Diurnal preference, clock gene polymorphisms and personality

by

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Abstract

Recent research has shown that morningness is negatively correlated with the personality variable impulsivity. Additionally, a relationship between conscientiousness and morningness has been reported. As several researchers have demonstrated links between clock gene polymorphisms and diurnal preference, this thesis aims to test the possibility that there is a direct link between these polymorphisms and personality. A total of 617 participants completed an online survey consisting of well known self report questionnaires relating to personality and diurnal preference. Conscientiousness was found to be the largest predictor of diurnal preference, with associations also evident with openness to experience and agreeableness. The relationship between impulsivity and eveningness was also confirmed. 174 participants were invited to donate buccal swabs and high and low conscientiousness groups were formed from the 20% extremes of conscientiousness score. A central 20% formed an intermediate group Genotyping of these subjects in relation to clock gene polymorphisms known to associate with diurnal preference was then undertaken. No differences in genotype were observed between conscientiousness groups in any of the polymorphisms. Further analysis of possible relationships between genotype and personality variables revealed associations between *CLOCK* rs11932595 and neuroticism, impulsivity and openness, before Bonferroni correction. 97 participants within the conscientiousness groups completed the GoStop behavioural test. No differences were observed between conscientiousness groups on the primary measure of the task. Furthermore, no relationship was observed between self-report and behavioural impulsivity. In relation to genotype, a longer mean stop latency was associated with *PER3* VNTR 5/5 genotype, before Bonferroni correction. In conclusion, conscientiousness is the primary personality dimension associated with diurnal preference and should be considered in future studies. Moreover, two possible

candidate clock gene polymorphisms that may relate to personality were also identified, the replication of which in future studies may contribute to the understanding of the underlying genetic basis of personality.

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Declaration of originality

This thesis and the work to which it refers are the results of my own efforts. Any ideas, data, images or text resulting from the work of others (whether published or unpublished) are fully identified as such within the work and attributed to their originator in the text, bibliography or in footnotes. This thesis has not been submitted in whole or in part for any other academic degree or professional qualification. I agree that the University has the right to submit my work to the plagiarism detection service TurnitinUK for originality checks. Whether or not drafts have been so-assessed, the University reserves the right to require an electronic version of the final document (as submitted) for assessment as above.

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Abbreviations

ADHD	Attention deficit/ hyperactivity disorder
ANOVA	Analysis of variance
ARAS	Ascending reticular activating system
ARMS	Amplification refractory mutagenesis system
BAS	Behavioural approach system
BFI	Big five inventory
BIS	Behavioural inhibition system
BIS	Barratt impulsiveness scale
CPT	Continuous performance task
EDA	Electrodermal activity
EPI	Eysenck personality inventory
EPQ	Eysenck personality questionnaire
ERN	Error related negativity
ERP	Event related potential
FFM	Five factor model of personality
GPSQI	Global PSQI score
GWPQ	Gray-Wilson personality questionnaire
HADS	Hospital anxiety and depression scale
I_7	Impulsiveness 7 subscale
IMT/DMT	Immediate and delayed memory tasks
IPIP	International Personality Item Pool
IQ	Intelligence quotient

IVE-7	Impulsiveness-venturesomeness- empathy-7 questionnaire
MANOVA	Multivariate analysis of variance
MFFT	Matching familiar figures test
NHL	Non-Hodgkins lymphoma
NEO-FFI	Neuroticism-extraversion-openness five factor inventory
NEO-PI	Neuroticism-extraversion-openness personality inventory
NEO-PIR	Neuroticism-extraversion-openness personality inventory revised
PCR	Polymerase chain reaction
PEN	Pyschoticism-extraversion-neuroticism
PSQI	Pittsburgh sleep quality index
RFLP	Restriction fragment length polymorphism
SCN	Suprachiasmatic nuclei
SIMP	Single item measure of personality
SRL	Skin resistance level
SRR	Skin resistance response
SSRT	Stop signal reaction time
TBE	Tris-Boric acid-EDTA
TDA	Trait descriptive adjectives
TIPI	Ten item personality measure
UPPS	Urgency-premeditation-perseverance-sensation seeking scale
VIF	Variance inflation factor

Chapter 1

1.0 Introduction

It has long been debated as to what extent human behaviour is a result of our genetic makeup as opposed to environmental influences. Sir Francis Galton was the first to conduct research that investigated the possible heritability of human behavioural traits. In 1869, he published work that investigated the families of 'highly gifted men' classifying them according to occupation and achievement. He found that the level of 'giftedness' or intelligence decreased the further removed a family member was to the subject (Galton, 1869). However, he was also concerned whether environmental factors influenced this trait and coined the term 'nature' versus 'nurture', where he tried to ascertain whether a person's achievements were due to their natural makeup, or through other means such as encouragement by others or help from relatives to reach an eminent position. Galton recognised the confounding effects of the environment in such studies and proposed that such research would be best carried out by the comparison of twins (Galton, 1875).

Galton's speculations about these studies have led to his work being built upon and expanded beyond intelligence and into research on personality. This has mainly been done through comparisons of monozygotic and dizygotic twins. Twin studies enable researchers to investigate the heredity of certain traits, by seeing whether monozyotic twins (who are genetically identical) are similar to dizygotic twins (who only share 50% of their genes). Environmental influences can also be tested through adoption studies that look at twins who have been reared apart. Genetically, monozygotic twins are twice as similar as dizygotic twins which means that heritability estimates, which tell us what proportion of a trait is the result of

genetic effects, can be calculated from theses studies by subtracting the correlational statistic of dizygotic twins from that of monozygotic twins and then doubling the result. The remainder is then considered the result of environmental influences.

Several studies have examined the personality traits of the five factor model of personality which include neuroticism, openness, agreeableness, conscientiousness and extraversion (see section 1.7.2). These dimensions were assessed in twins, in shared and non-shared environments, and it was found that the heritability among these factors were 0.48 ± 0.1, or 48% with the other 52% made up of environmental factors (Jang et al., 1996, Riemann et al., 1997).

Researchers investigating the genetic basis of personality have examined variation in various genes with those coding for neurotransmitters and their receptors proving the most popular (Noblett and Coccaro, 2005). Other avenues of research have recently emerged that could lead researchers to look at genes involved in other biological processes, such as circadian rhythms, as candidates for association with personality (Caci et al., 2004, Caci et al., 2005, DeYoung et al., 2007). These rhythms are biological processes that cycle for approximately 24 hours, differences in which can result in variation in diurnal preference, where an individual is either a morning type (where they wake early and retire to bed early) or an evening type (where they wake late and retire to bed late). These morning and evening types are already known to associate with genes involved in the generation of circadian rhythms (Archer et al., 2003, Carpen et al., 2005) and a recent study has identified an association between an individual's diurnal preference and how impulsive that person is (Caci et al, 2005). This study based its investigation on previous research that had implicated circadian rhythm disturbances in bipolar mood disorders (Mayeda and Nurnberger, 1998), which in turn have been linked to impulsivity (Moeller et al., 2001). Furthermore, regulation of the circadian

clock involves the neurotransmitter serotonin (Reiter, 1998) which is negatively associated to impulsivity (Soubrié, 1986). With this evidence of a link between circadian rhythms and personality, it is conceivable that the genes that underlie these rhythms may also influence an individual's personality.

1.1 Circadian rhythms and the circadian clock

Most organisms exhibit daily rhythms with a period length of close to 24 hours. Even under constant conditions and without any external time cues, these rhythms persist, indicating the presence of an autonomous intrinsic timing mechanism (Ko and Takahashi, 2006). Animals kept in constant darkness for extended periods function with a free-running rhythm as a result of internal de-synchrony, where their endogenous rhythm becomes uncoupled from that imposed by the light/dark cycle. In other words, they still exhibit a consolidated sleep-wake cycle without external cues. Depending on whether this endogenous period, τ (*tau*), is longer or shorter than 24 hours, their rest-activity cycle is pushed either forward or back from the endogenous one. The rhythm can be reset and synchronised or entrained by environmental cues known as *Zeitgebers*, the strongest of which is light.

This system is known as a circadian clock, where the term circadian is taken from the Latin *circa* (about) and *dies* (day). Biological rhythms with periods less than 20 hours are known as ultradian rhythms, for example heart rate, and those greatly over 24 hours, such as the menstrual cycle, are known as infradian rhythms. Figure 1-1 shows the characteristics of a circadian rhythm where the acrophase is the peak of the rhythm and nadir the trough. The amplitude is the acrophase to trough difference of the rhythm and the period is one complete cycle.

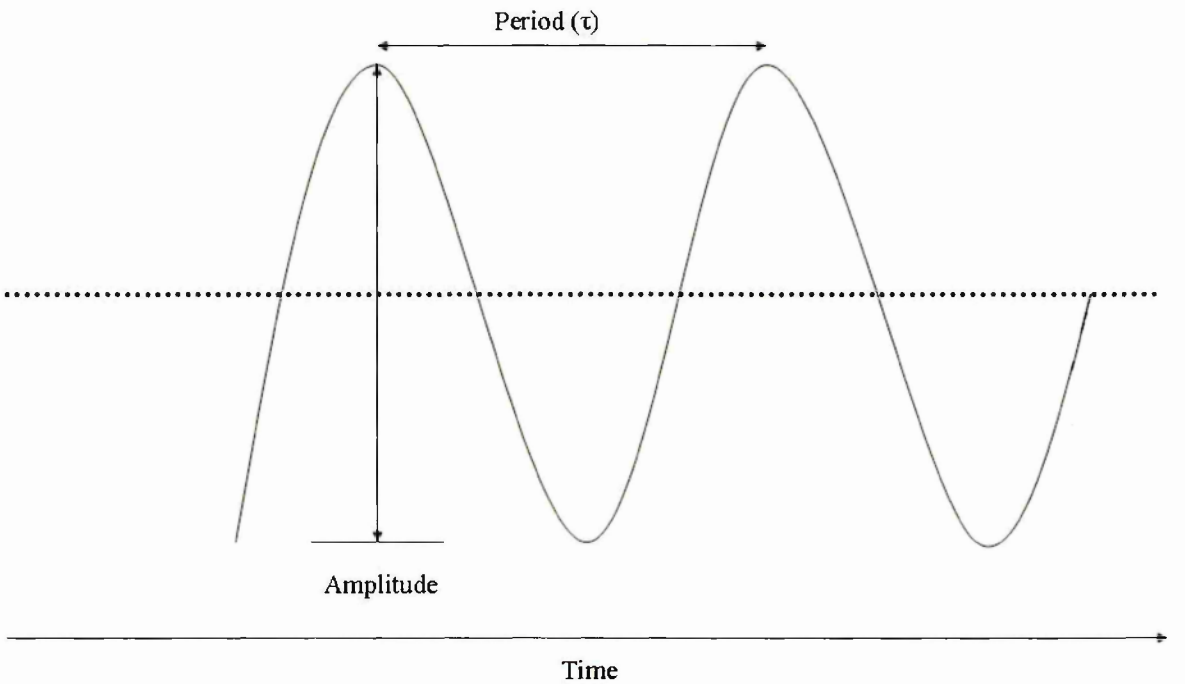


Figure 1-1 Characteristics of a circadian rhythm.

The circadian clock is present in numerous tissues and cell types that appear to be organised in a hierarchical manner in all mammals (Reppert and Weaver, 2002). The suprachiasmatic nuclei (SCN), a pair of neuronal clusters located in the hypothalamus, act as a master pacemaker and determine an individual's τ. This is reset mainly by external light detected by the retina and which in turn resets subservient clocks in peripheral tissues (peripheral clocks), such as the liver (Yamazaki et al., 2000). These peripheral clocks can synchronise independently of the SCN if there is a forced change of feeding time, indicating, (in addition to the indirect effect of light) the feeding signal may also be a dominant time cue (Mendoza, 2007). Indeed, restricted feeding causes many circulating macronutrients to become available

(Hastings et al., 2003) leading to either the resetting or induction of clock gene expression in peripheral oscillators (Hirota et al., 2002, Davidson et al., 2003).

1.1.1 Discovery of the circadian clock

1.1.1.1 The Suprachiasmatic nuclei (SCN)

In the 1920's, Curtis Richter performed some of the first experiments on rhythmic behaviours in rats and hypothesized that these behaviours occurred at specific times due to an endogenous clock mechanism (Foster and Kreitzman, 2004). He later identified an area in the brain, the hypothalamus, in which lesions caused arrhythmia, thus confirming his theory (Richter, 1967). Following on from this, research by Stephan and Zucker (1972) and Moore and Eichler (1972) revealed that the lesioning of the suprachiasmatic nuclei (SCN) resulted in the complete loss of behavioural and endocrine circadian rhythms. Subsequent experiments could not conclusively prove that the SCN was solely responsible for generating the endogenous period. This was until 1990, when Ralph and colleagues (Ralph et al., 1990) discovered a mutant hamster with a short period length. They transplanted the SCN from the mutant into a hamster whose SCN had been destroyed and found that circadian rhythms were restored in the normal animal but that it had also acquired the short period of the donor hamster. This was the final confirmation that the SCN contained the circadian clock.

1.1.1.2 The molecular clock

Whilst research into the location of the circadian clock was ongoing, Konopka & Benzer (1971) discovered mutants in Drosophila with altered endogenous periods. From this they identified a single gene that was the cause of the change in period length and named it *period*

(*per*). The resulting PER protein showed a rhythmicity of 24 hours (Siwicki et al., 1988) and gave the first indication of a possible molecular mechanism. In 1995, a second clock gene was discovered in Drosophilia, named *timeless* (*tim*) (Sehgal et al., 1995) which was expressed in a similar fashion to *per* and so resulted in the revision of the clock model.

This breakthrough in Drosophila led to the discovery of a similar mechanism mammals. In 1994, Vitaterna and colleagues identified a mutant mouse with a long circadian period, labelling the causative gene - circadian locomotor output cycles kaput (*Clock*) (Vitaterna et al., 1994). A search for homology between mice and drosophila pinpointed the same gene in Drosophila.

Following on from these discoveries, several more genes emerged to form part of the mammalian clock. A paralogue of clock, neuronal Per-Arnt-Sim protein 2 (*Npas2*) (King et al., 1997) was identified as well as brain and muscle arnt-like proteins 1 and 2 (*Bmal1* and *Bmal2*) (Gekakis et al., 1998). These genes were thought to form part of a positive limb whereas the negative limb was made up of proteins encoded by cryptochrome 1 and 2 (*Cry1* and *Cry2*) (Todo et al., 1996) as well as orthologues of the Drosophila *per* gene, the period genes 1, 2 and 3 (*Per1*, *Per2* and *Per3*) (Sun et al., 1997, Tei et al., 1997, Takumi et al., 1998). A secondary loop containing orphan nuclear receptors, retinoid orphan receptor (*Ror*) (Sato et al., 2004, Akashi and Takumi, 2005) and reverse of erythroblastic leukaemia virus α (*Rev-erb α*) (Preitner et al., 2002) was also identified.

With these genes established, a model of the clock mechanism has been proposed but with other elements being discovered all the time, it is likely to become ever more complex. An outline of the current model will be discussed together with details of the input and output pathways of the oscillator.

1.1.2 The circadian clock input pathway

In mammals, light is perceived by the retina, where the conversion of electromagnetic radiation into neural signals occurs in the photoreceptors. Until recently, it was thought that there were only two types of photoreceptors - rods and cones - but research has shown that there are others which mediate the entraining effect of light on the circadian clock. Foster and co-workers (Freedman et al., 1999) found that mice lacking both rods and cones can still be entrained by light. This led to the discovery of the photopigment melanopsin (Provencio et al., 2000, Hattar et al., 2002) located in mammals in a network of intrinsically photosensitive retinal ganglion cells. The axons of these cells form part of the retinohypothalamic tract (RHT), which projects directly into the retinorecipient region of the SCN where input from these axons plays a pivotal part in the entrainment pathway (Hattar et al., 2006).

This entrainment pathway is essential, as the endogenous period of the SCN is not exactly 24 hours and so needs to be synchronised to the daily light/dark cycle. Light at dawn and dusk stimulates the retinal ganglion cells and projects to the SCN through the RHT where the primary neurotransmitter glutamate, with pituitary adenylate cyclase activating peptide (PACAP) released as a co-transmitter, mediate the synchronising properties of light (Hannibal et al., 2002). Intracellular concentrations of Ca^{2+} and cAMP then increase, which induces phosphorylation of the Ca^{2+}/ cAMP response element binding protein (CREB) (Ginty et al., 1993) that bind to Ca^{2+}/ cAMP response elements (CRE) on a number of immediate early genes including *c-fos* (Rusak et al., 1990) as well as *Per1* and *Per2* (Albrecht et al., 1997) inducing their transcription. With prolonged exposure to light, further genes are induced (Reddy et al., 2002) and as a result, adjustments made through light at dawn and dusk maintain 24-hour periodicity (Hastings et al., 2003).

1.1.3 Positive and negative feedback loops

Within the SCN and periphery, a number of clock genes form positive and negative feedback loops integral to the circadian clock. Figure 1-2 shows how the expression of oscillating negative components is promoted by the transcription factors CLOCK and BMAL1. These proteins contain a basic helix-loop-helix (bHLH) and a Per-ARNT-Sim (PAS) domain, which enable DNA binding and heterodimerisation (the binding of two different proteins), respectively (Edery, 2000). The heterodimers appear to permanently bind to sequences known as E-boxes (CACGTG nucleotide motif) located in the promoter region of the *Per* and *Cry* genes (Gekakis et al., 1998, Hogenesch et al., 1998, Lee et al., 2001). This complex drives their expression in the nucleus through histone acetylation (Etchegaray et al., 2003) as well as that of the retinoic acid related orphan receptors *Rev-erbα* and *Rorα*. CLOCK has intrinsic histone acetyltransferase activity (Doi et al., 2006) but a conditional knock-out of CLOCK did not eliminate circadian rhythms in mice (Debruyne et al., 2006). This could be due to a paralogue of CLOCK, NPAS2, being able to compensate for the loss of CLOCK (Gallego and Virshup, 2007). NPAS2 is interchangeable with the CLOCK protein forming heterodimers with BMAL1 (Hogenesch et al., 1998). In a variety of tissues and organs, such as the heart, kidney and liver a paralogue of BMAL1, known as BMAL2 plays a similar role (Schoenhard et al., 2002). When a complex is formed between CLOCK and BMAL, CRY2 has been shown to inhibit the CLOCK/BMAL1 complex more strongly than the CLOCK/BMAL2 complex. Conversely, PER2 inhibited the CLOCK/BMAL2 stronger than CLOCK/BMAL1 suggesting the roles of the two paralogues may differ (Sasaki et al., 2009).

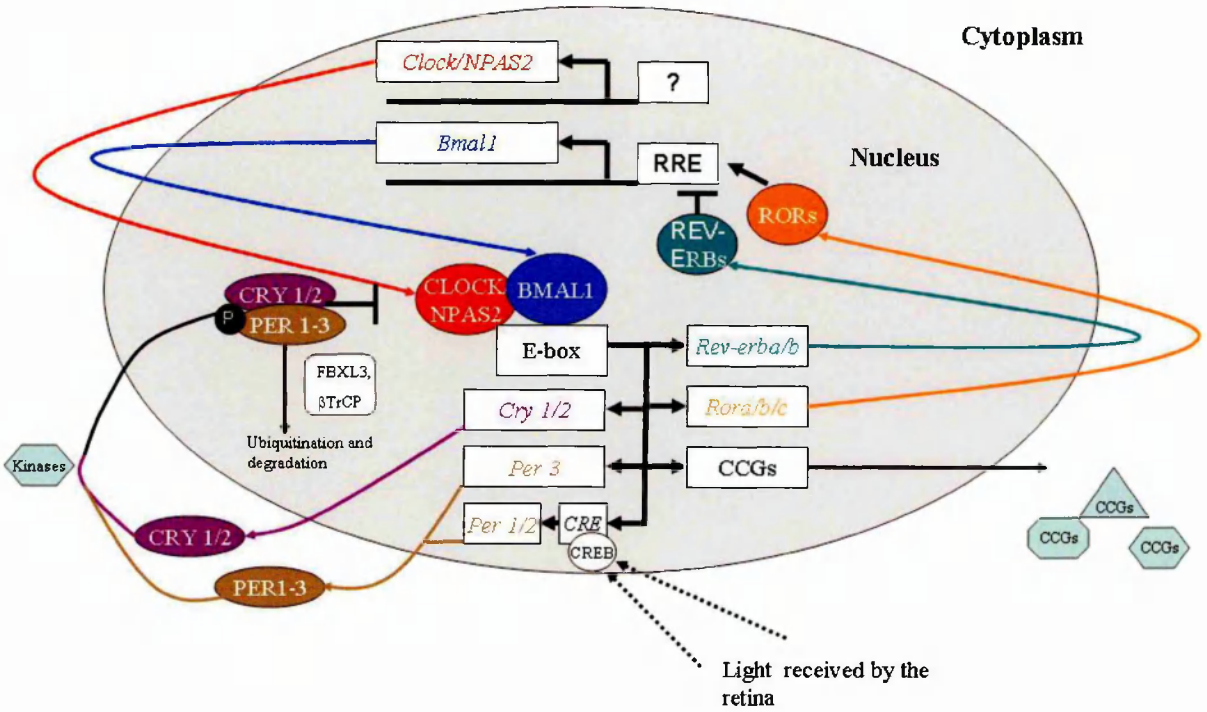


Figure 1-2. The circadian clock mechanism. Adapted from Barnard and Nolan (2008).

The negative loop involves three PERIOD proteins (PER1, PER2 and PER3) and two CRYPTOCHROME proteins (CRY1 and CRY2), which are translated in the cytoplasm from their respective transcripts. During the day, these proteins accumulate in the cytoplasm and in the evening trimerisation occurs, where a complex is formed between PER, CRY and the enzyme Casein kinase Iδ (CKIδ) or Casein kinase Iε (CKIε) (Lee et al., 2001). CKI adds a phosphate group (phosphorylates) to PER and then, at night, the complex is transported to the nucleus where it inhibits CLOCK/BMAL1 action (Griffin et al., 1999, Kume et al., 1999, Vitaterna et al., 1999). Additionally, CK2 phosphorylates PER2 whilst acting with CKI to promote degradation (Tsuchiya et al., 2009), as well as phosphorylating BMAL1 to aid nuclear accumulation (Tamaru et al., 2009). As PER and CRY do not contain bHLH regions, repression cannot be achieved through promoter attachment. Instead, CRY is able to bind to

the C-terminus of BMAL1, thereby inducing CLOCK/BMAL1 phosphorylation and reducing transcription of *Per*, *Cry* and *Rev-erbα* (Kiyohara et al., 2006, Satoh et al., 2006).

In addition to this, REV-ERBα and RORα are able to regulate *Bmal1* by competing to bind to retinoic acid-related orphan receptor response elements (RORES) in the *Bmal1* promoter (Guillaumond et al., 2005). This is achieved through REV-ERBs repression of the transcription of *Bmal1* (Preitner et al., 2002, Guillaumond et al., 2005) and RORs activation of it (Sato et al., 2004, Akashi and Takumi, 2005, Guillaumond et al., 2005).

By morning, regulated degradation of PER, CRY and REV-ERBα proteins leads to the activation of *Bmal1,* whereas *Clock* is expressed constitutively. *Bmal1* is then transcribed to produce new CLOCK/BMAL1 transcription factors that reinitiate a new circadian cycle, allowing the mRNA and protein levels of *Per* and *Cry* to begin oscillating again. Degradation of the PER/CRY complex is thought to be initiated through binding of F-box proteins to the clock proteins after phosphorylation. These proteins act as ubiquitin ligase adapter molecules, which lead to the formation of polyubiquitin chains that assist the binding of the targeted protein to a 26*S* proteasome, resulting in protein degradation (Sehgal, 2004).

