. Thank you, Sanjay.

S
I think we've covered everything. Thank *you,* M!

M
Oh, you were really good at asking questions, and exploring, Sanjay!

S
Yeah?

M
Yeah!

S
Oh, that's... *thanks*, M; that's always... do you know, it's always...

End of audio at 29.42

Appendix 14

Transcript Natasha and Glaucoma

FILE: P003_M W P2003.mp3

Key:

IV – Interviewer (female)

P – Participant (female)

Start of audio at 00.14

IV

Ok, now so you took part in the study. How many weeks gone now? I think it's a couple of weeks. It's been a good couple of weeks, isn't it?

P

Yes

IV

And in terms of, you know, your sort of main reasons for taking part and getting in contact with me, myself, and actually talking about you know varying sort of reasons why people may or may not take, what were your reasons for taking part?

P

I've been to a member of the IGA for a couple of years. I've got more interested in the condition then realize that there's a lot of research going on about the condition. So, I thought it was something I could do to help out with and get that one knowledge out there

IV

Yeah, absolutely. And have you been taking part in research before? I think you mentioned

P
I think I took part in a study from City University. They were working a way of detecting defects in the fields of view. Using a computer based laptop and filled desks with the idea that this would be a cheap way of doing it, which in any sort of countries I can't really afford these expensive field views, which means

IV
Of course, really be quite helpful as well. But anything in terms of this kind of research, in terms of cortisol research where you've taken many saliva samples in the morning and questioned well against questionnaires would be bad, but anything where you'd have to take any sort of saliva samples for research?

P
No.

IV
No. And now in terms of your general feelings about participation, what were they? If you want to sort of summarize how you felt about actually participating, perhaps after I spoke to you the phone and I mentioned all the aspect? What were your feelings?

P
I think this was quite, it wasn't a very destructive procedure, really a couple of mornings.

IV
Yeah

P
Push away. I have to rearrange how did things. It didn't seem to be any risk, made it clear. So I was quite happy with that. I thought it might be useful to put a bit of extra knowledge? *[inaudible, – 2.45]*

IV

Yeah absolutely. It's always very helpful. And now if I was to sort of explore your experiences of like the different aspects of the study and there was different. I mean this study was arranged a way where you have some aspects that you had to complete questionnaires, a diary. But then there was aspects of wearing on watch, putting the device near your bedside table. How did you feel, say in preparation for the night, the night before the actual study? When you were aware that you needed to provide, you know saliva samples the following morning? How were you then? Can you recall back to when you were at that initial stage that first night?

P
Okay. Well, I sort of arranged everything on my bedside table. So for going into bed, I had to watch a device from covering the light levels.

IV
Yeah

P
And then finally, the swab the tube. The bottle with slips I should say really put on bedside table. So I knew I had to do that in the evening. And I put the diary as well on my bedside table. So in the evening I had everything to hand. And I also took the tubes

IV
Oh yeah

P
I put and arranged with a *[inaudible, – 4.25]* the first one for the first day. I got them in order.

IV
Did you find that being organized for something that, you know, sort of helped, but it provided the study pack a good week in advance? And did you feel that that's helpful tools? *[inaudible, cross talk – 4.39]*

P
Yeah, I think so, because I could take it on board and look for it. Check out the tubes or devices

IV
Yeah. Oh good. And How did your bedtime routine or even experience change from your usual?

P
Ok. Well I switched the alarm clock on very early, I don't have to get up early because I'm retired now, but through woke up the radio, listen to the news programs. So I think a gradual waking experience, plus also why I put in an alarm so make sure I take my eye drops once in a morning and evening so

IV
Yeah

P
I am conscious of taking those exactly. So, I have a timer at seven o'clock in the morning *[inaudible, cross talk – 5.30]*

IV
Do you set a timer before going to bed?

P
Well it's on my phone

IV
Ah I see

P
[inaudible, cross talk – 5.35] It's actually got a message on it which tells people what drops to take or what eyes to put in. Cause it always changes, but I decided not to do that because I'm in discussion with you

IV
Yeah

P
Before we put any eyedrops in, they do tend to go through the *[inaudible, cross talk – 5.56]*. Sometimes you get a funny taste. Yeah.

IV
Yeah. Yes, of course

P

So I changed my routine. I switch off the alarm clock and I didn't take my eye drops too often. I'm taking on the swabs

IV I see. What about that.. sorry. In terms of bed time, do you take any eyedrops for bed as well before bed?