Recent research suggests that the F-box protein FBX13 is involved in the degradation of CRY. Godinho et al. (2007) found that a mutation in *Fbxl3* resulted in an extended τ and a further study involving the same mutation, revealed the extended period was due to the decreased ability of the mutant to bind and induce the proteolysis of CRY proteins (Busino et al., 2007). Another F-box protein, β-TrCP, binds to PER1 (Shirogane et al., 2005) and PER2 (Reischl et al., 2007) and facilitates degradation.

Further components have been identified that aid circadian clock function. WDR5 methyl transferase activity was shown to repress CLOCK/BMAL1-mediated transcription of the PER2 and CRY1 proteins. Furthermore, a reduction in the DNA and RNA binding protein NONO, achieved through RNA interference experiments, led to constantly low levels of *rev-erb* and Albumin D element binding protein (*dbp* - a clock controlled gene, see section 1.1.4) suggesting that it antagonises the repression of these genes (Brown et al., 2005).

As well as CKI, other kinases such as glycogen synthase kinase 3 (GSK3) and mitogen-activated protein kinase (MAPK) phosphorylate certain clock proteins (Sanada et al., 2002). BMAL1 is a substrate for MAPK (Sanada et al., 2002) and GSK-3 phosphorylates CRY2 (Harada et al., 2005), PER2 (Iitaka et al., 2005) and REV-ERBα (Yin et al., 2006). Phosphorylation by this kinase appears to have different effects depending on the clock substrate (Dardente and Cermakian, 2007). When CRY2 and REV-ERBα are phosphorylated, nuclear entry and/or retention in the nucleus are promoted (Dardente and Cermakian, 2007). CRY2 is then degraded (Harada et al., 2005) whereas REV-ERBα is stabilised and its degradation inhibited (Yin et al., 2006). These post-translational modifications are critical for establishing the 24-h periodicity of the clock (Lee et al., 2001).

1.1.4 The output pathway

The feedback loops regulate the expression of thousands of clock controlled genes (CCGs), which contain promoter E-boxes, D-boxes, RREs and CREs. Approximately 10% of all genes in tissues show circadian oscillation. These genes are able to confer the timing information of the circadian clock to the body by undergoing periodic transcriptional activation and repression by PER/CRY complexes (Hastings et al., 2003). This is achieved through the action of a number of factors. Among them are the secretory peptides arginine vasopressin

(Jin et al., 1999) and prokineticin 2 (PK2) (Cheng et al., 2002). The transcription of the genes encoding both of these is regulated by an E box enhancer. PK2 is directly regulated by CLOCK/BMAL heterodimers and has a pattern of circadian expression in the SCN (Cheng et al., 2002). Another output signal candidate is TGFα (Kramer et al., 2001) which, in the hamster, is also expressed in the SCN in a circadian fashion.

Transcription factors are also encoded by CCGs. These include *Dec1* and *Dec2*, which are thought to act as additional negative regulators as both proteins inhibit CLOCK/BMAL1 function. In addition to this, the CLOCK/BMAL1 transcription factor controls *Dec1* gene expression (Honma et al., 2002). *Dbp* is another such gene which coordinates downstream cascades of circadian gene expression (Reppert and Weaver, 2002). DBP activates genes through a D-box element and in turn conveys its rhythmicity (Lopez-Molina et al., 1997).

The neuroendocrine and autonomic systems are also essential in relaying circadian timing information to the rest of the body. The hormone melatonin transmits time of day and year information to the body and is secreted rhythmically, reaching its nadir (lowest point) during the day and acrophase (peak) at night (Skene and Arendt, 2006). Light suppresses melatonin secretion, which is received through the retina and relayed to the SCN (see section 1.1.2) The SCN controls melatonin synthesis via a multi-synaptic pathway ending in the release of noradrenaline in the pineal gland (Klein and Moore, 1979). The resulting rhythm is regulated through stimulation of melatonin synthesis at night by glutamate and inhibition during the day by Y-aminobutyric acid (GABA), another neurotransmitter (Perreau-Lenz et al., 2003). During the night, serotonin in the pineal gland is acetylated and then methylated to produce melatonin.

1.2 Variability in circadian phenotype and genotype

Variation in the endogenous period (τ) occurs naturally between species and within a species it follows a normal distribution. The average τ in humans is slightly greater than 24 hours (Czeisler et al., 1999), but individual differences have been attributed to age, with one study suggesting older subjects had a shorter period whereas another found the opposite (Weitzman et al., 1982, Kendall et al., 2001), season, with longer periods over the summer months (Wirz-Justice et al., 1984), sex, with women having a shorter τ (Wever, 1984) and race, with African Americans having a significantly shorter τ than other races (Smith et al., 2009).

Entrainment to the light/dark cycle occurs each day by phase advance of the clock if τ is longer than 24 hours and phase delay if shorter than 24 hours. Activity begins later in an animal with a longer τ relative to the light/dark cycle than in an animal of the same species with a shorter τ. In humans, a longer τ has been shown to correspond to a later habitual wake time (Duffy et al., 2001).

Measurement of τ has been achieved through forced desynchcrony protocols where subjects are required to maintain a 28 hour sleep/wake cycle, under constant conditions (Dijk and Czeisler, 1994). As humans cannot entrain to this cycle, the result is the uncoupling of the sleep/wake cycle from the endogenous period enabling researchers to measure it.

Extreme variation in τ may be linked to circadian rhythm disorders, which alter the phase of the sleep-wake cycle resulting in differences in sleep timing (Jones et al., 1999). Non-24 hour sleep/wake syndrome is one such disorder that is caused by an unusually long τ averaging in

one study 25.12 hours (Uchiyama et al., 2002). This results in patients being unable to sleep at normal times with their circadian rhythms unable to adjust to the light/dark cycle.

Two other disorders that may be caused by an altered τ are delayed sleep phase disorder (DSPD delayed sleep phase syndrome or DSPS) and advanced sleep phase disorder (ASPD, also known as advanced sleep phase syndrome or ASPS). Figures 1-3 and 1-4 show the circadian characteristics of these disorders in comparison to a normal rhythm, with their rhythm delayed and advanced respectively. DSPD is characterised by delayed sleep-wake timing, with delayed temperature and melatonin rhythms (Wyatt, 2004). Individuals with this disorder may have a long circadian cycle or might respond differently to light than a healthy person, although this has not been confirmed.

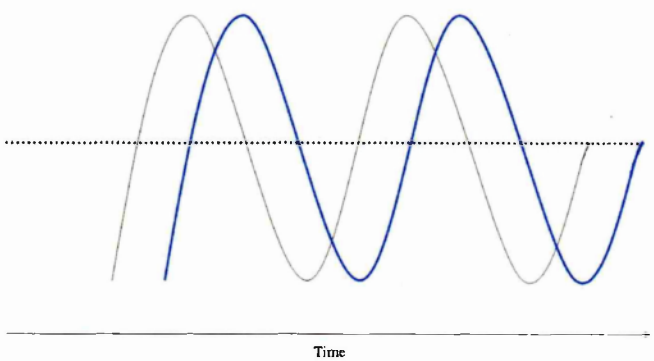


Figure 1-3 Circadian rhythm characteristics of an individual with a delayed circadian rhythm. The blue represents the delayed rhythm in comparison to an individual with a normal rhythm (black).

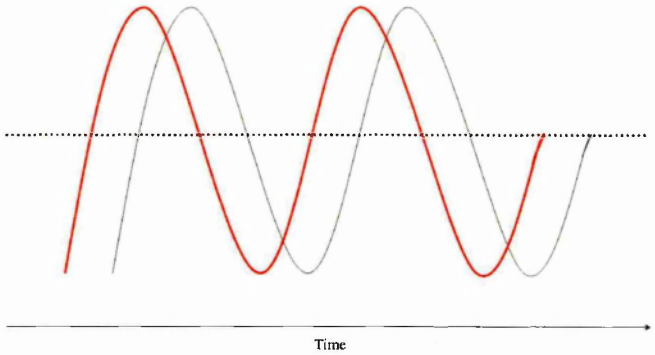


Figure 1-4 Circadian rhythm characteristics of an individual with an advanced circadian rhythm. The red represents the advanced rhythm in comparison to an individual with a normal rhythm (black).

ASPD is a disorder where patients will tend to fall asleep in the early evening and wake up in the early hours of the morning. Their core body temperature and melatonin rhythm are advanced but their sleep duration remains normal (Jones et al., 1999, Reid et al., 2001). One study investigated the endogenous period of an ASPD patient with a variant in the *PER2* gene and found a short τ of 23.3 hours (Jones et al, 1999). As not all ASPD patients have this variant, it is possible that the phenotype may alternatively be caused by altered sensitivity of the circadian clock to the synchronizing effects of light (Dijk and Archer, 2009).

Indeed, in humans, a light pulse in the early evening delays the circadian rhythm and one during the night advances the clock. One study replicated these effects in Drosophila and then tested whether the administration of serotonin would alter them in any way (Yuan et al., 2005). They found that adjustment to the rhythm was lessened after administration and suggested that serotonin may modulate the response of the body clock to light. Additionally, Drosophila that were kept in constant darkness for seven days had increased light sensitivity and lower levels of serotonin. If the same is true for humans, then serotonin could act to

lessen the effect of disturbances caused by light during the night, a mechanism which may be altered in patients with ASPD.

Evidence that a variant in the *PER2* gene led to a shorter τ means it is possible that differences in the length of τ between individuals could be caused by various mutations or polymorphisms in clock genes resulting in the altered oscillation of the circadian clock and circadian rhythm disorders. Indeed, several studies in animals have shown altered circadian period as a result variations in clock genes. The first mutation affecting the circadian clock in mammals was discovered in 1988 in the golden hamster, and was characterised by a τ of 20 hours in the homozygote (Ralph and Menaker, 1988). Following this Vitaterna et al (1994) used mice with a point mutation in the *Clock* gene and found that in constant darkness homozygotes for this mutation had a τ of 27.3 hours, compared to 23.3 hours in the wild type.

1.3 Diurnal preference

Research in chronobiology has shown that people can be placed along a continuum of high eveningness to high morningness, known as chronotype or diurnal preference. Morning types compared to evening types have been shown to have earlier entrained circadian rhythms and a shorter τ (Duffy et al., 2001).

Several parameters are known to affect diurnal preference of which genetic factors make a significant contribution. Research into the heritability of diurnal preference suggests that approximately 50% of the variance is accounted for by genetics (Vink et al., 2001). The affects of age and gender are also well documented. Morningness has been shown to increase with age in various studies (May et al., 1993, Adan and Natale, 2002, Robilliard et al., 2002) and is also higher in women (Adan and Natale, 2002, Mongrain et al., 2006). Further studies

have suggested that, in addition to age, work schedule is greatly influential with regards to diurnal preference (Paine et al., 2006) and that if both these factors taken into account this may negate gender differences (Adan and Almirall, 1992, Paine et al., 2006).

To date, most of the studies of diurnal preference have been performed using the Horne-Östberg questionnaire (HÖ) (Horne and Ostberg, 1976). Subjects who complete the questionnaire obtain a HÖ score, where a score above 70 indicates morningness and a score below 30 indicates eveningness, with scores in between classed as intermediates. Although criticised for being heterogeneous, where items on the scale are not thought to measure the same construct, (Torsvall and Akerstedt, 1980), it was later found that inter-item correlations indicated that the questions were homogeneous (Smith et al., 1989). Furthermore, it has been validated as a correlate of sleep-wake timing (Kerkhof and Van Dongen, 1996, Taillard et al., 2004) and correlates with the timing of core body temperature and melatonin rhythms (Duffy et al., 1999).

Nevertheless, a composite morningness scale was developed which has been proposed as a psychometrically sounder instrument (Smith et al., 1989), although most of its items were taken from the HÖ questionnaire. However, the use of cut-off scores, in all instances, on a seemly continuous variable is problematic. For example, a comparison of the HÖ, CSM and a shortened version of the HÖ, the rMEQ questionnaire (Adan and Almirall, 1991) revealed high correlations between all three measures but when using the cut off scores of each to form categorical variables there was little agreement between all three (Caci et al., 2009b).

Moreover, scores on the HÖ questionnaire become less valid in samples ranging from ages 30-49 years as exogenous factors, such as family responsibilities and work schedules, are more likely to influence diurnal preference. Scores in younger subjects are more closely

related to genotype, with a similar trend shown for those over the age of 50. The latter may be due to a weakening of the influence of exogenous factors and a return to a lifestyle in keeping with their endogenous circadian phenotype (Jones et al., 2007).

A questionnaire also aiming to assess chronotype was recently developed, the Munich Chronotype Questionnaire (MCTQ) (Roenneberg et al., 2003). This sought to address areas that had not previously been covered by other diurnal preference questionnaires. The authors believed that, in order to get a true measure of chronotype, free days or work days must be assessed separately. In a comparison between the MCTQ and HÖ questionnaire correlations were high but the authors suggested that the MCTQ collects additional information about sleep-wake behaviour (Zavada et al., 2005). Furthermore, the authors proposed that as the HÖ questionnaire represents preferences, it is not a direct measure of phase of entrainment and so does not strictly measure chronotype (Roenneberg et al., 2007). This, though, is debateable, as the research published which utilised the HÖ and found it to correlate with markers of circadian rhythms as well as sleep/wake timing, suggests otherwise. Additionally, the MCTQ has not been shown to associate with these variables, and due to this, the HÖ questionnaire is still considered the questionnaire-based measure of choice for determining diurnal preference.

1.4 Polymorphisms and mutations in human clock genes

Since the discovery of the structure of DNA in 1953 (Watson and Crick, 1953) advances in molecular genetics have made it possible to identify genes, and variations within genes, which may be associated with specific traits and disorders. Much research has centred on gene variations such as variable number tandem repeats (VNTRs), and single nucleotide polymorphisms (SNPs), VNTRs are composed of two groups, microsatellites which consist of repeat units of six base pairs or less and minisatellites which are repeats units of greater than

six base pairs. These repeat blocks vary between individuals and provide genetic markers for DNA analysis. Similarly, SNPs are also used as genetic markers and are changes in a single nucleotide which can result in a different in amino acid base (non-synonymous) or no difference due to the redundancy of the genetic code (synonymous). Both VNTRs and SNPs may also lead to a change in the translated protein's structure between individuals through either a change in an amino acid or in the case of VNTRs a lengthening or shortening of the amino acid sequence, both of which can then result in different phenotypes (an organism's observed characteristics). In fact, many genetic diseases are attributable to the expansion of VNTR repeats within protein coding regions including Huntington's disease and Machado-Joseph disease, both neurodegenerative disorders (Ashley and Warren, 1995).

As previously discussed, variations in τ may influence an individual's morning or evening preference (Duffy et al., 2001) and may be an important aspect of circadian sleep disorders (Jones et al., 1999). These differences in τ may be linked to variations in clock genes that in turn disrupt the positive and negative feedback loops of the circadian clock. Additionally, this variability may influence processes outside of the clock which are regulated by circadian clock genes. For example, data have emerged that suggest circadian genes also control the expression of tumour suppressor genes, cell cycle genes and genes that encode transcription factors and caspases. This involvement in cell proliferation and apoptosis has led to the discussion that polymorphisms in circadian genes may be associated with increased susceptibility to human cancers (Fu and Lee, 2003). Indeed, *Per1* and *Per2* have tumour suppressor activity and when these genes are mutated in cancer cells, the growth of the cancer comprising these cells is twice that of other tumour cells (Hrushesky et al., 2009).

The polymorphisms present in clock genes that have been implicated in different phenotypes are described below, a summary of which are in tables 1-1 and 1-2.

1.4.1 *CLOCK*

The first polymorphism reportedly linked to diurnal preference was in the *CLOCK* gene (Katzenberg et al., 1998). This is a C to T nucleotide substitution in position 3111 (rs1801260) of the 3'-untranslated region (3'-UTR). It was reported that subjects with 3111C had a lower mean HÖ score, indicating increased eveningness. It was also noted that as polymorphisms in the 3'-UTR have previously been shown to affect the half-life and stability of mRNA (Beelman and Parker, 1995), the presence of a polymorphism in this area could mean the levels of CLOCK protein translated are affected.

However, subsequent studies found no association between the same polymorphism and diurnal preference (Robilliard et al., 2002, Johansson et al., 2003). Robilliard et al (2002) investigated this polymorphism by genotyping subjects at the two extreme ends of diurnal preference, as opposed to Katzenberg et al (1998) who identified an association by genotyping the entire sample population. By choosing extremes, it would be expected that the C allele frequency would be higher in the selected evening group than in the intermediate and morning groups. Selecting extreme groups in this way may also mean that as less of the sample is used, any potential mediating factors may be missed. Nonetheless, no association was found.

Mishima et al (2005) used the same method of extreme diurnal preference selection and found an association with this polymorphism in *CLOCK* in a Japanese population. The authors suggested that inconsistencies in previous studies may be due to population stratification (non-random mating between groups) and/or small population sizes, but the results of this research show a small number of subjects with the C/C genotype (3%) as well as the inclusion

of shift workers, adding a further confounding factor. They also did not address the possibility of ethnic differences. It is also possible that another variation that is in linkage disequilibrium (the non-random association of genetic variants) with the polymorphism, induces functional change and causes the evening preference (Ebisawa, 2007). Clearly more research is necessary to establish the true role of this particular polymorphism in determining diurnal preference and future research should be considered to clarify the link in a Caucasian population.

Recent research has centred around the role of clock gene variants outside of the biological clock due to a number of studies in knock-out mice which have shown varying phenotypes. One study, in which mice lacked a functional *clock* gene, showed that these mice displayed an increase in cocaine reward and in the excitability of dopamine neurons in the midbrain ventral tegmental area (McClung et al., 2005). This suggests that *CLOCK* may play a role in the dopamine pathway and in drug addiction.

Other work has investigated a potential link between the *CLOCK* C3111T polymorphism and major depression (Desan et al., 2000, Bailer et al., 2005). Although neither study found an association, further work in mood disorder patients, revealed a higher recurrence of three types of insomnia in the homozygotes (a genotype containing two of the same alleles) for the C variant. These types of insomnia were initial, where there is difficulty getting to sleep, middle, when an individual has difficulty returning to sleep after awakening and early insomnia, where an individual awakens early in the morning and has difficulty returning to sleep. A similar trend regarding a decreased need to sleep was also identified in those with a C/C genotype and who had bipolar disorder (Serretti et al., 2003). Subsequently, the same group investigated the role of *CLOCK* 3111 in the regulation of diurnal mood fluctuations during a major depressive episode (Benedetti et al., 2003). After stratifying the sample to

include only patients who had had the illness for more than five years, a significantly higher recurrence rate was observed in homozygotes for the C variant. Further work has also recorded diurnal activity and nocturnal sleep through actigraphy in bipolar patients (Benedetti et al., 2007). This process entails the use of a sensor worn on the wrist and which measures motor activity and in some instances, light exposure. They found that carriers of the C allele had a similar degree of depression with higher activity levels in the evening, a delayed sleep onset and a reduced amount of sleep during the night, compared to T/T homozygotes.

Further research in depressed patients investigated the possibility of clock genes biasing 'non clock' functions, such as information processing and decision making, in other brain areas outside of the SCN where they are also expressed (Benedetti et al., 2008). Subjects underwent a moral valence decision task whereby they were required to push a button when words appeared with positive connotations and to ignore words with negative connotations, or vice versa. Blood oxygen-level dependent (BOLD) functional magnetic resonance imaging (fMRI) scanning took place throughout the task. Participants were genotyped for the C3111T polymorphism, and those carrying the C allele had a trend towards more rapid processing of information. Furthermore, in the posterior singulate area, carriers of the C variant showed lower neural responses for negative than for positive stimuli, with T/T homozygotes showing the opposite response. In the anterior singulate area carriers of the C allele showed higher responses for negative than for positive stimuli whilst again T/T homozygotes showed an opposite pattern of response. The authors proposed that these results provide evidence of the influence of clock genes on variables linked with human behaviour, but suggested that the results should be replicated in a larger sample.

Another area of research in which *CLOCK* has emerged as a possible candidate gene, is in obesity and related disorders. Scott et al (2008) carried out genotype and haplotype (a set of

polymorphisms on a single chromatid that are statistically as associated) analysis on three *CLOCK* SNPs, C236T (rs4864548), G2121A (rs3736544) and C3111T and their association with metabolic syndrome, which is the term for a group of risk factors linked to obesity which increase the risk of cardiovascular disease and diabetes.

Three common haplotypes were identified, CAT, TGT and CGC, with CGC less prevalent in subjects with metabolic syndrome and the CAT haplotype being significantly associated with it. A similar study investigating *CLOCK* haplotypes in obesity identified the haplotypes of rs1554483G and rs4864548A, as being associated with a 1.8-fold risk of being overweight or obese (Sookoian et al., 2008). Prior to this, Sookoian et al (2007) discovered a link between two polymorphisms in the *CLOCK* gene (rs11932595 and rs6843722) and Non-alcoholic fatty liver disease, a disorder in which fat is deposited in the liver and which is linked to metabolic syndrome.

Aside from the possible pathological influences of *CLOCK* polymorphisms, a potential role for this gene in an individual's personality has recently emerged. A genome wide association scan for personality identified a *CLOCK* SNP (rs6832769) as being associated with agreeableness (Terracciano et al., 2008). This result was replicated in two out of three replication samples, although the authors suggested larger sample sizes would be needed to confidently identify associated genetic variants.

These studies provide a plethora of evidence in support of a role for *CLOCK* outside of the circadian clock. In particular, the broad range of areas in which this gene has been implicated is especially interesting, with links proposed with various disorders in addition to the apparent associations with diurnal preference. This suggests that *CLOCK* may have a far greater influence on other biological processes than has been previously thought.

1.4.2 *NPAS2*

Variations in a paralogue of *CLOCK, NPAS2,* have also been the focus of recent research. A single nucleotide polymorphism (C1698T) in the gene, which results in an amino acid substitution of a valine to glycine residue, was linked with seasonal affective disorder (SAD) (Johansson et al., 2003). SAD is a mood disorder where sufferers experience depressive symptoms in the winter or summer. It was found that there was a significant difference between SAD patients and controls for the C1698T indicating a recessive effect of the T allele. This result was not confirmed when analysing a subset of the sample or in a second population based material. Subsequently, further work on the same SNP did identify an association with SAD (Partonen et al., 2007). Nievergelt et al (2006) assessed evidence for linkage and association involving polymorphisms in ten circadian clock genes, including *NPAS2*, with bipolar affective disorder (BPAD). No linkage between this polymorphism and BPAD was found.

Further support for the role of *NPAS2* role aside from the biological clock has recently emerged. Nicholas et al (2007) identified 40 out of 136 possible two marker haplotypes that were significantly associated with autism. The most significant of these involved markers rs1811399 and rs2117714. Moreover, areas of the brain that are altered in individuals with autism correspond to the areas of the brain in which *NPAS2* and *PER1* are expressed. This coupled with evidence that autistic children have atypical sleep architecture (the structure of an individual's sleep, defined by different sleep stages) (Elia et al., 2000) as well as altered levels of melatonin (Kulman et al., 2000) and serotonin (Anderson, 2002) clearly strengthens the argument for *NPAS2* as a candidate gene for autism.

Polymorphisms in *NPAS2* have also been implicated in cancer. Zhu et al (2007) examined a non-synonymous polymorphism which results in an Alanine to threonine change in amino acid sequence and found that genotypes hetero- or homozygous for the threonine amino acid had a reduced risk of developing non-Hodgkin's lymphoma. The authors suggest that a change in protein structure due to this polymorphism may affect NPAS2/BMAL1 heterodimerization as it is located within the PAS domain. This protein dimer has also been implicated in the regulation of expression of the major oncogene *c-myc* (Fu et al., 2002) further supporting this association.

The same group went on to establish a link between this same polymorphism and breast cancer (Zhu et al., 2008) where heterozygotes for the threonine amino acid had an increased risk of breast cancer compared to homozygotes for the alanine amino acid. The authors suggested that clock genes may serve as biomarkers for an individual's risk of human cancers.

1.4.3 *BMAL1*

Partonen et al (2007) investigated three circadian clock genes that may be associated with SAD. In this study, the SNP rs2290035, an A to T substitution located in the last intron of *BMAL1*, was found to significantly associate with SAD. In order to identify groups with the highest genetic risk for the disorder, the genotypes of all three genes were combined. The high-risk group included individuals who were either heterozygous or homozygous for *PER2* SNP 10870, heterozygous for *BMAL1* rs2290035 and homozygous for *NPAS2* C1698T. There was a ten-fold difference in risk between the highest and lowest risk groups, indicating that a combination of variants in these three genes are important in the pathogenesis of SAD.

1.4.4 *PERIOD* genes

1.4.4.1 *PER1*

Several studies have investigated variants in *PER1* as possible indicators of diurnal preference. Carpen et al (2006) identified a synonymous polymorphism T2434C (rs2735611), where the C allele was more frequent in subjects with extreme morning preference. As the SNP was in a non-coding region and had no affect on the secondary structure of RNA, it was proposed that the association may be caused by another SNP that was in linkage disequilbrium with T2434C.

Another synonymous polymorphism, G2548A (rs2253820), in the *PER1* coding region did not associate with diurnal preference (Katzenberg et al., 1999). Researchers also considered associations between C3071G (rs2585405) and prostate cancer but no link was established (Chu et al., 2007) . However, an investigation into autism discovered a significant relationship between variants of G2548A and C7310G (rs885747) and the disorder (Nicholas et al., 2007) (see section 1.4.2).

1.4.4.2 *PER2*

PER2 A2106G was the first mutation linked with pathologically extreme morningness (Toh et al., 2001). It is a missense mutation where individuals with familial advanced sleep phase syndrome (FASPS) have a serine to glycine substitution within the CKIε binding region of *PER2*. This removes a CKI phosphorylation site and is thought to lead to reduced phosphorylation, reduced proteosomal degradation and faster nuclear translocation. This results in a shorter τ, so individuals with FASPS show a four-hour advance of sleep,

temperature and melatonin rhythms. This was confirmed by the introduction of a *Per2* A2106G transgene into wild type and *Per2* knockout mice, which resulted in both displaying a shorter τ in line with the human FASPS (Xu et al., 2007). Moreover, mice who had serine to aspartate mutation introduced in order to mimic phosphorylation, displayed a longer τ, which lent support to the proposed mechanism behind the disorder.

This polymorphism was further investigated in a Japanese FASPS pedigree as opposed to Caucasian, but it was not found in any of the family members (Satoh et al., 2003). The sample size was small and the authors suggested that FASPS might be caused by genetic factors in some cases and non-genetic factors in others (genetic heterogeneity). Another polymorphism was identified during this study in the 5'-untranslated region of *PER2*, the SNP C111G. This was found in two affected family members and one unaffected but no explanation of the meaning of this discovery was offered. The polymorphism identified by Toh et al (2001) has not been found in any other FASPS pedigrees, but the research undertaken in mice appear to confirm the cause of the disorder in this particular pedigree.

Carpen et al (2005) investigated the C111G polymorphism further and found it significantly correlated with diurnal preference. The 111G allele (rs2304672) frequency was significantly higher in subjects with extreme morning preference. Because of its position, the polymorphism does not alter the encoded protein sequence but could have an effect on protein translation. A prediction of the secondary structure found a difference in the mRNA hairpin loop, which in turn could mean a difference in translatability. The authors also suggested the possibility of a link with ASPS, but too few ASPS subjects were available to make a significant association. It would therefore be important for further studies to investigate this link in a larger population.

Other possible effects of *PER2* variants have been recently assessed. Spanagel et al (2005) identified an A to G polymorphism, *PER2* 10870, and found it associated with increased alcohol consumption. This same SNP was later linked to winter depression (Partonen et al, 2007).

1.4.4.3 *PER3*

Ebisawa et al (2001) screened the *PER3* gene for polymorphisms that may be associated with DSPS and non-24 hour-sleep-wake syndrome (N-24). They found that one haplotype was significantly associated with DSPS. As T1940G resulted in an amino acid substitution, the rarer G allele was thought to be the causative variant. The authors speculated that this polymorphism might alter the CKIε dependent phosphorylation of PER3, thereby modifying the rate at which it translocates to the nucleus resulting in a lengthening or shortening of circadian rhythm. Although significant, the number of DSPS subjects was relatively small (27%) and 85% of DSPS patients did not carry that particular haplotype, the authors suggesting this is due to the possibility that DSPS is genetically heterogeneous.

Further research by Johansson et al (2003) found an association between the same polymorphism and diurnal preference. Interestingly, it was shown that those with at least one G allele were higher in morningness, as defined by the HÖ questionnaire, an opposite trend to the previous study. These results indicate that the T1940G may play a role in diurnal preference and sleep disorders but that other genetic factors are likely to influence an individual's susceptibility.

Ebisawa et al (2001) also identified four other non-synonymous coding region polymorphisms including a VNTR polymorphism which coded for either a 4 or 5 repeats of an 18 amino acid

motif (Figure 1-5). However, no associations between DSPS and any of these four polymorphisms were reported in that publication. Subsequent research found an association between the *PER3* VNTR and extreme diurnal preference as well as DSPS (Archer et al., 2003). In that study, the frequency of the 5-repeat allele was found to be significantly higher in those with morning preference and the 4-repeat allele was significantly higher in those with evening preference and DSPS, compared to a control population. The mechanism involved is thought to be similar to that of the *PER2* A2106G mutation, as *PER3* contains an arrangement of potential CK1ε phosphorylation motifs similar to that in *PER2*. This may lead to a difference in phosphorylation between the long and short variant of the VNTR and a subsequent difference in the rate of nuclear translocation.

The authors suggested that disparity between their results and those of Ebisawa and colleagues, may be due to ethnic differences and the previous use of carrier rather than allele frequencies. This may indeed be the case as their results were later partially confirmed by a study in Brazil (Pereira et al., 2005) where the findings were the same for diurnal preference but the opposite for DSPS with nearly 30% of the patients homozygous for the 5-repeat allele. The reason suggested for this anomaly was the latitude of the cities where the research was conducted and the resulting differences in day length and temperature throughout the year. In Sao Paulo there is only a difference of approximately 3 hours in day length between the longest and shortest days of the year, whereas London has an approximately 8 hour difference. Furthermore, variations in temperature and sun brightness are much less drastic in Sao Paulo than they are in London. As these factors affect entrainment it is possible that different genotypes exposed to different zeitgeber strengths could display different phenotypes (Pereira et al, 2005).

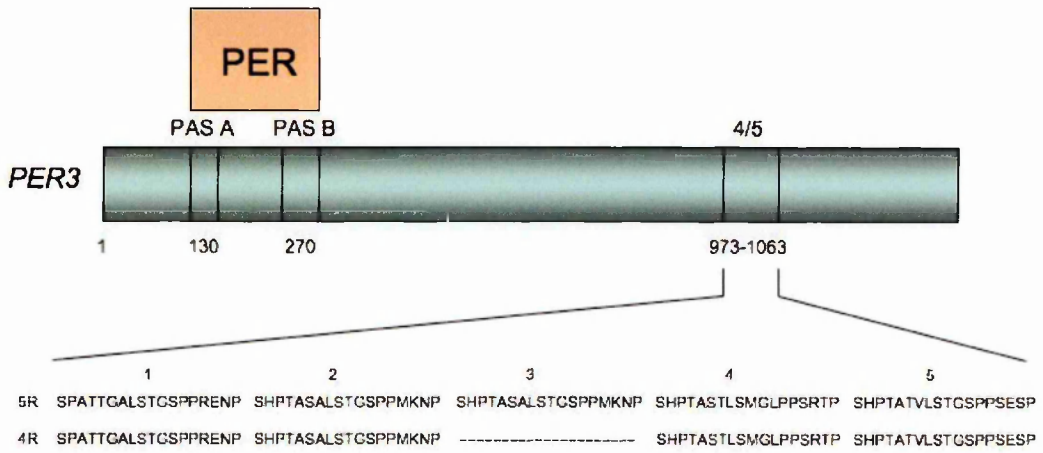


Figure 1-5 Schematic diagram of the human PER3 protein. Adapted from Archer et al., (2003).

Other research into the *PER3* VNTR investigated associations between habitual sleep timing and the phase of the rhythms of mRNA of *BMAL1*, *PER2* and *PER3* in human leukocytes. There was a stronger association between habitual sleep timing and *PER3* than with any of the other clock genes. Moreover, a comparison between 4/4 and 5/5 genotypes showed that 5/5 genotypes had a more robust association between circadian markers and sleep/wake timing than 4/4 genotypes. This suggests a more rigid circadian control in 5/5 genotypes than in 4/4s (Archer et al., 2008).

Another study compared sleep structure between individuals homozygous for the 4-repeat allele and those with the 5-repeat allele by recording sleep and waking performance under baseline conditions and in response to sleep deprivation (Viola et al., 2007). It was reported that participants homozygous for the 5-repeat had differences in sleep structure including shorter sleep latency, more slow wave sleep (SWS) and more slow wave activity (SWA)

compared to 4-repeat individuals during the first part of the nocturnal sleep episode. During sleep deprivation, 5/5 homozygotes had a more rapid increase of theta/alpha activity as well as increased slow eye movements, both indicators of sleepiness and inattention (Cajochen et al., 1999). Furthermore, 5/5 homozygotes had a higher alpha activity in REM sleep and wakefulness.

A comparison of 4/4 and 5/5 *PER3* genotypes during performance tasks after sleep deprivation showed a significantly greater decrement in cognitive performance in 5/5 subjects (Groeger et al., 2008). This occurred most obviously in the early morning approximately 2-6 hours after the peak in melatonin where 5/5 performance deteriorated markedly whereas 4/4 genotypes declined more modestly. After this time, the differences between genotypes became smaller and during the baseline day no large difference were seen between genotypes. Tasks measuring executive function were most affected by sleep deprivation, with 5/5 genotypes performing worse in comparison to the 4/4 genotype.

Comparisons during baseline sleep and during recovery sleep of the time course of sympathovagal balance, which reflects shifts between sympathetic and parasympathetic autonomic states, also revealed differences between *PER3* genotypes. Reduced heart rate variability (HRV, the variation in the interval between heart beats) reflects this shift in balance from parasympathetic to sympathetic control (Viola et al., 2008) and is a rick factor for cardiac disease (Dekker et al., 2000). Circadian rhythms and sleep are known to modulate sympathovagal balance whereby sympathetic activity is highest during wakefulness than during sleep. Sympathovagal balance changes during the NREM/REM sleep cycle so that sympathetic activity is lowest during NREM sleep and highest during REM sleep. Furthermore, sleep deprivation induces an increase in sympathetic activity and a reduction of HRV (Zhong et al., 2005).

When comparing *PER3* 4/4 and 5/5 genotypes, 5/5s had a lower global variability in heart rate as well as greater sympathetic predominance on autonomic nervous system control, especially during NREM sleep, in comparison to the 4/4 genotype. This may indicate that the *PER3* VNTR may be a genetic marker when sleep loss increases cardiovascular vulnerability. Moreover, during baseline sleep 5/5 genotypes displayed the same phenotype as those under sleep deprivation conditions, suggesting that these individuals may be in a state of sleep deprivation even under baseline conditions (Viola et al, 2008).

In order to see whether the differences displayed in response to sleep loss could be replicated in another sample, a comparison between genotypes in fMRI brain responses to an executive task were carried out in the morning and evening during the normal sleep-wake cycle and in the morning after a night without sleep (Vandewalle et al., 2009). The task required participants to state whether an auditorily presented letter was the same as that presented three letters earlier. Brain responses to the task decreased during the day in 5/5s but not in 4/4s and during the morning after no sleep the 4/4 genotypes had an increase in BOLD responses to the task, indicating increased brain activity, whereas 5/5s had a decrease, supporting previous research that identified differences between genotypes on executive function tasks (Groeger et al, 2008).

In a review discussing the implications of this research, Dijk and Archer (2009) suggest that the *PER3* VNTR can be considered a genetic marker to the susceptibility to the effects of total sleep deprivation and circadian misalignment and that 5/5 genotype is associated with a more rapid build up of sleep pressure (the biological pressure to sleep) during sleep loss.

The *PER3* polymorphism has also been implicated in processes outside of the circadian clock. One study showed that the polymorphism may affect breast cancer risk, especially among young women (Zhu et al., 2005). This is all the more interesting in light of the fact that the disruption of circadian rhythms have also been implicated in the disease (Stevens, 2009). Female shift workers are at greater risk of developing breast cancer (Davis et al., 2001) which may be caused by light at night which disrupts circadian rhythms and in particular leads to a reduction in melatonin synthesis and an increase in oestrogen production (Cohen et al., 1978). Other possible mechanisms have also been proposed, including alteration of clock gene functioning and desynchronisation of central and peripheral clocks (Stevens, 2009), leading to differing effects on cell cycle regulation (Sahar and Sassone-Corsi, 2007). It could be that the variants of the *PER3* VNTR are involved in this mechanism in some way, however, the study was carried out in a relatively small sample population and with only Caucasian subjects. Therefore, further research with a larger sample size and a more varied ethnic group would give a better indication of the *PER3* polymorphisms effects on the susceptibility to breast cancer.

Another study investigated the expression of the period genes in breast cancer and found differential expression patterns in cancerous cells in most of the breast cancer cases (Chen et al., 2005). The effect of *PER* mutations on expression was also investigated and it was concluded that the mutations do not contribute to the altered expression. This research did not investigate the *PER3* coding region VNTR, which would have provided an interesting comparison to Zhu et al's study and could be an avenue for future research.

More recently, Zou et al (2008) sought to determine whether the *PER3* VNTR was linked to heroin dependence. In a sample of 209 Chinese heroin-dependent subjects and 249 Chinese healthy controls, the frequency of the four- repeat allele was significantly higher in the mixed

gender heroin dependent group and in the male heroin dependent group than in the control groups. This indicates that the *PER3* VNTR may be a risk factor in the development of heroin dependence.

1.4.5 *CRYPTOCHROME (CRY)*

Recently emerging data have suggested a possible link between *CRY2* and prostate cancer through a G to C substitution in the *CRY2* gene (rs1401417) (Chu et al., 2007). Men with the C variant had a 1.7 fold increased prostate cancer risk compared to those with a G/G genotype, this risk increased to 4.1 fold in men with greater insulin resistance. The lack of other research investigating the role of *CRY* variants and the importance of this study suggests that further work is necessary in this area to fully understand its implications.

A further link with cancer was found when *CRY2* was investigated as a biomarker for non-hodgkins lymphoma (NHL) (Hoffman et al., 2009). Three SNP's were identified (rs11038689, rs7123390 and rs1401417) which were found to be associated with the disease. Additionally, by silencing the gene and performing a whole genome microarray, they were able to demonstrate that genes were predicted to have significant effects on several disease processes such as cancer were altered.

1.4.6 *CASEIN KINASE I (CKI)*

Takano et al (2004) identified a SNP in *CKIε* that causes an amino acid substitution of a serine (S) to asparagine (N) residue. This eliminates one of the putative autophosphorylation sites in the carboxyl-terminal extension of *CKIε*. When genotyping a group of DSPS and non-24h sleep-wake syndrome patients, the N408 residue was found to be less common in both

these groups. After further investigation it was revealed that the N408 residue was 1.8 fold more active than the wildtype, indicating that the enzyme may counteract the development of these disorders. A subsequent study in the Brazilian population found that this allele was much rarer (1.37%) than that found in the Japanese population (21.7%) and so has no influence on circadian rhythm disorder susceptibility (Castro et al., 2008). However, the authors suggested that genotyping in other populations was necessary to determine whether this polymorphism does have an influence on sleep and circadian disorders.

Xu et al (2005) reported a missense mutation in *CKIδ* that constituted an A to G change resulting in a threonine to alanine alteration in the protein. This mutation was found to result in FASPS and subsequent experiments revealed the mutant kinase had decreased enzymatic activity *in vitro*.

1.4.7 Conclusion

It is evident from the discussion above, that research into clock gene polymorphisms has expanded our knowledge of the possible functions of clock genes outside of the circadian clock. Their pleotropic effects appear to be far reaching, spanning mental and physical illness as well as personality and behavioural traits. Future work should elucidate further the mechanisms involved and advance our understanding of the circadian clock.

Clock gene	Polymorphism	Association
CLOCK	C3111T (rs1801260)	Diurnal preference (Katzenberg et al, 1998; Mishima et al, 2005) Bipolar disorder (Serretti et al, 2003; Benedetti et al, 2007) Major depressive episode (Benedetti et al, 2003, 2008) Agreeableness (Terracciano et al, 2008)
	Haplotype: rs4864548, rs3736544, rs1801260. Haplotype: rs1554483, rs4864548.	Metabolic syndrome (Scott et al, 2008) Obesity (Sookian et al, 2008)
	rs11932595 rs6843722	Non-alcoholic fatty liver disease (Sookian et al, 2007)
NPAS2	C1698T	SAD (Johansson et al, 2003; Partonen et al, 2007)
	Haplotype: rs1811399, rs2117714	Autism (Nicholas et al, 2007)
	A394T (rs2305160)	Non-Hodgkins lymphoma (Zhu et al, 2007) Breast cancer (Zhu et al, 2008)
BMAL	rs2290035	SAD (Partonen et al, 2007)
PER1	T2434C (rs2735611) Haplotypes: rs2553820, rs5885747	Diurnal preferences (Carpen et al, 2006) Autism (Nicholas et al, 2007)
PER2	A2106G	FASPS (Toh et al, 2001)
	C111G (rs2304672)	Diurnal preference (Carpen et al, 2005)
	10870	Increased alcohol consumption (Spanagel et al, 2005) SAD (Partonen et al, 2007)

Table 1-1 Polymorphisms in *CLOCK*, *NPAS2*, *BMAL*, *PER1* and *PER2* and their associated phenotype.

Clock gene	Polymorphism	Association
PER3	T1940G	DSPS (Ebisawa et al, 2001) Diurnal preference (Johansson et al, 2003)
	PER3 VNTR	Diurnal preference (Archer et al, 2003; Pereira et al, 2005) DSPS (Archer et al, 2003) Habitual sleep timing (Archer et al, 2008) Sleep structure (Viola et al, 2007) Cognitive performance (Groeger et al, 2008; Vandewalle et al, 2009) Symathovagel balance in cardiac control (Viola et al, 2008) Breast cancer (Zhu et al, 2005; Chen et al, 2005) Heroin dependence (Zou et al, 2008)
CRY	rs1401417	Prostate cancer (Chu et al, 2007)
	rs11038689 rs1123390 rs1401417	Non-Hodgkins lymphoma (Hoffman et al, 2009)
CKI	S408N	DSPS and non-24h sleep/wake syndrome (Takano et al, 2004)
	T44A	FASPS (Xu et al, 2005)

Table 1-2 Polymorphisms in *PER3*,*CRY* and *CKI* and their associated phenotype.

1.5 Genotyping techniques

1.5.1 Polymerase Chain Reaction (PCR)

PCR (Saiki et al., 1988) is now the standard procedure for amplifying specific regions of DNA. It involves a series of heat sensitive steps using two oligonucleotide primers that flank the DNA sequence to be amplified. One cycle consists of heat denaturation of the DNA, resulting in single strands, then the annealing of the primers to their complementary sequences, followed by the extension of the annealed primers with *Taq* polymerase (a thermostable enzyme). The target sequence is amplified through hybridisation of the primers to opposite strands of the DNA. This enables *Taq* polymerase to synthesise DNA across the region between the primers. An exponential increase in the amount of DNA results as each product then goes through the same cycle to produce more DNA.

1.5.1.1 Contamination

There are a number of factors that affect the efficiency and reliability of PCR reactions. Contamination can result in false positive results; therefore lab protocols address this issue through separation of pre and post PCR areas and cleaning of the lab areas. Additionally, autoclaving and/or crosslinking of laboratory equipment reduces the possibility of contamination as well as implementing the 'jackfish' method of pipetting.

1.5.1.2 Imperfect primer hybridisation

Primer design is also important, as bad design can lead to non-specific amplification. The introduction of hot start enzymes has gone some way to prevent non-specific binding as these enzymes are only activated when they reach an optimum temperature and so eradicate mispriming and primer dimerisation prior to PCR (Chou et al., 1992).

1.5.2 Analysis of PCR fragment size by gel electrophoresis

In order to visualise PCR products a common technique known as gel electrophoresis is often used. Depending on the size of the nucleic acid fragments to analyse, either an agarose or a polyacrylamide gel is used. Polyacrylamide, whilst more labour-intensive, gives a higher resolution and is usually used to distinguish smaller fragments. Both form a mesh that retards the movement of nucleic acid molecules as they move through it, according to their mass. As nucleic acids are negatively charged, an electric current can be passed through the buffer inside and outside of the gel causing the nucleic acid molecules to migrate towards the positive electrode. Smaller fragments migrate faster and so separation occurs. The molecules must then be stained with ethidium bromide in order for the resulting fragments to be visualised under ultraviolet light. Molecular markers containing fragments of known sizes are run along side in order to compare sizes with the unknown fragments.

1.5.3 Amplification refractory mutation system (ARMS)

ARMS (Newton, 1989) is a simple technique used to identify single nucleotide polymorphisms. It is based on the discovery that nucleotides with a mismatched 3'-residue do not function as primers under certain conditions during PCR. This is due to *Taq* polymerase's

lack of 3'-exonucleolytic proofreading activity. Three primers are therefore necessary for the reaction, two primers that are specific for the variant alleles and one reverse primer. Once PCR has been performed, gel electrophoresis is used to visualise the DNA fragments. When a variant allele is not present no fragment will be present on the gel, enabling accurate identification of genotype.

Comparison of ARMS and restriction fragment length polymorphism (RFLP) analysis, which involves the use of restriction enzymes to cut specific sequences present in genomic DNA, resulting in DNA fragments which are then separated by gel electrophoresis, found that both techniques were equally reliable but that ARMS was more rapid and economical (Gunesacar et al., 2005).

1.5.4 Pyrosequencing

Pyrosequencing (Ronaghi et al., 1998) is a method of sequencing short sections of DNA through detection of the activity of a DNA polymerase together with a chemiluminescent enzyme. Firstly, PCR must be performed on the genomic DNA in order to amplify it. The biotinylated PCR products are then immobilized onto streptavidin sepharose beads in order to obtain a single stranded template. This DNA is then hybridized to a sequencing primer and incubated along with DNA polymerase, ATP sulphurylase, luciferase, apyrase, adenosine 5′ phosphosulphate (APS) and luciferin.

Sequencing is performed through the addition of base pairs that are only incorporated into the DNA strand if it is complementary to the target DNA sequence. When incorporated, pyrophosphate (PPi) of the same molarity is released and is converted to ATP by ATP sulphurylases. This fuels luciferase-mediated conversion of lucifin to oxyluciferin generating

a light signal (see Figure 1-6). Between each cycle, apyrase degrades unincorporated nucleotides and ATP.