P
I don't take them before bed. I've got one. So one was for states 7 o'clock in the morning, 7 o'clock in the evening. And the other one, which is trouble post and from the discussion group suebash *[??, -06:32]*.

IV
Yes

P It works best when your eye pressure is high, this high during the early hours in the morning, so it's best to take it between about half nine and ten o'clock in the evening

IV
I see

P
Where it takes. So it comes into effect from the point where your eye pressure is higher. So I think I take that which is ten in the evening.

IV
Right. And did that crash with any sampling times?

P
No it didn't

IV
It didn't. So you were able to do the evening sample and then take the eye drop or

P
I did, I put the eye drops at quarter of 10 and did the evening sample after that

IV

Perfect, yeah that's okay.

P
Well I was far as I was taking the evening samples normally without level *[inaudible, -07:15]*.

IV
Sure, sure. Okay. I guess that would be the thing that sort of you didn't do anything differently, as it were then, other than

P
Well, because I eat breakfast as well.

IV
Oh yeah, well. That's where I'm going to come into actually. Because in terms of the experience if you tell me a little bit more about what the experience was like for taking the morning sample and it'll be a little bit more. Because I think you mentioned you touched the point of reset that routine changing but actually taking it. How long was it between when you woke up and collecting that very first sample? Do you recall that?

P
Yes. I seem to have a disturbed sleep pattern because sometimes I wake up in the middle of the night.

IV
Sure

P
And so usually I don't know when it's a middle of night. Then go back to sleep, but well as I wake up in the early morning, I did this in probably February. So it was still quite dark

IV
Right

P
Even when I felt like I was gonna get up. So there was a little bit of a range**,** what I was gonna get up now because I switched off the alarms.

IV
I see

P
So that was a bit difficult.

IV
Yes, of course.

P
When I had woken up, was it to go back to sleep again?

IV
Really, So what time was that? Do you recall about what time that might've been?

P
Well, it was probably around 7 o'clock, which is my usual wake up time.

IV
Okay

P
But sometimes it was earlier, sometimes it was later

IV
I see, fair enough

P
But I'll tell you just two days, but *[inaudible, cross-talking – 9.09].*

IV
Yes, of course. And in terms of sort of collecting the samples, as soon as you wake, did you know, if it was, so one of the days you said you might wake up a bit earlier. But do you know from one you wake up and open to take that first sample? Do you know If there is a gap in between?

P
Probably with a bit of gap

IV
Yeah. So can you sort of recall it?

P
Okay, maybe one a few minutes

IV
Yeah. Fair enough. No. Fair enough.

P So then I had to took out the first swab and chew that. I only had my first white bottle, the container

IV
Yeah

P
So I had that one. I also called the time *[??, – 10.01]*. I had a five minute time on my phone so I triggered that off. So I think about

IV
Yeah 15 min. Did you set the time as before going to bed or was that something that you just start it when you look up

P
Yeah, just started it

IV
Yeah. Fair enough.

P
But that was just a reminder

IV
Ah of course

P
I have a digital clock on my bedside table and so obviously recorded the time and took for a sample, I could just add 15 min

IV
Yeah

P
The timer was just the backup. Cause the time was setting good when you done the first chew a little bit like

IV
Yes. How did you feel of the time passing between the turn when you wake up and taking your first sample and 45 minutes. How was that?

P
I stayed in the bed actually

IV
Did you? Yeah

P
Yeah

IV
That's fair enough.

P
I didn't have to.. Other days I had to get up and do anything.. *[inaudible, cross-talk – 10.56]*

IV
Yeah and were you fairly sort of okay with doing every 15 minutes?

P
Yeah it was okay

IV
Okay good

P
I think about chewing the swab

IV
Yeah

P
I don't know. I've done it for like a long saliva on

IV
Yeah

P
But then *[inaudible , – 11.15]*. So I was chewing it and I wasn't sure how I had to chew it for

IV

Oh dear. Well it was only a couple of minutes suddenly fit in the guidelines. But how did you find the taste of it? It was the way you came with you?

P
I was just *[inaudible , – 11.36 - 11.44]*

IV
Good, yes of course. That's it. Well actually it's amazing because once you start to chewing it, you automatically produce more and more saliva.