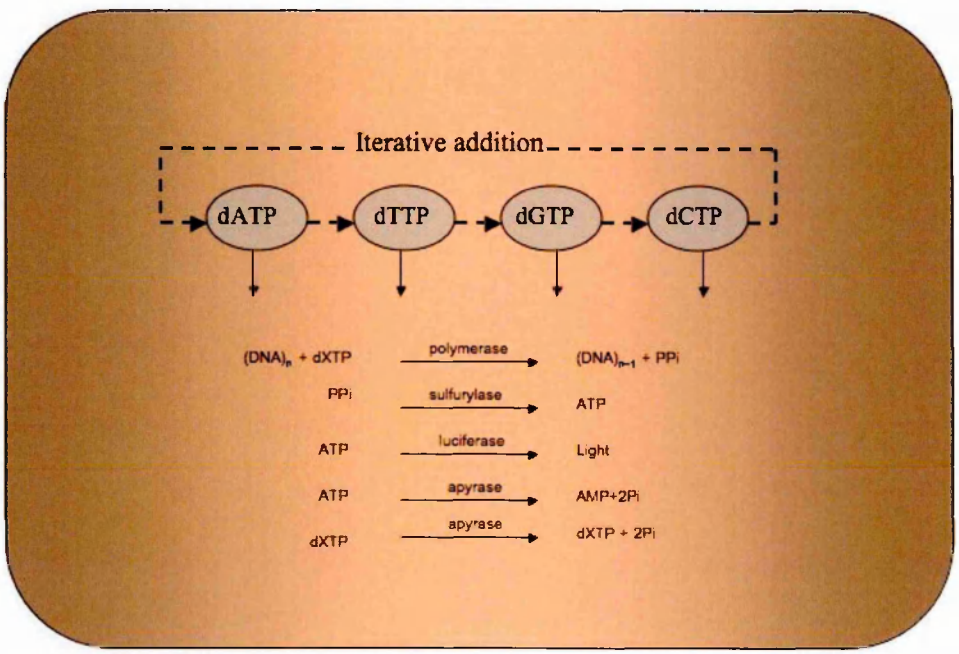


Figure 1-6 Pyrosequencing reactions. Adapted from Ronaghi et al., (1998)

Although applications for pyrosequencing are limited due to the short read length and relatively small throughput, the ease and speed at which sequencing occurs makes this technique advantageous over other sequencing methods (Ahmadian et al., 2006).

1.6 Biological theories of personality

For hundreds of years, scientists have argued that biological processes may be markers of a person's personality. A theory originally postulated by Hippocrates (460-377 BC), that was later built upon by Galen (130-200 BC), suggested that there were four types of personality known as humours - choleric, melancholic, sanguine and phlegmatic - and illness is caused by an excess of one of four related bodily fluids - Yellow bile, black bile, blood and phlegm (Maltby et al, 2007). It was not until the 20th century that other theories began to emerge. In 1940, Sheldon proposed that certain personality types were determined by a person's body type (Sheldon, 1940), although through controlled studies this theory was subsequently rejected (Strelau, 1998). Later research centred on distinct physiological processes such as heart rate, rather than the global systems which covered more general processes such as those just discussed. In addition to this, Jung proposed the existence of two personality types, introversion and extraversion, which are understood as a single continuum. Extraverts have an 'outgoing, candid and accommodating nature' and introverts a 'hesitant, reflective retiring nature' (Jung, 1964). This theory has formed the basis for a number of subsequent personality theories. Three of which will be discussed here.

1.6.1 Eysenck's theory of personality

In 1967, Hans Eysenck put forward a theory which aimed to provide a biological basis for personality (Eysenck, 1967). He proposed that the brain comprised two neural mechanisms, excitatory and inhibitory, the balance of which was regulated by the ascending reticular activating system (ARAS). The ARAS is located in the brainstem and was thought to manage the amount of information and stimulation that the brain receives maintaining attention, concentration and wakefulness. Arousal, within Eysenck's theory, is this information and

stimulation process and is thought to be managed by two circuits, which he linked to two main personality dimensions. The reticulo-cortical, which controls the level of arousal created by incoming stimuli, was linked to extraversion and the reticulo-limbic, which controls arousal to emotional stimuli, was linked to neuroticism. Eysenck defined neurotics as emotionally unstable individuals with traits such as anxiety and worry, conversely extraverts are sociable and impulsive (Eysenck, 1965).

Eysenck suggested that the differences between introverts and extraverts are due to the levels of arousal provided by the ARAS. In similar situations, introverts receive more arousal and extraverts less. As a consequence, introverts avoid stimulation as they are already aroused, whereas extraverts seek stimulation, as they are under aroused. When referring to the reticulo-limbic circuit, Eysenck proposed that neurotics have a higher level of arousal to emotional stimulation than emotionally stable individuals. These two traits were labelled superfactors and Eysenck developed the Eysenck Personality Inventory (EPI), a self-report questionnaire, which he used to measure them. Under the neuroticism superfactor, Eysenck identified a group of individuals who were free from fear and anxiety and who were not consistent with his theory. Consequently, he added the superfactor psychoticism to his model, which became termed the 'PEN' theory. Psychotics possess traits such as aggression and impulsiveness and who seem unable to appreciate the consequences of their actions (Eysenck, 1985). A new questionnaire was developed to measure this new model, the Eysenck personality questionnaire (EPQ). Less is understood about the biological basis of psychoticism, Eysenck suggests that aggressiveness is higher in men and may be linked to testosterone levels (Eysenck, 1990).

Support for Eysenck's arousal theory has been mixed. Gale (1973) reviewed a number of studies which both supported and opposed the hypothesis that introverts are higher in cortical

arousal than extraverts. They investigated this theory through the use of the electroencephalogram, which is a measure that involves the placement of electrodes on the scalp in order to measure brain activity, the result of which is five wave ranges known as alpha, beta, delta, gamma and theta. Beta and alpha are thought to be relevant to investigating Eysenck's theory, where beta corresponds to activity and alpha to low states of arousal. Gale concluded that the outcome of each study was dependent on the arousal inducing properties of the testing environment, such that a high arousal setting, for example those involving task performance demands, resulted in over arousal and so differences between introverts and extraverts would be difficult to detect. Therefore, he hypothesised that the best testing environment to assess Eysenck's theory would be one, which was moderately arousing so that differences in arousal levels would be more evident. After assessing a number of studies, Gale came to the conclusion that in such an environment, extraverts exhibited greater alpha activity and therefore lower arousal levels when compared to introverts (Gale, 1973). A further review by Gale (1983) led to the same conclusion.

Gale et al tested his theory in 2001 using the EPI (Gale et al., 2001), by asking participants to empathise with photographs of people showing positive or negative emotion and then to rate them accordingly whilst an EEG was being recorded. They were also asked to rate the task as either monotonous or overly demanding. The results supported Eysenck's theory of extraverts having lower cortical arousal than introverts with widespread enhanced alpha amplitude in extraverts. Further studies using EEG also showed support for Eysenck's theory (Tran et al., 2001, Fink and Neubauer, 2008).

Studies investigating the relationship between neuroticism and EEG have been inconsistent, with significant results reported but the direction of the association varying from study to study (Zuckerman, 1991).

Another commonly used measure is event-related potential (ERP), which again measures brain activity but is derived from measuring responses to stimuli. The waveform at 300ms (P300) has been suggested by Eysenck (1994) to be an indicator of cortical arousal. Whereas, most other researchers believe it is an index of cognitive response rather than arousal processes (Matthews and Gilliard, 1999). There is some research to support Eysenck's hypothesis, a number of studies have found higher amplitudes of P300 in introverts suggesting higher arousal (Wilson and Languis, 1990, Polich and Martin, 1992) although other studies showed opposite results (Cahill and Polich, 1992, Gurrera et al., 2005) Furthermore, one study found that high neuroticism individuals have a faster P300 latency (Stelmack and Houlihan, 1995). The conflicting results are thought to be due to differing experimental protocols (Gurrera et al., 2005), as extraverts habituate more rapidly to repetitive stimuli then do introverts (Ditraglia and Polich, 1991).

Further research into Eysencks theory used measures concerned with the autonomic nervous system such as electromodal activity. Electromodal (EDA) activity measures electrical activity in the skin via the application of electric current through an electrode. A second electrode then measures the skin resistance level (SRL) or inversely its skin conductance level (SCL). This method measures baseline activity, phasic EDA measures on the other hand, measure electrical activity in response to a known stimulus. The unit of measurement is known as skin resistance response (SRR).

EDA studies of baseline arousal have proved inconsistent (Matthews and Gilliland, 1999). For example, one study recorded skin conductance in groups of introverts and extraverts finding no differences between the two (Davis and Cowles, 1988) whereas Wilson and Languis (1990) found that introverts had higher SCLs than extraverts. A review conducted by

Matthews and Gilliland (1999) revealed an equal number of studies that showed a difference between introverts and extraverts and those that did not.

Studies utilising phasic EDA have proved more consistent, with several studies showing higher levels of EDA in introverts than extraverts at low levels of arousal stimulation(Fowles et al., 1977, Smith, 1983). This is not the case at higher levels of arousal where extraverts show higher levels of SCR. EDA tend to be insensitive to neuroticism (Zuckerman, 1991) and therefore extraversion tends to be a better predictor of EDA than neuroticism (Matthews and Gilliland, 1999).

One of the few studies utilizing fMRI technology found that higher scores on the extraversion trait of the EPQ led to a greater change in fMRI signal from rest to activity during behavioural tests (Kumari et al., 2004). Psychoticism score was negatively related to the resting fMRI signal in the globus pallidus-putamen which corresponds with research that found a negative relationship between psychoticism and dopamine D2 receptor binding in the basal ganglia (Gray et al., 1994). Neuroticism was negatively associated with resting activity in the left orbitafrontal cortex which may relate to the fact that activity in this area increases with positive emotion (Kumari et al., 2004).

1.6.2 Gray's theory of personality

In 1970, Gray proposed his own biological theory of personality based on earlier research in animals, originally intended as a modification of Eysenck's (Gray, 1970). He suggested that the interactions between two systems in the brain form the basis of personality. These systems are known as the behavioural approach system (BAS) and the behavioural inhibition system (BIS) which he proposed is a system separate from Eysenck's arousal system but suggested

they interact (Matthews and Gilliland, 1999). BAS leads to the tendency of an individual to seek rewards and such a person is described as impulsive. Individuals high in BIS tend to avoid punishment and are described by Gray as anxious. Gray agreed with Eysenck about third dimension aligned approximately with psychoticism that relates to fight/flight.

In comparison to Eysenck's model, anxiety and impulsivity are orientated at 30° to Eysenck's extraversion and neuroticism so that anxiety correlates with neuroticism and impulsivity with extraversion (Matthews and Gilliland, 1999). Gray believed that anxiety and impulsivity were the most important personality dimensions and that extraversion and neuroticism were the result of interactions between these two systems (Gray, 1981). Therefore an introverted person would have a more dominant BIS system compared to their BAS system and this would be reversed in an extravert.

Gray suggested that the core of BIS is a septo-hippocampal comparator. The septo-hippocampal region of the brain, which when lesioned, produces similar effects to anti-anxiety drugs (Gray, 1985), is thought to identify mismatches between the actual state of the world and its predicted state. The result is an increase in arousal and the inhibition of behaviour. Conversely, BAS is proposed as being associated with mesolimbic dopaminergic pathways ascending from nucleus A10 of the ventral tegmentum of the brain stem (Gray, 1987). Gray suggested the measurement of these systems through the Gray-Wilson personality questionnaire (GWPQ) (Wilson et al., 1989).

As in the case of Eysenck's theory, subsequent studies have lent some support for Gray's theory. In 1992, Stenberg et al found that more impulsive subjects had lower EEG arousal than low impulsive subjects (Stenberg, 1992), similar to what was seen in extraverts and

introverts. Furthermore, anxious subjects showed greater right side frontal theta activity and higher beta rhythm activation to the negative emotional stimuli.

Few studies have attempted differential tests of Gray's theory using ERP (Matthews and Gilliland, 1999). Although more recently, research has revealed a neural response to errors known as error related negativity (ERN), and these have been used to test Gray's theory. The ERN is a large negative shift in the response locked ERP occurring 50-100ms after subjects have made an erroneous response and are thought to reflect responses to punishment or non reward (Boksem et al., 2006). Two such studies have used Carver and Whites BIS/BAS scales to classify subjects (see section 1.8.1). In 2006, Boksem et al found that subjects scoring high on the BIS scale displayed larger ERN amplitudes, consistent with Gray's theory. Following this, Boksem et al (2008) carried out a similar study but included monetary reward and punishment. Subjects high in punishment sensitivity (BIS) displayed a larger ERN in the punishment condition compared to the reward omission condition, while subjects high in reward sensitivity (BAS-R) showed a larger ERN in the reward omission condition compared to the punishment condition. Boksem and colleagues suggested that these results indicate that subjects who score high on BIS are strongly motivated in avoiding punishment and those high on BAS are motivated to obtain rewards and so show high task engagement.

There have been a number of studies testing Gray's theory using EDA and cardiovascular measures. Fowles et al (1988) described a series of studies measuring heart rate responses and suggesting that heart rate acceleration increases with the amount of reward offered. From this, he proposed that heart rate was associated with BAS. Fowles et al also investigated EDA measures, which they found increased during extinction trials. These trials involve the association of an originally neutral stimulus, i.e. one that does not evoke a response, with an unconditioned stimulus i.e. one that does evoke a response (known as conditioning). When

the neutral stimulus is presented repeatedly without being followed occasionally by the unconditioned stimulus there is a progressive weakening of the conditioning. In this study, EDA measures increased when expected monetary incentives were discontinued, therefore indicating that it is related to BIS. These conclusions were further supported by another study in 2000 which showed that heart rate increased when participants were given a reward and EDA increased in response to punishment, consistent with BAS and BIS respectively (Arnett and Newman, 2000). As stimuli were introduced during these studies they do not indicate baseline differences between BIS and BAS on the respective measures.

Recent genetic evidence has also emerged in support of Gray's theory. Combinations of polymorphisms in *DRD2* and *COMT* genes that were associated with high BAS scores also had high dopamine activity compared to those with low BAS scores (Reuter et al., 2006).

1.6.3 Comparison of the two theories

Table 1-3 shows a comparison of Gray and Eysenck's theories. Overall, there is evidence in support of both theories which means that neither can be considered the definitive theory of personality. It has been suggested that they may be oversimplified (Matthews and Gilliland, 1999) and that a combination of them may best explain the biological basis of personality (Maltby et al., 2007). Indeed, the traits outlined in each theory fall into similar categories, where behavioural activation is correlated to extraversion and behavioural approach. Furthermore, behavioural inhibition in Gray's model is correlated to neuroticism in Eysenck's theory. Clearly, many of the discrepancies between studies have been due to the difficulties in developing adequate protocols, and so in order to investigate the biological basis of personality further it is essential to resolve this issue.

Theory	Gray		Eysenck		
Dimensions	Impulsivity (BAS)	Anxiety (BIS)	Psychoticism	Extraversion	Neuroticism
Characteristics	Sensitive to potential rewards	Sensitive to punishment	Aggressive, impulsive, unable to appreciate the consequences of their actions	Sociable, seek excitement	Anxious, worrisome
Biological basis	Mesolimbic dopaminergic pathways	Septo-hippocampal comparator	Testosterone	ARAS	Reticulo-limbic circuit

Table 1-3 Comparison of Gray and Eysenck's theories of personality

1.7 Trait dependent theories of personality

Other theories of personality have come about through what is known as the lexical approach and factor analysis, which is based on statistical principles that similar characteristics will come together to form clusters . Sir Francis Galton (1884) suggested that the socially relevant personality descriptors will tend to become encoded in language as single words, and was the first to group words by personality trait using a dictionary. This lexical approach assumes that the frequency of use and number of words relating to a specific trait corresponds to their level of importance.

In 1936, Allport built upon this by identifying 18,000 words, 4,500 of which described personality traits (Allport and Odbert, 1936). Allport and Odbert then split the words into four mutually exclusive categories. This early research provided a starting point for other researchers to go on and identify a more comprehensive system.

1.7.1 Cattell's theory of personality

During the 1940's, Cattell narrowed Allport and Odbert's list of 4,500 words by removing all synonyms leaving 171 traits (Cattell, 1943, 1945). He then asked a large sample of individuals to rate the degree to which the attributes relate to them and then used factor analysis, a method by which the underlying relationships between variables are identified which in turn reduces the data, to identify 36 surface traits. Surface traits are trait descriptors that group together in various individuals and situations, with underlying source traits accounting for the observed variance in the surface traits. Another ten surface traits were added after further assessment and subsequent factor analysis identified 16 source traits. According to Cattell, each person would have differing levels from high to low of each factor, and a combination of all 16 would make up an individual's personality (Cattell, 1965).

In order to assess his theory of personality, Cattell developed the sixteen-personality factor questionnaire (16PF), the latest version of which is the fifth edition (Cattell and Cattell, 1995), and although widely used, some issues have arisen over the validity of his theory. The clustering solution he used to narrow down the list of trait descriptives has been considered neither well-informed nor exhaustive, and together with a number of undocumented subjective decisions it is unlikely that the results could be replicated (John et al., 1988). In fact, re-analysis of Cattell's studies by other researchers has not produced the same number of factors Cattell proposed (Kline and Barratt, 1983, Noller et al., 1987). Further research consistently led to the emergence of five factors underlying personality (John and Srivastava, 1999).

1.7.2 The five factor model of personality

The earliest evidence in support of a personality theory made up of five factors was in research undertaken by Thurstone in 1934 (Thurstone, 1934). He subjected 60 personality traits to factor analysis and found five common factors, but his work was largely ignored. Following this, various failed attempts to reproduce Cattell's 16 factors led to only five replicable factors (Fiske, 1949, Norman, 1963, Tupes and Christal, 1992). In 1985, Costa and McCrae published their manual, the NEO-PI personality (Costa and McCrae, 1985), which was based on research on how personality changed with age, in which five broad factors emerged through factor analysis. They termed the five factors: openness, conscientiousness, extraversion, agreeableness and neuroticism, with each dimension placed on a continuum. The model they proposed is hierarchical and each dimension is composed of lower order facets (Table 1-4). These factors have also been validated across a large number of cultures (McCrae, 2001, McCrae, 2002, McCrae and Terracciano, 2005).

Openness	**Conscientiousness**	**Extraversion**	**Agreeableness**	**Neuroticism**
Fantasy	Competence	Warmth	Trust	Anxiety
Aesthetics	Order	Gregariousness	Straightforwardness	Anger hostility
Feelings	Dutifulness	Assertiveness	Altruism	Depressions
Actions	Achievement	Activity	Compliance	Selfconsciousness
Ideas	Self-discipline	Excitement seeking	Modesty	Impulsiveness
Values	Deliberation	Positive emotions	Tendermindedness	Vulnerability

Table 1-4 Facets of the five factors (Costa and McCrae, 1992).

In 1990, Goldberg attempted to further strengthen the argument for the "Big five", as they have become known, by using what was described as the 'most comprehensive pool of English trait-descriptive adjectives ever studied empirically' (Goldberg, 1990). The results of

his research concluded that there were indeed five factors, which were termed surgency (equivalent to extraversion), agreeableness, conscientiousness, emotional stability (equivalent to neuroticism reversed) and intellect. These domains are essentially the same as Costa and McCrae's factors, although there are differences between the fifth domain. In Costa and McCrae's theory, openness to experience excludes characteristics that describe intellectual ability but which are included in Goldberg's corresponding Big Five intellect factor.

Eysenck argued that his three factor model better described personality, but in fact both theories share neuroticism and extraversion dimensions, with Eysenck's psychoticism dimension a combination of low agreeableness and low conscientiousness. Eysenck believed that agreeableness and conscientiousness were lower order facets of psychoticism and so should not assume their own dimension (Eysenck, 1992).

Despite other evidence in support of the five factor model, there is still some debate over a number of points. Firstly, it has no theoretical base, as the model has been postulated purely on the data obtained from research. This has therefore made it open to criticism (Briggs, 1989). There are also disagreements over the best way to label the five factors, with different researchers defining them differently. The five factor model also does not include evaluative traits which according to Ashton and Lee (2001) contradict the logic of lexical studies of personality structure. They put forward their own model of personality containing six factors (an addition of the dimension honesty-humility), arguing that their model predicts several personality phenomena that are not explained by the five factor model (Ashton et al., 2004). Another study suggested that evaluative terms and descriptions of temporary states such as mood should have been included in the original lexical analyses. They proposed that the model of personality should be composed of seven factors; these were the dimensions negative valence, positive valence, positive emotionality, negative emotionality,

conventionality, agreeableness and dependability. Many of the factors align with the big five; Positive emotionality is similar to extraversion, negative emotionality to neuroticism, agreeableness the same as the big five dimension, dependability similar to conscientiousness and conventionality more aligned to the obverse of Costa and McCrae's 'openness to experience' than to Goldberg's 'intellect'. The upper extreme of positive valence is the grandiose sense of self importance and specialness whereas negative valence taps self perceptions of evilness and awfulness (Waller, 1999). These seven factors also emerged in a later study, lending some support to this theory (Almagor et al., 1995).

The seven factor model was later refuted by McCrae and John (1992) who argued that the two new dimensions could be assumed under the five factors. More recent research has proposed that the five factor model can form an even more basic structure as the factors have been consistently shown to be intercorrelated (Digman, 1997, DeYoung et al., 2002). Although, not suggested as an alternative to the big five, Deyoung et al (2002) established a higher order theory in which two metatraits comprised the dimensions of personality. Agreeableness, conscientiousness and neuroticism reversed were included under the metatrait stability, whereas openness to experience and extraversion were assumed under plasticity. These studies indicate that the five factor theory is not perfect and suggests that further work in the area should look beyond the model rather than looking to confirm it.

Although not a biological based theory, several researchers have linked the five factor model to certain biological processes. Deyoung et al (2002) proposed that stability was associated with the serotonergic system and plasticity to dopaminergic system. They later confirmed an association between openness and the dopaminergic system (DeYoung et al., 2005). Other research has supported this theory, Brummet et al (2008) found higher serotonin levels in females with low neuroticism scores but the opposite was evident in males. Furthermore,

another study established a negative correlation between serotonin and neuroticism and a positive association with conscientiousness.

The investigation of polymorphisms in genes involved in both these systems have also provided further evidence for an association. Variation in the dopamine D4 receptor (*DRD4*) has been linked to both conscientiousness (Dragan and Oniszczenko, 2007) and neuroticism (Tochigi et al., 2006). Two studies have also proposed links between the alcohol dehydrogenase (*ADH*) group of genes and different personality variables (Luo et al., 2007, Zuo et al., 2009) which, apart from the oxidization of alcohol, are thought be involved in the dopamine and serotonin pathways (Hoog et al., 2001).

1.7.3 Measures of the five factor model of personality

Several measures have been developed to evaluate the five factor model of personality. The neuroticism-extraversion-openness personality inventory (NEO-PI) was first developed in 1985 and the 240-item revised version (NEO-PIR) (Costa and McCrae, 1992) is now one of the most popular measures of the five factor model. It encompasses not only the five factors but also six facets within each dimension, which contain eight items, allowing researchers to measure on both general and more specific levels of personality. Both internal consistencies for each of the scales and test retest reliabilities have been reported to be good (Costa and McCrae, 1992). Additionally, the short term stability was assessed and found that all dimensions and fair to good stability with coefficients ranging from 0.68 – 0.74 (Carter et al., 2001). Due to the length of the questionnaire and the time taken to administer it, Costa and McCrae introduced a 60 item reduced version the NEO-FFI, which used the 12 items in each domain which loaded highest on their respective factor. Again the questionnaire has shown good internal consistency (Costa and McCrae, 1992) and retest reliability (Robins et al., 2001)

and is highly correlated with the NEO-PI-R. Although widely used, some studies have suggested caution over its application. Hrebickova et al (2002) compared the NEO-FFI across six cultures and found weak internal reliability in the openness and agreeableness scales caused by a number of items not representing the dimension adequately. This led to McCrae and Costa's attempt to revise the NEO-FFI, but it only yielded modest improvements, with the authors suggesting that the original version should suffice (McCrae and Costa, 2004).

The commercial nature of the NEO-PI-R has led some researchers to propose alternative measures that are available within the public domain. Goldberg (1999) argued that copyrighted measures cannot be freely used by other scientists and so contributions to further developments and refinements of the measure can not be made. He put forward a pool of items which cover the many facets of the big five, termed the International Personality Item Pool (IPIP) which built upon his trait descriptive adjectives (TDA) (Goldberg, 1992) (which could be used as a measure of the big five), and on a pool of Dutch items which had been previously identified (Goldberg, 1999). Scales were constructed that were similar to well known measures such as the NEO-PI-R, and whose reliabilities either matched or exceeded the parent scale. The IPIP-NEO (the NEO-PI-R version) is available as 50, 100 or 240 item questionnaire with reportedly high correlations with the corresponding conscientiousness, extraversion and emotional stability/neuroticism scales of the NEO-PI-R (Gow et al., 2005). Although, it has been argued that the high correlations does not mean the versions are equivalents (Costa and McCrae, 1999).

The IPIP also contains items known as the big five markers (Goldberg, 2001), which were derived from trait-descriptive adjectives previously identified (Goldberg, 1992) and correlated with the NEO-PI. These adjectives were used to form questionnaire items and a 50-item and 100-item scale resulted, which both have high internal consistencies. A 20-item questionnaire,

known as the mini-IPIP (Donnellan et al., 2006), has also be proposed and which is also psychometrically acceptable.

The IPIP scales have been translated into 25 languages but in relation to other measures, few studies have published data utilising these scales. There is also a lack of published research utilising the English scales, although Gow et al (2005) did confirm the internal consistencies of the 50 item scale and found that it correlated highly with the appropriate scales of the NEO-FFI and the Eysenck Personality Questionnaire (EPQ) (see section 1.6.1).

Another public domain personality measure is the Big Five Inventory (BFI), and like the IPIP it contains short statements in order to assess the five factors. It is the shortest of the questionnaires described so far, with only 44 items but still has good psychometric properties (John and Srivastava, 1999). In a comparison between the NEO-FFI, TDA and BFI, John and Srivastava (1999) found that the longer TDA had the highest reliability and the NEO-FFI the lowest, with the agreeableness and openness scales of all three tending to be the least reliable. The NEO-FFI openness scale had the lowest reliability of all scales, where the value and action facet items did not correlate well with the overall scale. The authors suggest this may be due to the use of college students in their sample as opposed to the older adults used by Costa and McCrae (1992), further questioning the stability of the measure. Conversely, the facets of the NEO-FFI had the strongest item total correlations. Corrected convergent validity correlations across the instruments was 0.91 and pair-wise comparison between the TDA and BFI showed that they shared nearly all their reliable variance. Convergence between the BFI and NEO-FFI was also high but correlations for extraversion and openness were lower than the other dimensions. Moreover, similar results were found for the TDA with John and Srivastava suggesting that the conceptualisation of the dimensions is not equivalent in both measures.

In fact, when inspecting the construction of the scales some differences come to light. The NEO-FFI warmth scale was included in the extraversion dimension by Costa and McCrae but it also correlates with the agreeableness domain. Also, the fifth domain has been interpreted differently by Goldberg, which he refers to as intellect or imagination. In addition to this the BFI scale does not include items related to NEO values and actions facets located under the openness domain.

Several other very short measures of the Five Factor model have also been proposed. Gosling et al (2003) put forward a ten item questionnaire known as the ten item personality measure (TIPI), Woods and Hampson (2005) suggested a single item measure of personality (SIMP) and Rammstedt and John (2007) proposed a short version of the Big Five inventory containing only ten items. Although, all of these questionnaires are psychometrically inferior to the longer measures, they are useful when time is limited and personality is not the primary focus of the investigation (Gosling et al., 2003). A comparison of four of the measures revealed that the TIPI achieved slightly better validity than the other measures (Furnham, 2008).

1.7.4 Conclusion

Biological and trait dependent theories have attempted to define personality, but a definitive answer is still elusive. There is a general consensus that there are five factors but with an ongoing debate over what these factors incorporate and how they should be labelled. This means that a deluge of measures have been proposed but from different perspectives of what the five factor model encompasses, making comparisons between studies all the more

difficult. Until there is agreement over what fundamentally constitutes personality, these issues will continue to plague personality research.

1.8 Impulsivity

Impulsivity has played an integral part in a number of theories of personality as previously discussed. It has also been important in the assessment of a variety of psychopathologies and social problems (Arce and Santisteban, 2006). Research on its links with substance abuse have been well documented (Perry and Carroll, 2008). Recent research suggests that both alcohol abuse and problem gambling are associated with impulsivity (Lawrence et al., 2009) and that impulsivity is a risk factor for illegal activities used to fund gambling (Martins et al., 2004). Other risk taking behaviours such as sexual risk have also been associated with impulsivity (Hoyle et al., 2000). It is also a main dimension of suicidality where Swann et al (2005) found that subjects with a history of suicide attempts were more impulsive. Research conducted on children has also linked impulsivity to attention deficit/hyperactivity disorder (ADHD) (Cormier, 2008) which is also characterised by hyperactivity and inattention.

These investigations have measured impulsivity using a variety of definitions and measures and this heterogeneity between measures has led to impulsivity being conceptualised as consisting of multiple, independent facets, varying in definition between researchers. Impulsivity is now generally viewed as a multi-dimensional construct and several researchers have suggested it comprises of at least two related factors such as reward drive, a heightened sensitivity to reward and rash impulsiveness, the tendency to engage in rash, spontaneous behaviour and not consider future consequences (Moeller et al., 2001, Dawe et al., 2004, Reynolds et al., 2006). Nevertheless, a large number of measures have been developed but from different perspectives of what defines impulsivity, leading to difficulties in the

comparison of results from different studies. This means that researchers must be cautious in the selection of a measure and understand its theoretical basis when reporting their findings in the context of other studies.

1.8.1 Measures of trait-dependent impulsivity

A number of self-report questionnaires have been developed in order to measure impulsivity in individuals. Eysenck created a number of scales firstly based on his two dimensional theory and later on his Psychoticism-Extraversion-Neuroticism theory of personality (PEN). When the third dimension, psychoticism was added, the EPQ was developed and the placement of impulsivity had to be reassessed. Having originally being part of the extraversion dimension, impulsivity had been split into four factors: narrow impulsiveness, non-planning, liveliness and risk taking. Under the new model, these factors correlated differently with each trait and so factor analysis was undertaken. It resulted in two factors: impulsiveness and venturesomeness, with impulsiveness aligned with psychoticism and venturesomeness with extraversion.

It was at this point that Eysenck developed an impulsiveness questionnaire to complement the EPQ (Eysenck and Eysenck, 1978), and after several previous attempts the impulsiveness-venturesomeness-empathy 7 questionnaire (IVE-7) was created. Since then it has become one of the most widely used self-report measures to assess impulsivity (Parker and Bagby, 1997) and it has been shown to be valid and reliable (Eysenck et al., 1985) as well as stable across cultures (Caci et al., 2003). Eysenck's PEN model specifies the biological mechanisms underlying personality traits and so his scales have both practical and theoretical importance as results can be attributed to specific biological pathways.

A number of different scales have been devised to assess Gray's theory of personality, which consists of two dimensions: impulsivity (BAS) and anxiety (BIS) as previously discussed (see section 1.6.2). The most recent of the scales produced are the BIS/BAS scales (Carver and White, 1994) which have been found to have valid psychometric properties (Campbell-Sills et al., 2004). The scales consist of a 20-items designed to measure the sensitivity of the two motivational systems. It contains four subscales: a BIS scale that contains seven items and three scales for facets of BAS (drive, reward responsivity, and fun seeking). When relating the scales to the measurement of impulsivity, several concerns have come to light. Gray's conceptualisation of impulsivity as a personality dimension in its own right means that it encompasses many subfacets which may or may not be related directly to impulsivity as defined by other theorists such as Eysenck. In fact, the BAS is only directly related to one of the types of impulsivity that Gray himself outlines (Gray, 1983). When devising scales to measure the BIS and BAS domains Carver and White (1994) split the BAS dimension into three further scales and it is questionable whether a combination of scales measures impulsivity directly or one scale in particular. Impulsivity may be a combination of BIS insensitivity as well as BAS sensitivity (Carver and White, 1994) and so the measurement of impulsivity with these scales could prove problematic.

Unlike most of the other scales discussed, the Barratt Impulsiveness Scale (BIS) (Barratt, 1959) is independent of a comprehensive theory of personality. Barratt, after reviewing correlations between various personality scales, identified an impulsiveness trait, which at the same time had a low correlation with anxiety. He thought that impulsiveness may be related to oscillatory inhibition (intra-individual variability of performance in perceptual motor or learning tasks), and as the inventories available at the time were not broad enough to measure this factor, he rewrote items from several scales and created the first BIS. He also suggested that impulsiveness was not unidimensional, and took this into account during further

development of his scale (Stanford et al., 2009). Further revisions were made to ensure anxiety and impulsiveness items did not correlate and the final version (BIS 11) was developed (Patton et al., 1995).

The BIS 11 scale has been used in a number of different populations (Stanford et al., 1996, Kirby et al., 1999, Crean et al., 2000) and consists of 30-item self-report questionnaire which measures three subtraits of impulsiveness: Motor impulsiveness, non planning impulsiveness and attentional impulsiveness. Although the scale has been found to be internally reliable (Patton et al., 1995), the lack of a theoretical base undermines the validity of the measure.

In an attempt to place impulsivity within a theory of personality, Whiteside and Lynam (Whiteside and Lynam, 2001) brought together the various theories and definitions of impulsivity and using the five-factor model of personality as a framework (FFM), combined them into a comprehensive scale. Using these dimensions, they identified four distinct facets of personality associated with impulsivity. This first is urgency or the tendency to experience strong impulses which is associated with the impulsiveness facet of the neuroticism dimension. The second is (lack of) premeditation or the tendency to think and reflect on the consequences of an act before engaging in that act, which relates to the (low) deliberation facet of conscientiousness Thirdly, (lack of) perseverance or the ability to remain focused on a task that may be boring or difficult which associates with the self discipline facet of conscientiousness. Lastly, sensation seeking which is a tendency to enjoy and pursue activities that are exciting and an openness to trying new experiences that may or may not be dangerous, which associates with the excitement seeking facet of extraversion. These four facets make up the four scales of the urgency-premeditation-perseverance-sensation seeking scale (UPPS). The construct validity and internal reliability was found to be good (Whiteside, 2005) and this four factor model of impulsivity was later supported by other studies

(Whiteside and Lynam, 2003, Billieux et al., 2007). As the scale is still in its infancy future work would determine how useful this tool is in impulsivity research.

1.8.2 Behavioural measures of impulsivity

Very few studies have used both psychometric and behavioural measures of impulsivity (Reynolds et al, 2006). The results of those studies have been ambiguous, with some finding correlations between behavioural indices such as performance on delay or probability discounting tasks and self-report measures such as sensation seeking, extraversion, or impulsivity and venturesomeness (Kirby et al., 1999, Richards et al., 1999, Swann et al., 2002) and others finding no such relationship (White et al., 1994, Mitchell, 1999, Crean et al., 2000). Together, these findings indicate that psychometric and behavioural tasks may be measuring different constructs which share only a minimal overlap, or at the very least, components of impulsivity. Correlations between behavioural measures themselves also vary (Reynolds et al., 2006), suggesting that different measures may reflect separate underlying processes (Sonuga-Barke, 2002).

There are various behavioural measures that have been developed for the measurement of impulsivity. Most of these instruments are based on response inhibition where there is difficulty in withholding inappropriate responses. A positive significant correlation between behavioural impulsivity and measures of inhibitory control has also been established (Logan, 1994).

The Stop signal paradigm, is one such method and is based on a well established theory of response inhibition, known as the Race Model (Logan, 1994). It has become one of the most important methods for the study of general inhibitory problems in adults and children and is a

valid and reliable measure of the inhibition process. The Stop signal paradigm consists of a primary go task and a secondary stop task which is normally an auditory stimulus. The task requires the inhibition of the response to the primary go stimulus when a stop signal appears. Whether the participant inhibits or not depends on a race between the go and stop tasks. If they finish the stop task first, they inhibit their response, if they finish the go task first they fail to inhibit and respond as they would if no stop signal had been presented. Therefore, inhibitory control depends on the latency of the response to the go signal (go reaction time) and the latency of the response to the stop signal (stop signal reaction time) (Logan et al., 1997).

As the stop signal reaction time is not directly observable it is estimated through the use of adjusting stop intervals. This is where the stop signal delay (the time between go signal onset and stop signal onset) changes dynamically after each stop signal trial so that it is increased for each successful inhibition (increasing the difficulty), or decreased for each failure to inhibit (decreasing the difficulty). This method allows a stop signal delay to be generated that represents the time delay required for a participant to successfully withhold a response in the stop trials 50% of the time. This stop signal delay represents a threshold where a 'tie' in the race occurs and the go and stop processes finish at the same time (Figure 1-7). It is also the average point in time at which the stop process finishes. This delay can then be used to calculate the stop signal reaction time by subtracting it from the go reaction time.

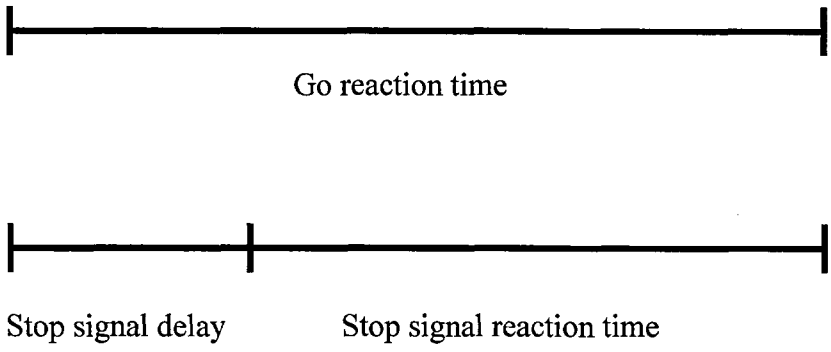


Figure 1-7 The diagram represents the tie in the race between the go and stop signals. The top line corresponds to go reaction time and the bottom line to stop-signal delay in addition to stop signal reaction time. As the stop signal delay is adjusted so that subjects inhibit 50% of the time it means the race is tied and results in the two lines finishing at same point in time. Stop signal reaction time can then be estimated by subtracting the stop signal delay from the mean go reaction time, both of which are observable (Adapted from Logan et al, 1997).

A variant of the stop signal paradigm known as the GoStop task has recently been introduced (Dougherty et al., 2005). Like other versions, it presents participants with a series of visual stimuli which they must respond to when a go signal appears and then must withhold a response to when a stop signal appears. The go signal in this instance is a number presented in black, and matches the previous number identically. Unlike other versions of the stop paradigm, which used an auditory stimulus as a stop signal, the GoStop uses a matching number that changes from black to red, the time delay of which varies according to the settings used.

By using a combination of go and stop signals, three trial types emerge; no-stop, stop and novel trials. During the no stop trial only the go signal is presented whereas the stop trial contains a percentage of go signals which randomly include a stop signal. The novel trials consist of randomly generated non-matching numbers presented in black. Both inhibition in the novel trials and latency in the no stop trials (also known as go reaction time) are indicators

of attention and so can be used to assess whether differences between groups on the primary measures of the GoStop are due to lapses in attention rather than response inhibition.

There are two primary measures of the GoStop task, percentage inhibition and stop latency (also known as stop signal reaction time). Percentage inhibition is the percentage of stop trials where no response occurs. It has been shown that high impulsive participants inhibit less than low impulsive individuals throughout a range of ages (Williams et al., 1999). This is used as the primary measure when fixed stop signals are employed, rather than adjusting, and mean stop latency cannot be calculated. It is also calculated, when adjusting stop signals are used, in order to check whether the tracking procedure has been implemented successfully.

Research has shown that stop latency is slower in impulsive individuals due to their poor inhibitory control (Logan et al, 1997). This is due to slow responding to the stop signal rather than responding too quickly to the go signal. The difficulty in inhibiting prepotent responses (a response that has priority over other response tendencies) is therefore not because of the quickness of their prepotent responses but because their inhibitory responses are slow (Logan et al., 1997).

The stop signal paradigm has been found to be a reliable measure of response inhibition after test-retest reliability was examined (Soreni et al., 2009). Studies in pathological populations have provided consistent results (Alderson et al., 2007), but those in non-pathological populations which have compared the stop signal paradigm to self report impulsivity measures have varied, with participants high on trait impulsivity only showing marginally slower SSRTs (Lijffijt et al., 2004).

In relation to theories of personality, the stop signal paradigm was found to be associated with the impulsivity subscale of the Eysenck personality inventory (Logan et al., 1997) but not to any of the scales of the Eysenck personality questionnaire (Avila and Parcet, 2001), although a link was found between this task and sensitivity to reward and punishment as defined in Gray's theory. Furthermore, associations were seen between the GoStop and the I_7 subscale of the IVE-7 questionnaire one study (Marsh et al., 2002) but not in others (Lijffijt et al., 2004, Edmonds et al., 2009). These differing results may be due to the questionnaire used, for example Logan et al used a questionnaire specific to impulsivity whereas Avila and Parcet used the broader EPQ. It could also be due to the population sampled, as during Marsh and colleagues study, subjects were recruited from probation centres in order to boost the number of impulsive subjects.

Less research has been undertaken using measures of the five factor model and the stop signal paradigm although one study did identify a relationship between conscientiousness and the GoStop (Edmonds et al., 2009). Further work is needed in this area to fully understand the behavioural aspects of theories of personality.

The Matching Familiar Figures Test (MFFT) is the most widely used test to evaluate cognitive style (Cataldo et al., 2005), which is the way in which a person learns and processes information. It can be used as a measure of impulsivity as subjects are classified into four groups: reflective, impulsive, fast and accurate, and slow and inaccurate. During the test, participants must match a standard figure to one of six variants. One of these variants is identical to the standard one and the other five are similar. Performance is assessed by the time taken to give the first response (latency) and the total number of errors with lower latency scores and higher error scores indicative of impulsivity. However, it has been suggested that the MFFT reflective-impulsive taxonomy does not predict a generalised

impulsive style in all types of tasks (Cataldo et al., 2005) and that the slow-accurate classification is a better way of predicting impulsivity (Victor et al., 1985). This disagreement over the correct way to measure impulsivity suggests that the results from the MFFT may not be completely reliable.

Another commonly used measure of impulsivity is the Continuous Performance Task (Beck et al., 1956) which requires sustained attention for the detection of and response to infrequent targets. Impulsivity is measured through commission errors (responses that occur when no response is required) and inattention through omission errors (no response when a response is required). Several problems have arisen with this test. Firstly, although it is able to identify children with behavioural impulsive problems such as ADHD, few abnormalities have been found in adults and adolescents on most continuous performance tasks (O'Toole et al., 1997). Secondly, concerns have been expressed at the correlation between performance on the CPT and intelligence quotient (IQ, the measure of a person's intelligence), given that adolescents with disruptive behaviour disorders often obtain lower IQ scores than control subjects (Thompson et al., 2006).

Dougherty et al (2002) has developed a variant of the CPT, the immediate memory test/delayed memory test (IMT/DMT), which helps to address these issues. Most research has centred on children or severely impaired patients, fewer associations having been found in higher functioning groups and so this may mean that the tasks were not sufficiently difficult (Dougherty et al., 2003). The IMT/DMT is more difficult than previous CPT tasks, with a more restrictive criterion to determine commission errors, and has shown differences in responding in higher functioning groups (Dougherty et al., 2003). Research found that commission errors in the IMT and DMT were usually more common in adolescents and adults with psychiatric disorders characterised by impulsivity (Dougherty et al., 2000, Swann et al.,

2001, Mathias et al., 2002). With regards to IQ, Dougherty et al (2003) found a negative correlation between commission errors on the IMT/DMT and IQ in adults. Whilst Thompson et al (2006) found no correlation between the IMT/DMT and IQ, but interestingly found that the IMT and the impulsiveness scale of Eysenck's Junior I_6 impulsiveness questionnaire were highly correlated. Further work is necessary to confirm this finding in children as well as assessing whether the same result would be significant in adults, but as previous research investigating the relationships between behavioural and self-report measures of impulsivity has been ambiguous, the IMT is an important step forward.

Another instrument used to measure impulsivity is the Circle tracing task (Bachorowski and Newman, 1985). Participants are instructed to trace with their preferred hand over three-quarters of a pre-drawn circle as slowly as possible and impulsivity is measured by the time take to complete the tracing. Wallace et al (1991) found that impulsive subjects traced the circle more quickly than normal controls when instructed to trace it slowly but other studies contradict this result (Bachorowski and Newman, 1990, Scheres et al., 2004) suggesting that performance anxiety may influence motor speed.

There are various versions of the Stroop Task (Stroop, 1935), which is a computer based test. It is a choice reaction test and tests the ability to identify colours in which names of colours are written. In one version of the test three conditions were used (Keilp et al., 2008). In the first, the speed of word reading is measured by the written names of colours being presented in black and the participant responding by pressing a computer key that is related to that colour. The second measures colour naming speed by the presentation of a row of X's in a specific colour, the participant responds in the same manner as previously. The last condition measures the number of errors the subject makes when they are required to name colours whilst ignoring the words, this is achieved through the presentation of written words

corresponding to a colour but which appear in a colour that does not correspond to the written word. The interference score is considered the measure of impulsivity through resistance to automatic interference. As reading is an automatic process and not easily inhibited, the task assesses the difficulty participants have with resistance to distractions. As interference control has been implicated in ADHD (Doyle et al., 2000), van Mourik et al (2005) analysed previous studies and found that the Stroop task did not provide evidence for a deficit in interference control in ADHD. Some researchers consider this a measure of inhibitory control (Kindlon et al., 1995) but others reject this and believe that resistance to automatic interference measures a different process (Nigg, 2000, Avila et al., 2004).

The Wisconsin card-sorting test (Berg, 1948) measures the ability to adjust a strategy to changing demands (set shifting). During the test a number of stimulus cards are presented to the participant. They are then given additional cards and asked to match each one to one of the stimulus cards, although they are not told how to match the cards only whether a certain match is correct or wrong. The dependent variables measuring impulsivity are the percentage of perseverative responses (responses to a previously correct category) and the percentage of perseverative errors (card sorts throughout the test which match an incorrect category). Impulsivity is most related to the number of perseverative errors and the test is independent of inhibitory control. Bowden et al (1998) conducted a study to examine the reliability and validity of the test and concluded that there was a lack of internal validity and inconsistencies in the scoring system used.

The Differential Reinforcement of Low rate responding task (DRL) is another behavioural measure of inhibitory control. There are a number of different variations of the test, in the computerised version to obtain the highest number of points, participants are instructed to press a button, wait and then press it again. Impulsive subjects will not wait as long as control

subjects and the measure of impulsivity is therefore the correct number of responses divided by the total number of responses. However, this test along with the CPT, the Stroop test and the WCST have all been criticised for their poor construct validity or reliability (Kindlon et al, 1995).

1.9 Rationale for current study

In 1967, Blake found that introverts and extraverts had differing body temperature changes in the morning and evening (Blake, 1967). Introverts temperature increased more quickly in the morning and dropped earlier in the evening. From this he predicted that introverts would perform better in the morning because they are more aroused and extraverts would perform better in the evening. A number of studies after this investigated the time of day effect in relation to personality but most did not take diurnal preference or gender into account (Revelle et al., 1980, Matthews, 1987).

Eveningness has been linked to various disorders such as depression (Chelminski et al., 1999), drug addiction (Adan, 1994) and bulimia (Kasof, 2001). A recent publication has also suggested a negative correlation between morningness and impulsivity but not venturesomeness (Caci et al., 2005). Other studies have reported a relationship between conscientiousness and morningness (Jackson and Gerard, 1996, Gray and Watson, 2002, Cavallera and Giampietro, 2007, DeYoung et al., 2007, Randler, 2008, Tonetti et al., 2009). Further research found that neuroticism is associated with a weakened circadian rhythm (Murray et al., 2002).