P
Yes

IV
Yeah. As we suggest a couple of minutes and then you just spit it back out into the tube. Now, how did the experience, you took one sample in the evening. How did the experience in taking samples in the morning? The first waking sample differ from the evening sample? Would you, I mean those big difference in terms of day and night but how did that differ for you?

P
It wasn't really that much different. The only thing was the one going to bed, I did it and then off to the otherside claiming to go to bed again. So it's just sloppy. Yeah *[inaudible , – 12.40]*

IV
Yeah. Fair enough. And then tell me about the sort of other thought that's in terms of your thoughts about the other aspects of the study, for example, wearing the watch and putting that light on your bedside table. Tell me a little bit more about that in terms of going to sleep with that, you know, little things like that.

P
I didn't have any problems with the thing on the bedside table. Watch. I don't normally wear a watch or anything. So that was different, but it wasn't the heavy thing. There was no display on it. It was sort of structure or anything

IV

Oh good, I was glad that

P
I had to push a button to tell me the time

IV
Yes exactly

P
[inaudible, – 13.31]

IV
Good good. And in terms of the sort of did you find it effected your sleep to wear the watch?

P
No

IV
And in terms of generally how was your sleep between during these two days?

P
Well, it was probably similar to normal, I do wake up occasionally during night, so *[inaudible, – 13.53]*

IV
Yeah fair enough. And that's the normal?

P
Yes

IV
Yeah, that's fair enough. Okay. So one of the most crucial aspects of the study was making sure participants get a saliva sample from the very moment of awakening. How do you determine your moment of awakening? How was it? How did it happen that?

P
That's … *[inaudible , – 14.27]* isn't it?

IV
Yeah

P
You know that’s a sort of, you know when you're awake you know that you’re awake

IV
So that’s it? I mean, how would you say it? And also, it's very you know, it's an interesting question that because it must differ from one person to the other, which is what I'm assuming but it could be a general thing. But how would you determine your own personal awakening? How would you know that okay this is the moment I am, I me?

P
Yeah, right. Probably when I can aware of the things outside, I can not dreaming or something else, I guess. Consciousness is really surrounded

IV
Yeah, fair enough

P
So it's awakening … *[inaudible , – 15.17 – 15.21]*

IV
Sure. In comparison, for example to you mention that you do have little sleep disruption of the night. How would you compare those sleep disruptions that you are and you notice that sleep disruptions because you are awake? How would you compare that to morning awaking? Is there anything in particular that you do to sort of?

P
If I have to go physical thinking, it might be the check the time

IV
Yeah

P
And also I just turn over, around over

IV
Fair enough

P
[inaudible , – 16.03]

IV
Yeah fair enough. But then how would you sort of after gaining consciousness sort of how long did it take you to feel fully alert? Even if you go back to this morning

P
Yeah

IV
So from gaining consciousness how long would that take you to sort of you're okay, right I'm ready for the day?

P
I probably wait for something, someone alarms and I sort of doze off for the bed. I sort of hear the radio in the background. And sometimes it merges with the dream because I dream about some similar. There's something strange there. Probably this is for radio. Its influences so this is similar to a transitional period.

IV
Yeah fair enough

P
Eventually you do wake up and you realize what were you dreaming about? And then you realize it's something that you really then you realize your conscious

IV
Yes fair enough. That was brilliant and so let me see. In terms of some sort of *[inaudible , – 17.24]* on the actual wakening experience and trying to understand it from your perspective a little more. What is your usual week so what do you normally do in the morning? What's your usual routine for waking up?

P

Okay well I said normally I have a radio little bit earlier so I listen to that

IV
Yeah

P
Probably *[inaudible , – 17.47]*

IV
And the radio, does it come automatically?

P
Yeah.

IV
And what time does that automatically come?

P
Quarter to six.

IV
Quarter to six and would it be something that you would obviously listen to?

So you've got that on a time out around quarter to six?

P
Yes

IV
What do you *[inaudible , – 18.03]*

P
… *[inaudible , – 18.04]*

IV
… *[inaudible , – 18.05].* So has it been a time that the radio hasn't turned on?

P
Sorry?

IV
Has it been a time that the media hasn't turned on?

P
Well, I have switched off that for the study actually.

IV Ah did you?

P
Yeah

IV
So actually that's I didn't know that. So you switched off it for the study. How did you find waking up in those two day?