With a number of publications already implicating clock gene polymorphisms in determining diurnal preference, it is plausible that facets of an individual's personality could be linked

directly to these polymorphisms. A study involving mice also points to this conclusion, with a mutation within *Clock* resulting in increased exploratory behaviour and activity in female mice when exposed to a novel or stressful environment (Easton et al., 2003). This was later confirmed, by another study involving mice carrying a dominant negative mutation in the *Clock* gene (Roybal et al., 2007). They displayed a behavioural profile similar to human mania, including hyperactivity and decreased sleep amongst other symptoms.

The current study therefore attempts to test the hypothesis that there is a link between personality, diurnal preference and clock gene polymorphisms through the use of a number of measures. Comparisons between diurnal preference and personality will be made by utilising the HÖ questionnaire, as it is the most widely used measure and correlates with biological parameters (see section 1.3). This will be used in conjunction with the NEO-FFI, which measures the five-factor model of personality, and has been selected primarily due to it being well validated and stable across cultures but also because it has been widely used and is (due to time constraints) short. Although other measures are available (see section 1.7.3) they are by no means widespread in the literature and as previously discussed, may not measure exactly the same constructs as the NEO-FFI. In order to make true comparisons to previous work it is necessary to ensure the dimensions identified are consistent with earlier research. These measures will be used to confirm whether conscientiousness, in addition to other personality dimensions, is indeed associated with morningness, as has been proposed previously.

Furthermore, the reported association between impulsivity and morningness (Caci et al., 2005) will be tested with the use of the I_7 subscale of the IVE-7, as was used in that study. This is in order to provide consistency in the definition of impulsivity and to make the comparison of the results more meaningful. The I_7 has played a significant role in impulsivity

research, it also does not suffer the same defects such as criteria contamination, reliability and construct validity issues as do a number of other measures. Furthermore, it has been translated into other languages which share similar internal reliabilities (Caci et al., 2003, Lijffijt et al., 2005) and so has cross-cultural stability. It was also linked to the motor impulsivity subscale of the Barratt impulsiveness scale and so may be associated with behavioural aspects of impulsivity (Caci et al., 2003).

In order to fully assess personality, it is necessary to include a behavioural measure. From the instruments discussed, the stop signal paradigm seems to be the most effective measures of behavioural impulsivity and its disorders than the various other tests investigated in previous studies. Additionally, the GoStop variant of the stop signal paradigm has been linked to conscientiousness (Edmonds et al., 2009) and so will provide a behavioural measure of this dimension. From previous research, it can be hypothesised that mean stop latency will be slower in participants low in conscientiousness and high in impulsivity.

The results obtained from these measures can then be used to identify associations between personality traits and clock gene polymorphisms known to associate with diurnal preference (see section 1.4). It also provides the opportunity for further investigation of possible predictors of diurnal preference as well as clarification of the placement of impulsivity within the personality framework.

Chapter 2 Materials and Methods

2.1 Data collection

2.1.1 Questionnaire development

The following questionnaires were selected in order to assess personality, diurnal preference, sleep disturbance and depression. In addition to this, a self-report questionnaire which acted as a screening tool, was developed to determine whether the subjects fulfilled the inclusion/exclusion criteria, and in order to select participants.

2.1.1.1 The Neuroticism-Extraversion-Openness Five Factor Inventory (NEO-FFI)

The NEO-FFI (Costa and McCrae, 1992) is the shortened version of the revised NEO personality inventory (NEO-PIR) (Costa and McCrae., 1992) and consists of 60 items designed to measure the dimensions of the Five Factor Model of personality. Participants are asked to respond, using a 6-point likert scale. This measures a subjects level of agreement with a statement they are given and ranges from strongly disagree to strongly agree, with no opinion in the middle. This results in five broad domain scores falling between 12 and 72.

2.1.1.2 Impulsiveness 7 questionnaire (I_7)

The impulsiveness subscale (I_7) of the Impulsiveness-venturesomeness-empathy questionnaire (IVE-7) (Eysenck et al., 1985) was used to assess impulsivity. It is a 19-item questionnaire in

a yes/no format. Scores range from 0 to 19, where high scores indicate increased impulsiveness.

2.1.1.3 The Horne-Östberg (HÖ) questionnaire

The HÖ questionnaire (Horne and Ostberg., 1976) is a common measure of diurnal preference and consists of 19 items, 14 of which are multiple choice and 5 which require the subject to enter information relating to time of day. Scores range from 16 to 86, where 70-86 are definite morning types, 59-69 are moderate morning types, 42-58 are neither type, 31-41 are moderate evening types and 16-30 are definite evening types.

2.1.1.4 The Pittsburgh Sleep Quality index (PSQI)

The PSQI (Buysse et al., 1989) is screening tool used to measure levels of sleep quality and disturbance in the last month. It consists of 19 questions covering 7 areas: sleep latency, subjective sleep quality, sleep duration, habitual sleep efficiency, use of sleep medication, sleep disturbances and daytime dysfunction. 15 items on the scale are multiple choice where scoring is based on a four point likert scale (0-3). The other four items require the participant to write answers relating to bedtime and wake-up time, sleep duration and sleep latency. The seven component scores are combined to produce a global PSQI score that ranges from 0 to 21, where anything above 5 is indicative of poor sleep.

2.1.1.5 The Hospital Anxiety and Depression Scale (HADS)

The HADS (Zigmond and Snaith, 1983) consists of 14 items, 7 of which relate to depression and 7 to anxiety. Each item is a four point (0-3) multiple choice question, and scores range

from 0 to 21 for anxiety and from 0 to 21 for depression. The subscales can be used independently, where scores of 0 to 7 are regarded as normal, 8 to 10 suggest the presence of the mood disorder and 11 or higher indicating its probable presence.

2.1.2 Website construction

A webpage was constructed as part of the University website with details of the study. An account was then created at www.Zapsurvey.com and the above questionnaires were incorporated into one online survey, not randomly but in their original form. The University web page was linked to the online survey and data collected by the website. The data were downloaded in the form of a CSV file but due to some difficulties importing the file into SPSS, a PHP file was created, which cleaned the original file and enabled it to open in SPSS successfully. Duplicate and incomplete entries were deleted and multiple entries through one IP address were barred using the website's settings.

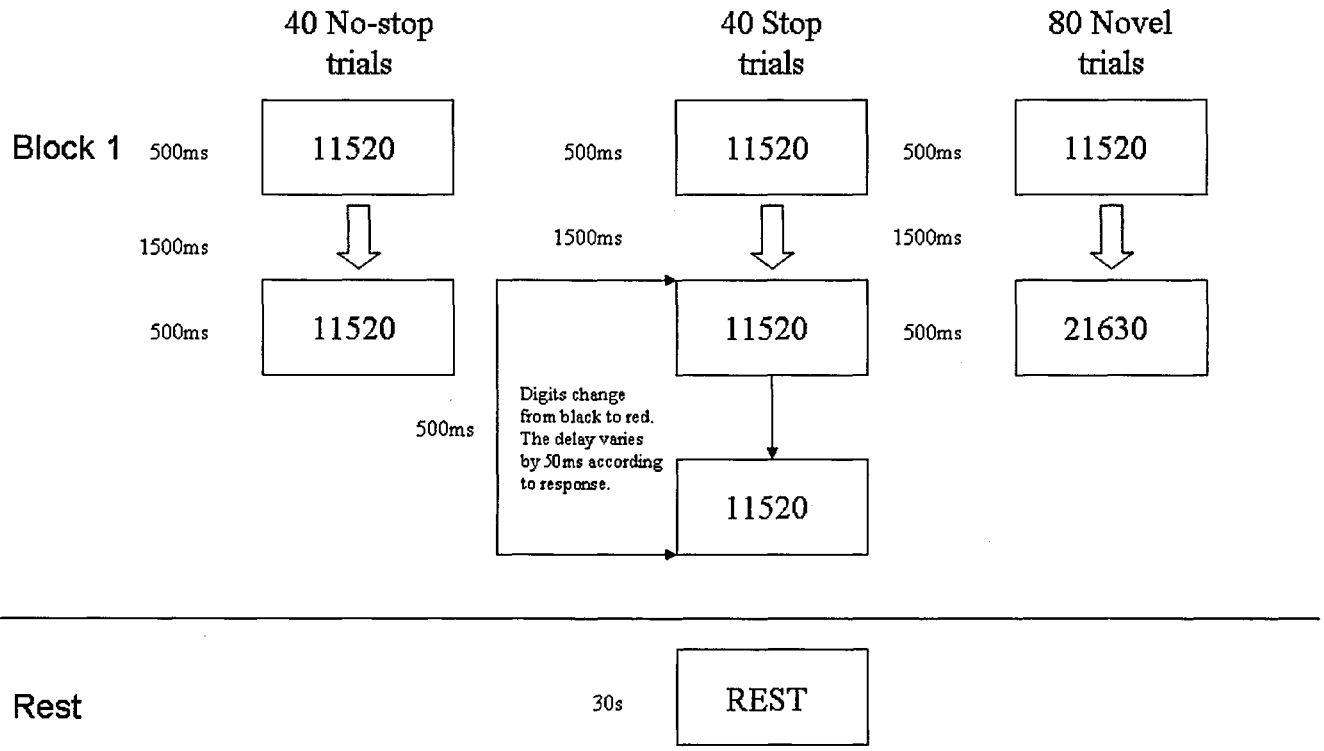
2.1.3 Behavioural test

Subjects who were selected to be in high, low or intermediate conscientious groups (see section 2.2.3) were asked to undergo the GoStop Paradigm version 1.01 (Dougherty et al., 2005). 105 participants agreed, with 7 then excluded after examining the results for outliers (see section 5.3), leaving 97 participants (mean age 25.5 ± 5.9yrs, 65 females [67.0%]). High, intermediate and low conscientiousness groups consisted of 40 (mean age 26.3 ± 5.9, 35 females [87.5%]) , 31 (mean age 24.4 ± 4.9 yrs, 22 females [71.0 %]) and 26 participants (mean age 25.6 ± 6.9 yrs, 8 females [30.8%], respectively. The test required participants to respond to a 'go' stimulus (a five digit number presented in black that is identical to the previous number) by clicking a mouse and to withhold a response to a 'stop' stimulus (a

matching five digit number that changes from black to red). The test parameters were set so that an adjusted stop signal paradigm was selected where stop intervals varied dynamically according to whether or not the response was correct. The initial stop interval was set at 250ms with an increase of 50ms for a correct response and a decrease of 50ms for an incorrect response. The duration of the stimulus was set to 500ms, blackout 1500ms and rest 30s. There were two blocks consisting 40 stop trials (trial containing go and stop signals), 40 no-stop trials (trial containing only a go signal) and 80 novel trials (non-matching numbers presented in black, included to maintain attention), all of which were randomly presented (Fig. 2-1).

The tests took place at the University except for 9 participants whose test took place at the participant's home, with the time of day and season of test recorded in each case. Instructions were read to each subject using cards provided with the test, and they were asked to click the mouse using their dominant hand. Following completion of the test, latency, stop latency and percentage inhibition were calculated by the program and this, as well as additional data, was saved as a text file automatically by the program. These variables were then entered into SPSS and the means of each variable were calculated for each participant.

Figure 2-1 GoStop Impulsivity Paradigm trial structure



2.1.4 DNA sample collection

Participants selected to form part of the high, low and intermediate conscientiousness groups (see section 2.2.3) were asked to donate a buccal swab (Epicentre Technologies, Madison, WI, USA). They did this, after participating in the behavioural test, by running the swab 20 times along the inside of each cheek under supervision of the investigator. This was then air dried for 15 minutes before being stored at -20°C. A proportion of those agreeing to take part could not spare the time or lived too far away to complete the behavioural test. In these incidences, swabs were sent out via the Royal Mail along with instructions and a return envelope. As no supervision was possible, it was necessary to rely on the participants compliance with the instructions.

2.2 Subjects

2.2.1 Exclusion criteria

Ages were restricted to those between 18 and 39 due to known age related changes in HÖ score above that age range and due to diurnal preference in this age range being more closely linked to genotype (Jones et al., 2007). Subjects were also excluded if they took medication that affected serotonin or dopamine levels due to the association between diurnal preference and depression (Chelminski et al., 1999). The effects of this medication may prevent an accurate assessment of diurnal preference. Additionally, for the same reason, participants were excluded if they had a chronic illness or sleep disorder.

2.2.2 Recruitment

An ethics application was submitted to the University of Surrey ethics committee before recruitment took place and the study proposal received a favourable ethical opinion (EC/206/02/SBMS). Subjects were recruited through a poster campaign entitled 'Genes and Behaviour', which requested healthy participants between the ages of 18 and 39 years and offered them a £5 voucher if selected to take part in the behavioural test. Advertisements were also sent out via email and an advert was placed in the local press. All adverts directed prospective participants to a website containing an online questionnaire, furthermore, contact details were also given so those without internet access could request the questionnaire in paper form.

2.2.3 Selection of extreme conscientious groups

Power calculations were performed in order to determine the number of subjects required for each group, whilst ensuring that genetic differences could be detected. G power was used to calculate 25% v. 35%, based on the general allele frequency of the *PER3* VNTR 5 repeat being 0.3 and wishing to be able to detect a 10% difference in prevalence. Power was set to 80%, using a two-tailed test at $p = 0.05$. This gave a sample size of 330 alleles per group (165 individuals per group). Therefore in the initial phase we needed to recruit 1650 participants.

Using the conscientiousness scores of the NEO-FFI, 10% extremes formed high and low conscientiousness groups, as well as selecting the centre 10% of the sample as an intermediate group. After the initial recruitment period, it was decided that the selection of the extreme groups should be expanded to 20% due to the limited number of respondents. New power

calculations used G power to calculate 20% v. 40%, to be able to detect a 20% difference in prevalence. Power was set to 80%, using a two-tailed test at $p = 0.05$. This gave a sample size of 91 alleles per group (46 individuals per group). Therefore in the initial phase we needed to recruit 460 participants.

The website containing the questionnaires received 1198 visits. Of those visits, 342 did not start to complete the survey, 13 participants entered deliberately incorrect details and so their entries were deleted, 64 were duplicate entries, 134 dropped out before reaching the end of the survey (drop out rate 17.2%) and 28 were excluded due to taking medication affecting serotonin or dopamine levels or because they had a chronic disease or sleep disorder. This left a total of 617 participants (mean age 25.2 ± 5.5 yrs, 412 females [67%]).

When the high, low and intermediate groups were selected a total of 174 participants (mean age 25.5 ± 5.4, 117 females [67.2%]) responded to the invitation to take part in the behavioural test and/or donate a buccal swab. This resulted in group sizes for high, low and intermediate conscientiousness of 65 (mean age 26.3 ± 5.3, 50 females [76.9%]), 52 (mean age 25.4 ± 6.0, 27 females [51.9%]) and 57 (mean age 24.8 ± 4.9, 40 females [70.2%]), respectively.

2.3 Genotyping of subjects

2.3.1 DNA extraction

When ready to extract, the swabs (see section 2.1.4) were defrosted and each twisted in a labelled 1.5ml Eppendorf tube containing 400µl of Quick Extract solution (Epicentre Technologies). The tubes were then vortexed for 10 seconds before being heated for 1 minute

at 65°C, after which they were vortexed again for 15 seconds and then heated for 2 minutes at 98°C. Lastly, they were vortexed for 15 seconds and then stored at -20°C along with the used swabs.

2.3.2 Polymerase chain reaction (PCR)

PCR was used to amplify specific regions of DNA in order to visualise them. The method works through a series of cycles consisting of varying temperature changes. Each reaction must contain template DNA, primers which have been designed to anneal to either side of the region of interest (Table 2-1), dNTPs, $MgSO_4$ buffer solution and *Taq* polymerase.

When genotyping the *PER3* VNTR, Green GoTaq Mastermix (Promega, Madison, WI) was used. This is a premixed solution containing bacterially derived *Taq* polymerase, dNTPs, $MgCl_2$, reaction buffers and two dyes. 20µl reaction solutions were prepared consisting of 10µl Green GoTaq Mastermix, 1.5µM of each primer, 5µl DNase free water and 2µl DNA. In cases that were difficult to amplify, 30µl reactions were made containing 3µl DNA. The reactions were amplified for 95°C for 5 minutes and then 40 cycles of 95°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds and then an elongation stage of 72°C for 5 minutes. The amplification of a number of samples failed using GoTaq Mastermix, and consequently a *KOD* hot start enzyme (Novagen, Madison, WI) was used. This enzyme is not activated until it is heated to 95°C which prevents non-specific amplification prior to cycling. The PCR was carried out in a volume of 20µl and contained 2µl 10x reaction buffer, 1.2µl $MgSO_4$, 2µl dNTPs, 11.2µl DNase free water, 0.6µM each primer, 0.4µl *KOD* enzyme and 2µl DNA. The reactions were amplified at 95°C for 2 minutes, then 40 cycles of 95°C for 20 seconds, 58°C for 10 seconds and 70°C for 3 seconds.

Polymorphism	Primer sequence
PER3 VNTR	(AF) Forward 5' GTCTTTTCATGTGCCCTTACTTTC 3' (AR) Reverse 5' ATCCGAATACGAAAGACAGCATAC 3'
PER2 10870	(F1) Forward 5' TACTCGAAGCCGACTTTGCC 3' (R1) Reverse* 5' ACACCTACGAAGGGTGAAGAATG 3' (S1) Sequencing 5' ACTTTGCCTGAGTCTTG 3'
PER1 T2434C	ARMS primers: T specific forward 5' GTATGGATGTGTTGACCCCTGAA 3' reverse 5' CTGGGCCTGGGGCTAGA 3' C specific forward 5' TGGAGGACCTGCCTGGC 3' reverse 5' CCCCCAACAATCCAGTCCTA 3'
CLOCK C3111T rs1801260	ARMS primers: C specific 5' AGGTGATCATAGGGGCAC 3' T specific 5' AGGTGATCATAGGGGCAT 3' Reverse 5' GAGGTCATTTCATAGCTGAGC 3' Pyrosequencing primers: (F1) Forward 5' ATTAAATACCAGCCAGCAGGAGG 3' (R1) Reverse*5'CCATCAAAAAATATCCAGGCACCTAA 3' (S1) Sequencing 5' GGAGGTGATCATAGGG 3'
CLOCK rs12648271	(F1) Forward 5' CACTCTTTCGGATTATTTGAAGC 3' (R1) Reverse* 5' AATTTCTCTCAGGGCGTTTTGT 3' (S1) Sequencing 5' AGTTATGTTTTATAAAAGCC 3'
CLOCK rs11932595	(F1) Forward 5' CATCTTGAGTGCATTGGTTTAGAC 3' (R1) Reverse* 5' TTGAAGGACAGGGAGACTGGT 3' (S1) Sequencing 5' GTTTAGACCCCTGCC 3'
PER2 C111G rs2304672	(F1) Forward 5' GTGTGCTTGTTAATGCGTGACAG 3' (R1) Reverse* 5' GAAATTCCGCGTATCCATTCA 3' (S1 new seq) Sequencing 5' CTCTGTTTGCCAGCT 3'

* Biotinylated at 5' end

Table 2-1 Primer sequences.

2.3.3 Agarose gel electrophoresis

In order to resolve the difference in size between the four and five repeats in the *PER3* VNTR, a 2% Tris-Boric acid-EDTA (TBE) gel was prepared. The molecular marker PhiX174DNA/*HaeIII* (Promega) was loaded into the first well in order to determine the size of each PCR product. The total volume of the PCR reaction was then loaded into each well. The gel was run at 80V for 2 hours in 1 X TBE. Visualisation of the PCR products was carried out using an ultraviolet transilluminator and recorded using Polaroid film.

2.3.4 GenomiPhi

Samples that had failed to be genotyped were amplified using Genomiphi (GE Healthcare, Little Chalfont, Bucks, UK). This method uses the bacteriophage Phi29 DNA polymerase to amplify DNA templates non-specifically via a strand displacement reaction, and is useful for increasing limited sample sizes.

Firstly, 9µl sample buffer was added to 1µl DNA and then heated at 95°C for 3 minutes and cooled on iced to 4°C. Mastermix was then prepared on ice and contained 9µl reaction buffer and 1µl enzyme mix for each sample. 10µl master mix was then transferred to each sample and incubated at 30°C for 1.5 hours. The enzyme was then inactivated by heating the samples to 65°C for 10 minutes and cooled to 4°C on ice. The resulting sample was treated as genomic DNA and amplified by PCR as previously described (see section 2.3.2).

2.3.5 Taqman genotyping assay

Pre-designed Taqman genotyping assay's (Applied Biosystems, Foster City, CA), were purchased for *PER2* C111G, *CLOCK* rs12648271 and *CLOCK* rs11932595. These contained two un-labelled PCR primers and two minor groove binder (MGB) probes for each allele and were used together with ROX Mastermix (Thermo Scientific, Epsom, Surrey, UK). Each well contained 6.25µl 2x mastermix, 0.31µl 40x assay, 4.94µl DNAse free water and 1µl DNA. PCR was carried out using a GeneAmp PCR system 2700 thermal cycler (Applied Biosystems) and amplified for 95°C for 15 minutes, then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Allele detection was carried out using an ABI 7500 realtime PCR machine (Applied Biosystems). Due to difficulties in distinguishing alleles most samples were also genotyped using pyrosequencing (see section 2.3.7).

2.3.6 Amplification refractory mutation system (ARMS)

Previously designed primers (Carpen et al., 2006; Robilliard et al., 2002) were used, that terminated with either the normal or variant nucleotide, to amplify the region immediately prior to the *CLOCK* C3111T and *PER1* T2434C according to ARMS protocol (Newton et al., 1989). The same protocol was used for both polymorphisms. Two 20µl reactions were made for each sample, the first containing the C variant primer pair and the second, the T variant. Each reaction contained 10µl Green GoTaq Mastermix, 5µl DNAse free water, 1.5µM of each primer and 2µl DNA. For CLOCK C3111T the reactions were amplified at 94°C for 3 minutes and then 35 cycles of 94°C for 45 seconds, 60°C for 45 seconds and 72°C for 1 minute. For *PER1* T2434C the reactions were amplified at 94°C for 4 minutes and then 30 cycles of 94°C for 30 seconds, 64°C (T allele) or 61°C (C allele) for 30 seconds and 72°C for

1 minute The PCR products were then run on a 1% agarose gel at 80V for 1 hour, and visualised as previously described (see section 2.3.3).

2.3.7 Pyrosequencing

Samples in which genotyping for *CLOCK* C3111T had failed or that were difficult to determine using ARMS, were sequenced using a PSQ-96 pyrosequencer (Biotage, Uppsala, Sweden) and based on work carried out by Ronaghi et al. (1999). Primers were designed using pyrosequencing assay design software version 1.0 (Biotage), which also produced a report containing the sequence to be analysed. As only single SNP assays were used, a simplex file was created (rather than a multiplex) in which the sequence to analyse was entered. The software then generated the appropriate nucleotide dispensation order for the relevant assay. When ready to run, this file was selected for the appropriate well in order for the SNP to be identified.

In order to find the most efficient annealing temperature, the primers were optimised using temperature gradient PCR on a DNA engine DYAD Peltier Thermal Cycler (MJR research, Waltham, MA). Eight 0.2ml tubes containing 5.3µl DNase free water, 6.3µl Green GoTaq mastermix, 0.5µl DNA and 0.3µM each primer were amplified at 95°C for 5 minutes, and then 50 cycles of 95°C for 30 seconds, eight temperatures ranging from 50°C to 70°C for 30 seconds, 72°C for 30 seconds, and then a final hold of 72°C for 5 minutes. The samples were then run on a 1.6% agarose gel (see section 2.3.3), with each sample corresponding to a particular temperature. The band most visible and therefore the ideal annealing temperature, was found to be 58.4°C as defined by the sequence of the most abundant specific amplification product.

50µl reactions were made for each sample and contained 25µl Green GoTaq Mastermix, 2µl DNA, 1µM of each primer and 21µl water. The reactions were amplified as above. Following amplification, 3µl of streptavidin sepharose (GE Healthcare) and 37µl of binding buffer (Biotage) was added to each reaction. 40.5µl annealing buffer (Biotage) and 4µl sequencing primer was then added to each well of the pyrosequencing plate (Biotage).

A vacuum manifold was used to extract the solution from each tube simultaneously, which was then washed in each of the following for 5 seconds, 70% ethanol, 0.2M NaOH, pyrosequencing wash, and distilled water. The vacuum was then turned off and the manifold placed onto the pyrosequencing plate, with each pin placed into its respective well. The plate was heated for 2 minutes at 80°C before being placed into the pyrosequencer. A reagent cartridge was then loaded into the pyrosequencer containing enzyme solution, substrate solution, and four nucleotides, according to the number of samples and sequence to be analysed. The results were visualised as pyrograms, where peaks indicated a nucleotide was present.

This process was also used to genotype *PER2* 10870, *PER2* C111G, *CLOCK* rs12648271 and *CLOCK* rs11932595. Primers were designed for each SNP (Table 2-1) and optimised to find the ideal annealing temperature (Table 2-2). Simplex files were created for each SNP using the report created during primer design. Samples that were difficult to genotype were amplified using the *KOD* enzyme in 50µl reactions containing, 5µl 10x buffer, 3µl $MgSO_4$, 5µl dNTPs, 31µl DNase free water, 1.5µM primer, 1µl *KOD* enzyme and 2µl DNA. The reactions were amplified for 95°C for 2 minutes, then 50 cycles of 95°C for 20 seconds, annealing temperature for 10 seconds and 70°C for 1 second. The genotyping of *CLOCK* rs12648271 failed completely and due to the position of the polymorphism in the gene, a different primer could not be designed and no further analysis could be undertaken.

Polymorphism	Annealing temperature (°C)
PER2 10870	53.2
PER2 C111G	58.4
CLOCK rs12648271	60.2
CLOCK rs11932595	61.8

Table 2-2 Annealing temperatures.

2.4 Statistical analysis

Data analysis was conducted using SPSS software version 11.0.4 (SPSS Inc., Chicago, IL). Responses to the PSQI, depression scale of the HADS, NEO-FFI, HÖ, I_7 and the self report questionnaire were all adjusted to the correct format so that they could be scored accurately. The questionnaires were then scored according to the instructions given. In order to perform parametric tests, the distribution of each scale was checked for normality by plotting histograms of their distributions. Transformations were performed on those that did not have normal distributions. For specific details of the transformations applied see chapters three, four and five. The following analyses were performed on the resulting data.

2.4.1 Pearson correlation

Pearson correlations were used in Chapter 3 initially, to measure the strength any relationships between variables and to identify any preliminary associations which could be investigated further with subsequent analyses. The Pearson correlation is standardised (the variable is converted to a standard unit of measurement by dividing the observed deviation by the standard deviation) so that different variables can be compared to each other. Correlation shows this relationship by assessing the amount and direction of variance the variable deviates from its mean. Therefore, a positive relationship would be one where two variables deviate from their mean by a similar amount and in the same direction.

In Chapters 4 and 5, Pearson correlations were carried out again on the same variables in order to observe whether the same relationships were present in the smaller samples. These correlations though, do not indicate the cause of the association, so a third variable may be affecting the results as a result of overlapping variance. For this reason in Chapter 3, when investigating the relationship between personality and diurnal preference, the dimensions of the NEO-FFI, impulsivity and HÖ score were subjected to a partial correlation where age, gender, sleep disturbance, night shift work and depression, were all held constant in order to identify whether the correlations were still significant. The effects of these variables were controlled for by the removal of any overlapping variance, giving a more accurate correlation value.

2.4.2 Comparing means

In Chapters 3, 4 and 5 independent *t* tests were used to compares two means from independent samples to determine whether they vary significantly. In Chapter 3, gender differences in each

variable were assessed using this method and subsequently in Chapters 4 and 5, *t* tests were carried out on the smaller samples in order to determine whether the same gender differences were evident in the smaller samples. Additionally, variables in high and low groups were compared using this method in Chapters 4 and 5. In Chapter 5, *PER2* 10870 and *PER1* T2434C no longer had three genotypes due to the smaller sample size, therefore independent *t* tests were conducted between the two remaining genotypes to assess mean stop latency.

An ANOVA enables the comparison of more than two means and uses an *F* ratio to determine the overall fit of the model. This method was used in Chapter 4 to confirm that there was indeed a difference in conscientiousness scores between the three conscientiousness groups and in Chapter 5 to compare mean stop latency between three genotypes. As an ANOVA only indicates that the model accounts for more variation than external factors and not where the association lies, planned contrasts were performed in Chapter 4 (which are used when there is a specific hypothesis of the outcome) to identify which groups differed from one another and *post hoc* tests in Chapter 5 as there were no specific hypotheses.

Multivariate analysis of variances (MANOVAs) were used in Chapter 4 to assess the differences on the dependent variables between conscientiousness groups and also between genotype. These were used instead of multiple ANOVAs as they take into account the relationships between the dependent variables and to avoid inflating the familywise error rate (the probability of making a type I error or getting a false positive result, in a family of tests). Each significant MANOVA was followed up by an ANOVA for each variable and *post hoc* analyses (where there is no specific hypothesis) as well as a multiple regression. This was to determine whether, in addition to relationships between dependent variables, there may also be independent variables that differ between groups.

2.4.3 Regression

In Chapter 3 hierarchical multiple regressions were performed in order to establish which independent variables predicted a person's diurnal preference (dependent variable). The hierarchical method was used so that known predictors from previous research could be entered first followed by new predictors, in order to see how they contribute to the overall model.

A multinomial logistic regression was used in Chapter 4 as unlike in multiple regression the dependent variable can be categorical and the predictor variables continuous or categorical. It was used to find which variables best predicted genotype (three categories). Similarly, in Chapter 5, multinomial logistic regression was used to find which behavioural variables best predicted genotype. In this type of regression one group is compared to the other two which means that there is no comparison between two of the groups. Due to this a binary logistic regression, which allows only two groups to be compared, was performed following each multinomial logistic regression in order to compare the remaining two groups.

2.4.4 χ^2 tests

χ^2 tests were used in Chapter 4 to determine whether genotype frequencies differed between conscientiousness groups and in Chapter 5 to check that allele frequencies were consistent with the previous chapter. This method is used when comparing two categorical variables in order to determine whether there were significant differences between the two. It works by comparing observed frequencies with the expected frequencies that you may get by chance. When greater than 20% of the expected frequencies were less than 5 or when any of the expected frequencies were less than one, the χ^2 test becomes inaccurate and so a fishers exact

test was performed which is designed for small sample sizes. In these instances allele frequencies were compared between only the high and low groups rather than genotype frequencies as it is only possible to do a 2 x 2 contingency table with this test.

Chapter 3 Diurnal preference and personality

3.1 Hypotheses

3.1.1 Hypothesis 1

Recent research has identified an association between impulsivity and diurnal preference, where impulsivity is higher in evening types (Caci et al., 2005). This study utilised the IVE-7 scale to measure impulsivity, venturesomeness and empathy as well as using the HADS as a screening tool. They did not take into account other possible confounding factors such as sleep disturbance and night shift work which may have affected the results. The present study therefore sought to firstly confirm this previous research but also to establish whether this association was still present after controlling for factors that may influence diurnal preference. The first hypothesis is therefore:

- Impulsivity will be correlated with eveningness

3.1.2 Hypothesis 2

Several studies have investigated the relationship between the dimensions of the five factor model of personality and diurnal preference and reported an association between conscientiousness and morningness (Jackson and Gerard, 1996; Gray and Watson, 2002; Cavallera and Giampietro, 2007; Deyoung et al., 2007; Randler, 2008; Tonetti et al., 2009) but again none controlled for all the confounding factors or used the same scales as was used in the present study. Associations between diurnal preference and the other dimensions of the

five factor model have been inconsistent. Furthermore, as impulsivity is thought to be comprised of neuroticism, conscientiousness and extraversion (Whiteside and Lynam, 2001), it is likely that the association between diurnal preference and impulsivity identified by Caci et al was due in part to variance shared between impulsivity and conscientiousness which may mean that conscientiousness was the underlying dimension responsible for the association. It could be hypothesed that consciousness would have a greater association with diurnal preference than impulsivity. The second hypothesis is therefore:

- Conscientiousness will have a greater association with diurnal preference than the other dimensions of the five factor model of personality and impulsivity.

3.2 Methods

For full details of methods see Chapter 2. Methodologies specific to this study are outlined below.

3.2.1 Study design

Participants were asked to complete an online questionnaire comprising the NEO-FFI, the depression scale of the HADS, HÖ, PSQI, I_7 and a self-report questionnaire which requested information in relation to the exclusion criteria (see section 2.2.1). This was in order to collect data for the following variables: Gender, age, shift work, depression, diurnal preference, sleep disturbance, impulsivity, neuroticism, extraversion, openness, agreeableness and conscientiousness. Subjects with sleep disorders, chronic illnesses or those taking medication that affected serotonin or dopamine levels were excluded, for an explanation of the exclusion criteria see section 2.2.1.

3.2.2 Statistical analysis

Statistical analysis was performed using SPSS version 11. Correlations used Pearson's *r* (see section 2.4.1) and were performed for the variables diurnal preference, age, depression, sleep disturbance, impulsivity, neuroticism, extraversion, openness, agreeableness and conscientiousness. Differences between gender on the same variables used an independent *t* test (see section 2.4.2), and predictors of diurnal preference were calculated by a hierarchical multiple regression (see section 2.4.3) with gender, age, sleep disturbance and depression added in block one, impulsivity in block 2 and neuroticism, extraversion, openness, agreeableness and conscientiousness added in block 3.

3.3 Results

After cleaning the data and excluding participants who did not fulfil the exclusion criteria (for a full breakdown see section 2.2.3) this left a total of 617 participants (mean age 25.2 ± 5.5 yrs [standard deviation], 412 females [67%]) who had completed the online questionnaire. The scores on the NEO-FFI, the depression scale of HADS, HÖ and PSQI all followed a normal distribution. Only age (Fig. 3-1) and I_7 score (Fig. 3-2) did not follow a normal distribution and so the data from these were converted to *z* scores in order to produce a distribution with a mean of 0 and a standard deviation of 1. Gender and shift work were dichotomous and therefore did not need to be converted.

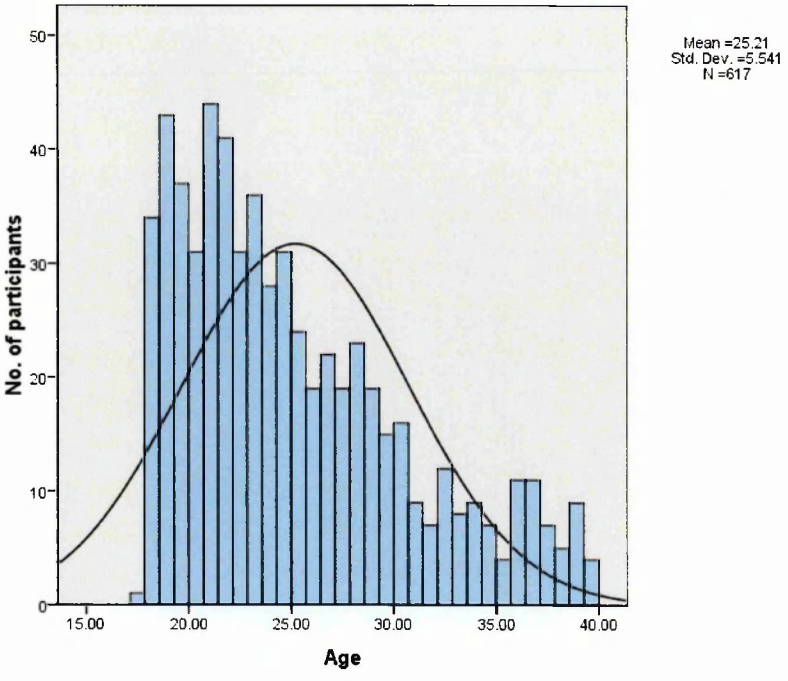


Figure 3-1 Histogram showing the number of participants and their ages, with a normal distribution curve to highlight the lack of normality.

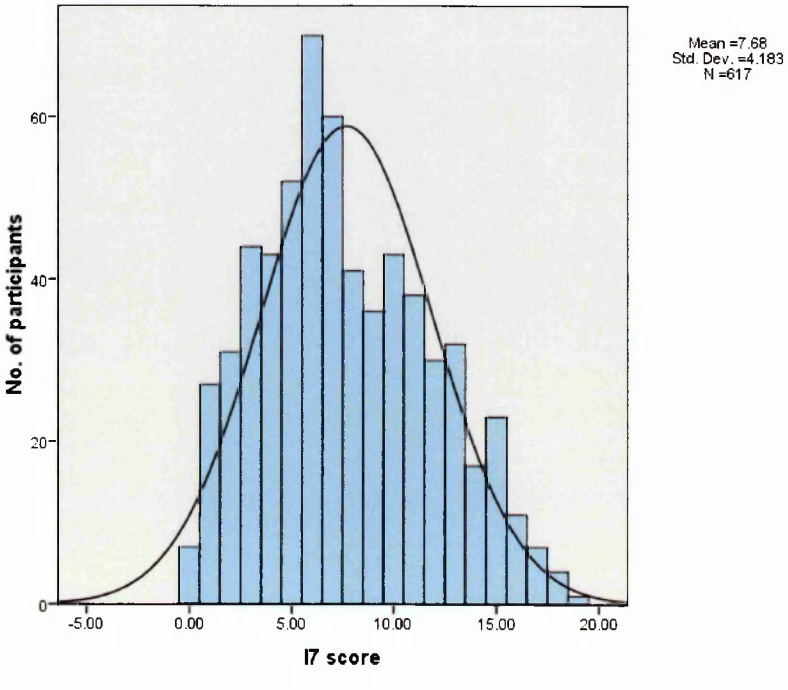


Figure 3-2 Histogram showing the number of participants and their I_7 scores, with a normal distribution curve to highlight the lack of normality.

3.3.1 Reliability of questionnaires

Internal reliability of the scales was determined using Cronbach's alpha (Table 3-1). The PSQI had the lowest reliability score of 0.68, which is slightly lower than reported in other studies (Buysse et al., 1989, Beck et al., 2004), with all other scales having values above 0.7 and the conscientiousness scale of the NEO-FFI the highest at 0.88.

Questionnaire	**Alpha**
PSQI	0.68
HADS: Depression	0.80
HÖ	0.87
NEO-FFI:	
Neuroticism	0.79
Extraversion	0.76
Openness	0.71
Agreeableness	0.76
Conscientiousness	0.88
I_7	0.80

Table 3-1. Reliability scores

Variables	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1. Age	—									
2. Depression	0.14**	—								
3. HÖ score	0.16**	-0.10**	—							
4. Impulsivity	-0.08	0.15**	-0.19**	—						
5. GPSQI	0.03	0.39**	-0.21**	0.21**	—					
6. Neuroticism	-0.04	0.53**	-0.03	0.18**	0.35**	—				
7. Extraversion	-0.15**	-0.52**	0.05	0.11**	-0.21**	-0.49**	—			
8. Openness	0.11**	-0.08	-0.14**	0.13**	0.05	-0.06	0.14**	—		
9.Agreeableness	0.05	-0.30**	0.18**	-0.26**	-0.17**	-0.28**	0.25**	0.00	—	
10. Conscientiousness	0.10*	-0.32**	0.33**	-0.37**	-0.26**	-0.28**	0.30**	-0.12**	0.26**	—

* $r < 0.05$ level, ** $r < 0.01$ level (2-tailed).

Table 3-2 Correlations between demographic factors, control variables and personality variables.

Variables	*1*	*2*	*3*	*4*	*5*	*6*	*7*
1. Neuroticism	—						
2. Extraversion	-0.31**	—					
3. Openness	-0.02	0.13**	—				
4. Conscientiousness	-0.15**	0.20**	-0.17**	—			
5. Agreeableness	-0.18**	0.15**	-0.03	0.14**	—		
6. Impulsivity	0.09*	0.22**	0.14**	-0.30**	-0.21**	—	
7. HÖ	0.07	0.02	-0.15**	0.26**	0.12**	-0.13**	—

* $r < 0.05$ level, ** $r < 0.01$ level (2-tailed).

Table 3-3. Partial correlations controlling for age, gender, sleep disturbance, night shift work and depression.

3.3.2 Analysis of the sample

A correlation matrix was constructed (Table 3-2) including the variables age, depression, HÖ score, impulsivity, GPSQI, neuroticism, extraversion, openness, agreeableness and conscientiousness. This enabled the preliminary identification of any relationships evident between the variables. This was followed by a partial correlation matrix (Table 3-3) which included the five dimensions of the five factor model, HÖ score and impulsivity whilst controlling for age, gender, sleep disturbance, night shift work and depression. This was undertaken in order to establish whether the relationships were still evident even after taking into account possible confounding factors and therefore strengthening the association.

3.3.2.1 Associations between personality variables

Impulsivity correlated positively with neuroticism (r = 0.18), openness (r = 0.13) and extraversion (r = 0.11) and negatively with agreeableness (r = -0.26) and conscientiousness (r = -0.37) (all $p < 0.01$) in the sample (Table 3-2). Correlations between dimensions of the five factor model revealed that the dimensions were intercorrelated and not orthogonal.

3.3.2.2 Age related differences

Depression (r = 0.14, $p < 0.01$), morningess (r = 0.16, p <0.01), openness (r = 0.11, p <0.01) and conscientiousness (r = 0.10, $p < 0.05$) all increased with age, whereas extraversion (r = -0.15, $p < 0.01$) decreased with age.

3.3.2.3 Associations between diurnal preference and personality

Pearson correlations revealed associations between diurnal preference and both age ($r = 0.16$, $p < 0.01$) and depression ($r = -0.10$, $p < 0.01$), indicating that morningness increases with age and participants with depression are more likely to be evening types (Table 3-2).

Further investigation of the correlations revealed relationships between personality and diurnal preference. Both agreeableness ($r = 0.18$), and conscientiousness ($r = 0.33$) (Fig. 3-3) were positively correlated with HÖ score, whereas openness ($r = -0.14$) and impulsivity ($r = -0.19$) (Fig. 3-4) (all $p < 0.01$) were negatively correlated with HÖ score. This suggests that morning types are higher in agreeableness and conscientiousness and evening types higher in openness and impulsivity. Although still significant, partial correlations led to a diminished relationship between HÖ score and conscientiousness ($r = 0.26$), agreeableness ($r = 0.12$), and impulsivity ($r = -0.13$), but not openness ($r = -0.15$) (Table 3-3). Neuroticism ($r = -0.03$, $p = 0.40$; Partial: $r = 0.07$, $p = 0.10$) and extraversion ($r = 0.05$, $p = 0.19$; Partial: $r = 0.02$, $p = 0.61$) were not associated with diurnal preference in either analyses.

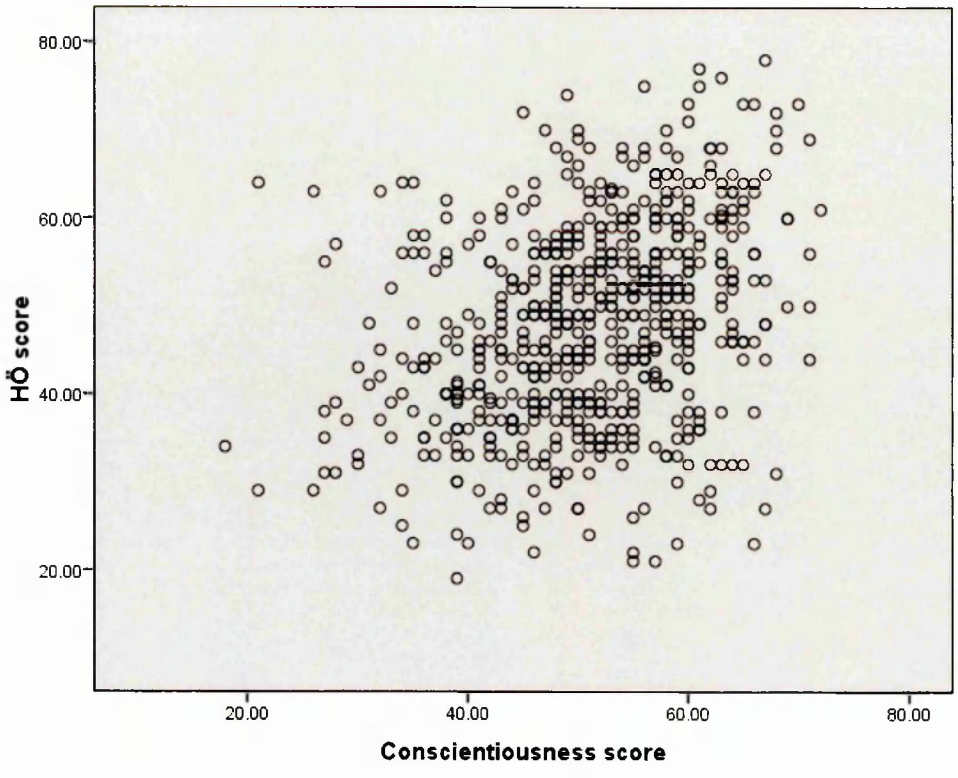


Figure 3-3 Bivariate scatterplot of HÖ score against conscientiousness score

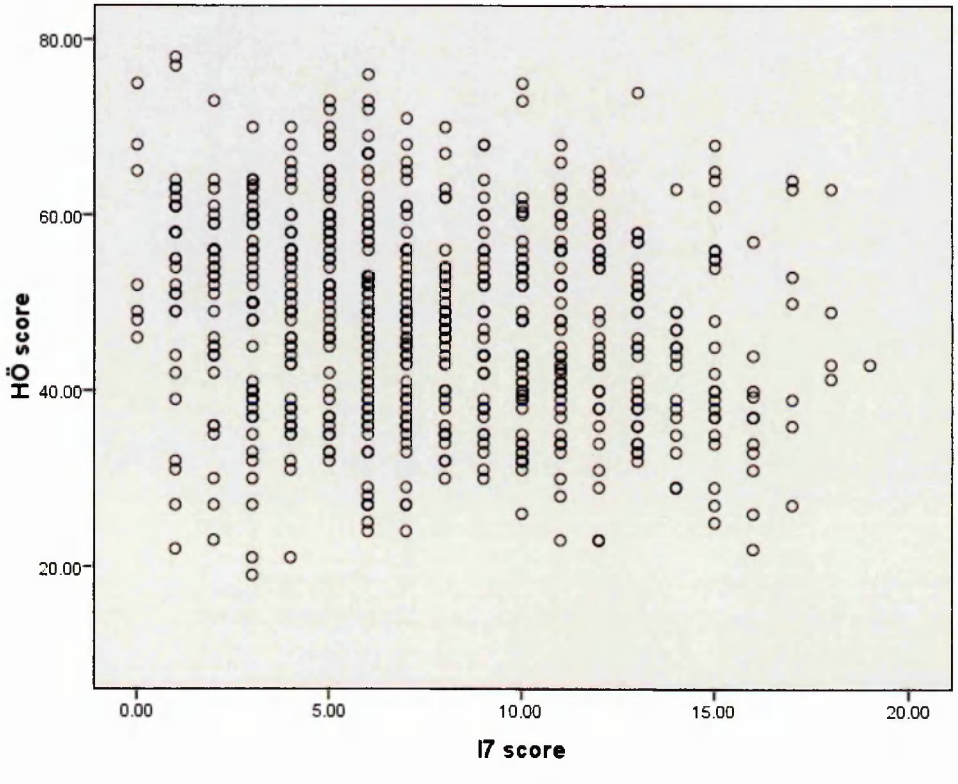


Figure 3-4 Bivariate scatterplot of HÖ score against I_7 score.