P

[inaudible , – 18.31] So I sort of became conscious, normally start waking up, which is not the time the radio goes off. Well, it's not the time only get up. That's a good thing because I only have this radio quite early and then when the alarm goes off to take my eyedrops and I have a coffee comes onautomatically at that time

IV
Sure, ah so the coffee automatically starts making as well

P
Yeah but I switched all of them off for the study

IV
I see

P
So I looked at the time

IV
I see. What was the difference in terms of waking up during the study time when you found switched off everything in comparison to when you normally wake? Was there anything comparisons? Can you make comparisons?

P
Well, I seem to assume to get out of bed or realize it's fully awake about the same time, which is around 7 o'clock

IV

Hmm I see, that's interesting. And did you feel any different?

P
Yeah, it was coffee

IV
That's problem, the coffee *[laughs – 19.35]*

P
My coffee *[inaudible, laughs – 19.37]*

IV
Of course. It's really interesting and actually tell me a little bit more when you do wake up and getting out of bed and carrying on with your day to day business, how does that you should take you? Or you want to sort of ..

P
You know only fairly sort of structured really because

IV
Yes

P
Seven o clock, take the eyedrops, coffee is on then I can get breakfast.

IV
Fair enough. So is it very much an active waking that you have and it's a routine that you obviously have

P
Yes

IV
And did you find that the study affected your sleep? I mentioned, asked you that before, but did it effectual to your sleep any sort of way other than your normal disruptions?

P
No

IV
Okay, that's good to know.

P

IV
Yeah just wanted to see if you have any sort of con many thing

P
I felt only sort of stressed out

IV
Good, good. Don't worry, the last thing we want to do consider your stress, oh my gosh *[laughs – 21.04]*. And actually we came sort of the end of it. Actually we're trying to understand a little bit more about the awakening process and even this speaking about it, it is very interesting. Did you want to add anything in terms of morning awakening, in terms of first of all just taking that sample or waking up, or even gaining consciousness, taking samples, getting up? Do you have any sort of thoughts on that for yourself?

P
Not really. I don't think I was sort of dozing off between the sample

IV
Sure

P
So once I waking up and taking the first sample, I was sort of quite conscious of it. I feel I need to pull back to sleep again. I don't know If they're affected subsequent samples

IV
No that's okay at all

P
So

IV
Oh and then anything else you'd like to add to the general study and then anything else that you feel might be..?

P

Yeah, it was the questions to fill in. There were couple of questions which I answered to do with my eyesight. What was that? Did you find difficulties at night?

IV
Yeah

P
I felt like I did. I'm not sure that's affected the glaucoma or maybe

IV
Oh I see

P
Just the lights on the car generally, oncoming traffic, prior headlights, etc. And the other thing was

IV
So that was driving at night, yeah?

P
Yeah

IV
Yeah, fair enough.

P
The other thing when going downstairs you asked a question

IV
Yeah

P
I have a little bit difficulty with a lot of wearing vocal glasses

IV
Ah you mentioned that last time yes. Ah yes fair enough

P
Okay

IV

And yet you're absolutely right. Best to have that write down. Anything else that you also think of?

P
Umm, your questions or how I felt over the year that a seasonal variation? Well, the only thing I know seasonal is that it affects my time of waking

IV
Sure

P
But it's lighter in the summer months, I wake earlier

IV
Yes, and how are you finding now there's a transition with such transitioning into spring? How do you tend to find that your waking is affected? Do you find that you're waking earlier?

P
No, sunrise still surround the seven o'clock. So I go *[inaudible, – 23:49].* It's not normally that much light so I have more time

IV
Oh fair enough

P
That's the only seasonal variation, you have to think about right at *[inaudible, – 24:05].* I haven't noticed any variation of that.

IV
Yes

P
I don't tend to hide *[inaudible, – 24:13]*

IV
Fair enough, a lot of people do. And I just meant one of the main reasons we ask that actually is just due to the fact that some may be so seasonal and it is quite prominent in it. They have marked changes. So it's really interesting to understand what is going on there as well. See, that's one of the reason and also way of measuring the lighting in the environment. I'm

also, you know, obviously recording the times that you've conducted to the study. So I'll know you'd be winter slash spring. Whether that has anything to do with, you know, the amount of light that's in the room at the time. So it's always good to have some sort of form of control. So, yeah. Oh okay Mark, Thank you so much

End of audio at 25.05