3.3.2.3.1 Predictors of diurnal preference

As correlations do not provide evidence of causality nor do they indicate whether one variable predicts another, a hierarchical multiple regression was performed to determine whether impulsivity or the dimensions of the five factor model were actual predictors of diurnal preference.

Firstly, it was necessary to check whether the assumptions of the regression model were met and therefore a number of tests were undertaken. To check the model for multicollinearity, the average variance inflation factor (VIF) was calculated as 1.38, indicating that the regression was unlikely to be biased. Furthermore, the largest VIF value was less than 10 (Myers, 1990) and all tolerance values were above 0.2 in both models suggesting that multicollinearity was not a problem.

In order to check whether the variance of the residuals was the same at each level of the predictor variables (homoscedasticity) and that each of the models were linear, a graph was plotted of the standardised residual values against the standardised predicted values (Fig. 3-5). A residual is the difference between the models predicted value and the observed value in the data. As the points on the graph are distributed randomly and evenly around zero in both cases, this indicates that the assumptions of homoscedasticity and linearity were met. The residuals were also checked to ensure they followed a normal distribution (Fig. 3-6).

To check whether the residuals were uncorrelated, a Durbin-Watson test was undertaken for each model, the result of which was a value of 2.11 which indicates that the residuals were indeed uncorrelated.

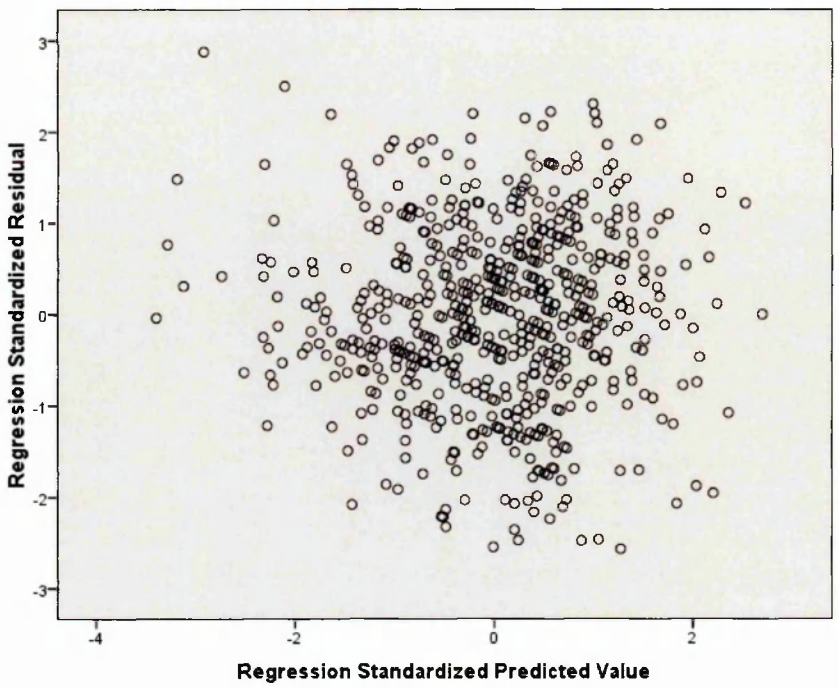


Figure 3-5 Scatterplot of standardised residual values against the standardised predicted values.

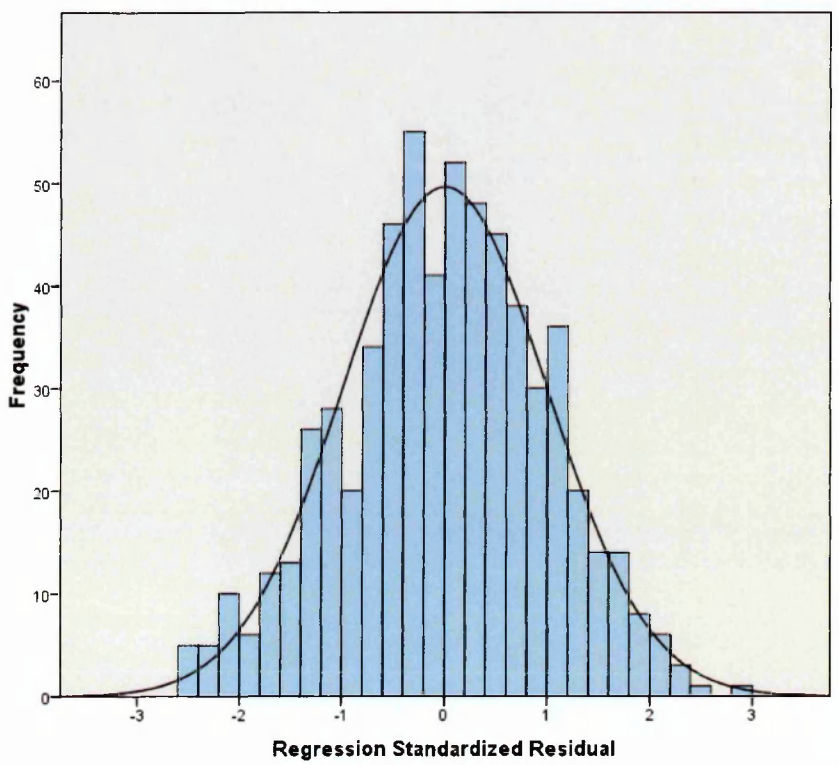


Figure 3-6 Histogram of residuals showing they follow a normal distribution.

A hierarchical multiple regression was chosen as variables that are known predictors from previous research can be entered into the model first, in order of importance. For this reason the first block included the control variables: age, gender, sleep disturbance, night shift work and depression all of which are known to influence diurnal preference, the second block included impulsivity which reportedly associates with eveningness and the third contained neuroticism, extraversion, openness, agreeableness and conscientiousness for which some associations have been reported but not as consistently as with the other variables. In the first step, the control variables accounted for 8% ($AR^2 = 0.08$) of the total variance, with gender ($\beta = 0.09$), sleep disturbance ($\beta = -0.19$) and age ($\beta = 0.17$) all significant predictors of HÖ score (Table 3-4). The addition of impulsivity in step two increased the variance accounted for to 9% ($AR^2 = 0.09$), with impulsivity ($\beta = -0.13$) also a significant predictor. In step three, the addition of the dimensions of the five factor model increased the amount of variance accounted for by the model to 16% ($AR^2 = 0.16$) and resulted in the loss of significance of gender and impulsivity, whereas neuroticism ($\beta = 0.15$), openness ($\beta = -0.10$), agreeableness ($\beta = 0.10$) and conscientiousness ($\beta = 0.25$) all emerged as significant predictors of HÖ score. This meant that with all the variables taken into account, conscientiousness was the biggest predictor of HÖ score indicating that if a participant had a high conscientiousness score it could be predicted that they would also have a high HÖ score. The other dimensions were predictors but were less significant and so may be less accurate as predictors of HÖ score.

		B	*SE B*	*β*
Step 1				
	Gender	2.18	0.96	.09*
	Shift work	0.28	1.66	.01
	Depression	-0.14	0.14	-.04
	Sleep disturbance	-0.80	0.18	-.19***
	Age	1.93	0.45	.17***
Step 2				
	Gender	1.97	0.95	.08*
	Shift work	0.57	1.65	.013
	Depression	-0.10	0.14	-.03
	Sleep disturbance	-0.71	0.18	-.17***
	Age	1.80	0.45	.16***
	Impulsivity	-1.46	0.46	-.13**
Step 3				
	Gender	-0.07	0.97	-.003
	Shift work	0.56	1.58	.01
	Depression	-0.06	0.17	-.02
	Sleep disturbance	-0.68	0.17	-.17***
	Age	1.82	0.45	.16***
	Impulsivity	-0.41	0.50	-.04
	Neuroticism	0.18	0.06	.15**
	Extraversion	0.03	0.08	.02
	Openness	-0.16	0.06	-.10**
	Agreeableness	0.17	0.07	.10*
	Conscientiousness	0.30	0.06	.25***

($R = 0.42$, $R^2 = 0.18$, Adj $R^2 = 0.16$)
* $p<.05$
** $p<.01$
*** $p<.001$

Table 3-4. Hierarchical multiple regression analysis of personality traits as predictors of diurnal preference.

3.3.3 Gender differences

Independent *t*-tests were carried out for each questionnaire score as well as for age in order to determine whether there were differences between males and females in each variable (Table 3-5). The results showed that males had a significantly higher GPSQI score indicating greater sleep disturbance ($t[615] = 2.47$, $p < .05$), although both male and females were above the recommended cut off of 5. Males were also more impulsive ($t[615] = 2.44$, $p < .05$). Females had significantly higher scores on neuroticism ($t[615] = -2.41$, $p < .05$), agreeableness ($t[615] = -5.77$, $p < .01$), conscientiousness ($t[346] = -5.24$, $p < .01$) and HÖ score ($t[615] = -3.07$, $p < .01$). Age ($t[615] = -1.64$, $p = 0.10$) and depression ($t[615] = 1.88$, $p = 0.06$) did not differ significantly between sexes.

	Males	Females	Combined	Males vs females
Age	24.7 ± 5.3	25.5 ± 5.7	25.2 ± 5.5	$t(615) = -1.64$
Depression	4.2 ± 3.6	3.6 ± 3.4	3.8 ± 3.4	$t(615) = 1.88$
GPSQI	6.1 ± 2.7	5.5 ± 2.8	5.7 ±2.8	$t(615) = 2.47$*
Neuroticism	38.5 ± 8.9	40.4 ± 9.3	39.8 ± 9.2	$t(615) = -2.42$*
Extraversion	49.8 ± 7.4	49.7 ± 7.5	49.7 ± 7.5	$t(615) = 0.19$
Openness	49.3 ± 7.4	49.1 ± 7.4	49.2 ± 7.4	$t(615) = 0.39$
Agreeableness	49.2 ± 7.2	52.7 ± 7.0	51.5 ±7.2	$t(615) = -5.77$***
Conscientiousness	48.1 ± 10.4	52.5 ± 8.5	51.0 ± 9.4	$t(346) = -5.24$***
HÖ	45.9 ± 10.9	48.9 ± 11.7	47.9 ± 11.5	$t(615) = -3.07$**
I_7	8.3 ± 4.3	7.4 ± 4.1	7.7 ± 4.2	$t(615) = 2.44$*

t, independent t test, *p < 0.05 ,** p < 0.01, *** p < 0.001.

Table 3-5 Descriptive statistics.

Variables	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1. Age	—									
2. Depression	0.18**	—								
3. HÖ score	0.12	-0.08	—							
4. Impulsivity	-0.03	0.14*	-0.17*	—						
5. GPSQI	-0.03	0.37**	-0.27**	0.17**	—					
6. Neuroticism	0.02	0.52**	-0.09	0.20**	0.35**	—				
7. Extraversion	-0.24**	-0.50**	0.14	0.07	-0.25**	-0.53**	—			
8. Openness	0.10	-0.04	-0.07	0.13	0.14*	-0.09	0.03	—		
9.Agreeableness	0.03	-0.33**	0.13	-0.26**	-0.19**	-0.29**	0.25**	0.05	—	
10. Conscientiousness	0.00	-0.33**	0.28**	-0.43**	-0.26**	-0.35**	0.38**	-0.20**	0.27**	—

* $r < 0.05$ level, ** $r < 0.01$ level (2-tailed).

Table 3-6 Correlations between demographic factors, control variables and personality variables in males.

Variables	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1. Age	—									
2. Depression	0.13**	—								
3. HÖ score	0.17**	-0.09	—							
4. Impulsivity	-0.09	0.15**	-0.18**	—						
5. GPSQI	0.06	0.39**	-0.17**	0.22**	—					
6. Neuroticism	-0.07	0.55**	-0.03	0.19**	0.36**	—				
7. Extraversion	-0.12*	-0.53**	0.02	0.13**	-0.19**	-0.47**	—			
8. Openness	0.12*	-0.10*	-0.17**	0.12*	0.01	-0.05	0.19**	—		
9.Agreeableness	0.04	-0.26**	0.18**	-0.24**	-0.13**	-0.32**	0.27**	-0.02	—	
10. Conscientiousness	0.13**	-0.29**	0.33**	-0.31**	-0.23**	-0.30**	0.27**	-0.07	0.20**	—

* $r < 0.05$ level, ** $r < 0.01$ level (2-tailed).

Table 3-7 Correlations between demographic factors, control variables and personality variables in females.

3.3.3.1 Associations between diurnal preference and other variables between the genders

Sleep disturbance was significantly correlated with HÖ score in both males (r = -0.27, p < 0.01) (Table 3-6) and females (r = -0.17, p < 0.01) (Table 3-7) suggesting evening orientated individuals of both sexes experienced more sleep problems. Age was also correlated with diurnal preference in females (r = 0.17, p < 0.01) but not males (r = 0.12, p = 0.10), indicating that morningness increases with age in females.

When investigating correlations between HÖ score and personality variables, HÖ score was significantly associated with agreeableness (r = 0.18, p < 0.01), openness (r = -0.17, p < 0.01) and conscientiousness (r = 0.33, p < 0.01) (Fig. 3-9) in females but only conscientiousness (Fig. 3-7) in males (r = 0.28, p < 0.01). Impulsivity was significantly associated with HÖ score in both sexes (males: r = -0.17, p < 0.01; females: r = -0.18, p < 0.01) (Figs. 3-8 and 3-10), suggesting that evening types are more impulsive.

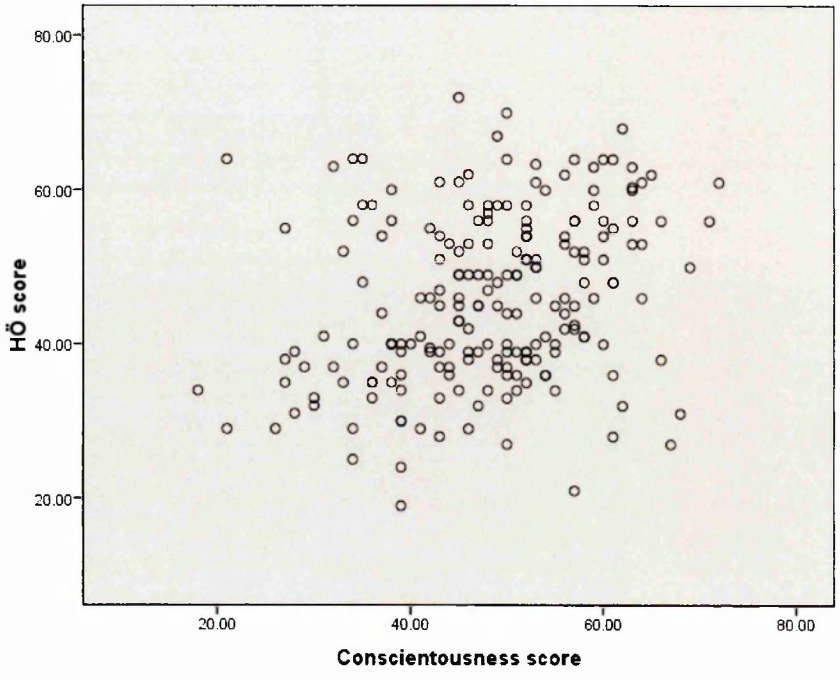


Figure 3-7 Bivariate scatterplot of HÖ score against conscientiousness score in males.

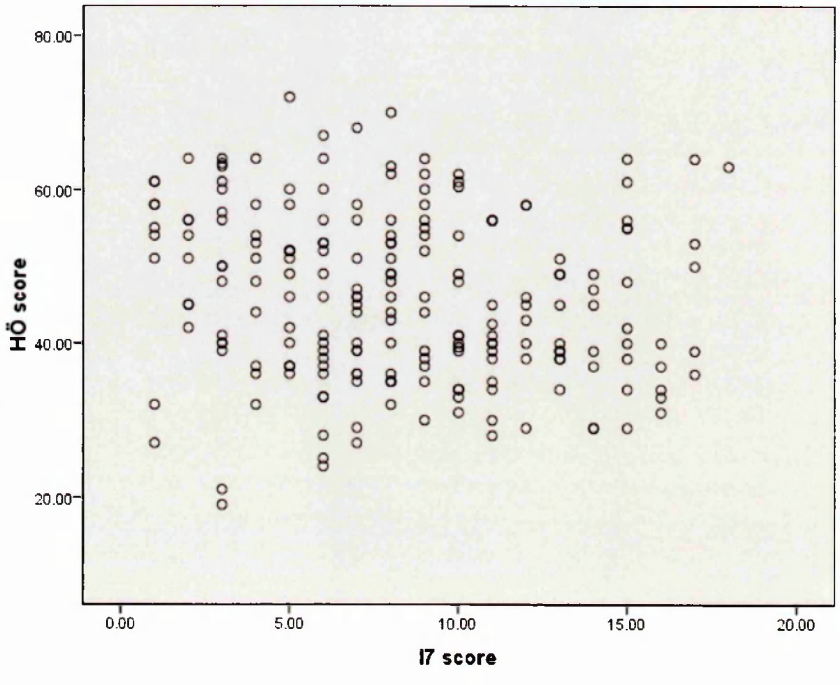


Figure 3-8 Bivariate scatterplot of HÖ score against I_7 score in males

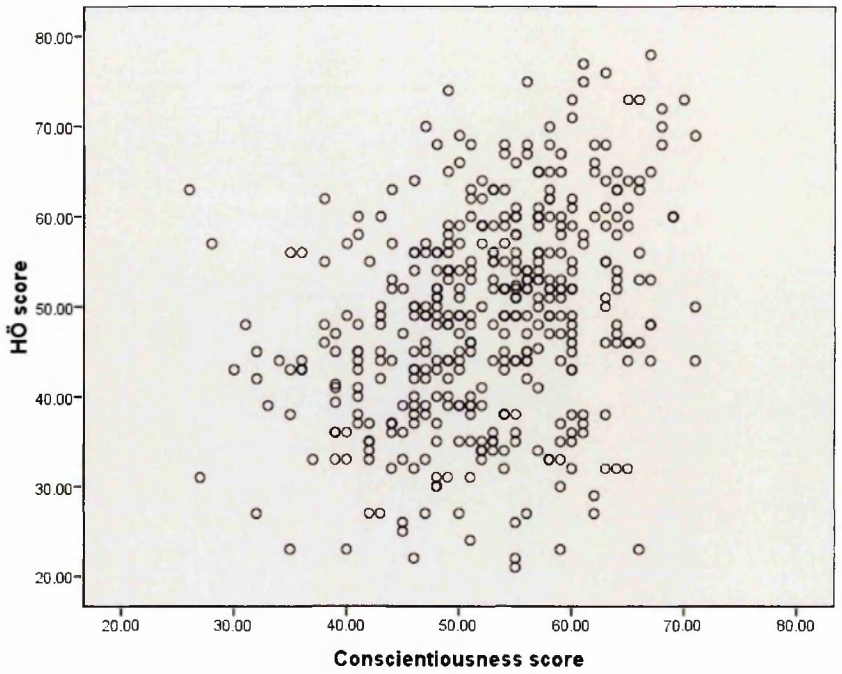


Figure 3-9 Bivariate scatterplot of HÖ score against conscientiousness score in females.

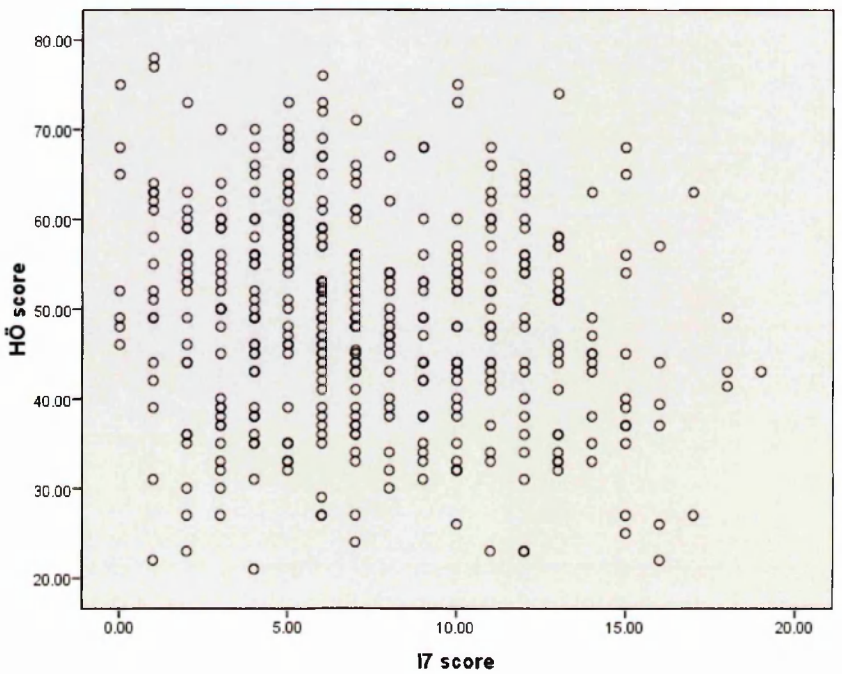


Figure 3-10 Bivariate scatterplot of HÖ score against I_7 score in females

3.3.3.2 Predictors of diurnal preference in males and females

As in section 3.3.2.3.1, a hierarchical multiple regression was chosen to determine which variables best predicted diurnal preference. Regression analysis was performed for each sex and as in the previous regression model described, each of the assumptions of the regression analysis was checked. The average VIF was calculated as 1.38 in the female regression model and 1.45 in the male model and the largest VIF value was less than 10. Additionally, all tolerance values were above 0.2 indicating there was no issue with multicolinearity in this model. Homoscedasticity, linearity and normality were also checked for males (Figs. 3-11 and 3-12) and females (Figs 3-13 and 3-14) and the results indicated that the assumptions were met. The Durbin-Watson test gave a value of 2.12 for the female model and 2.00 for the male model, suggesting that the residuals were uncorrelated.

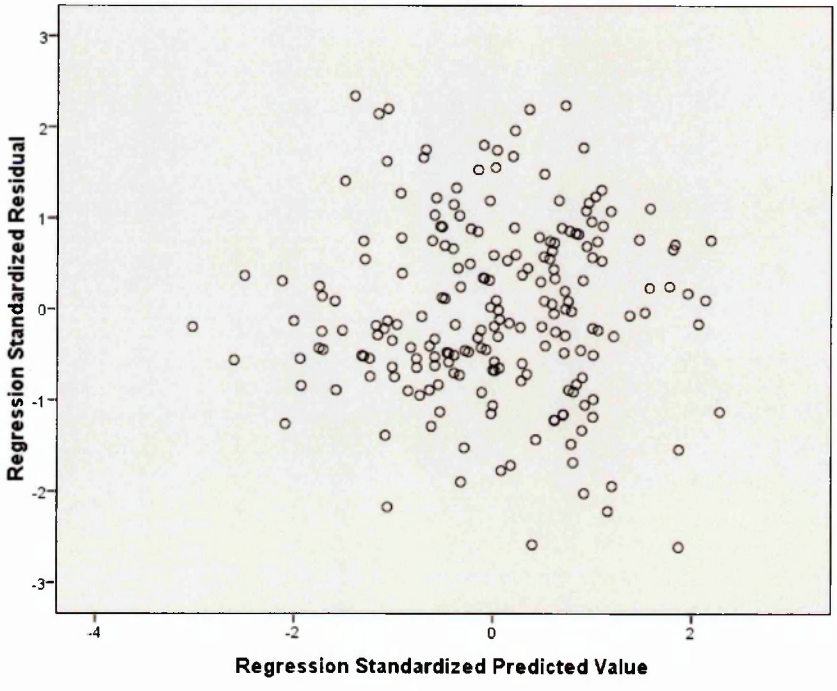


Figure 3-11 Scatterplot of standardised residual values against the standardised predicted values in the male regression model.

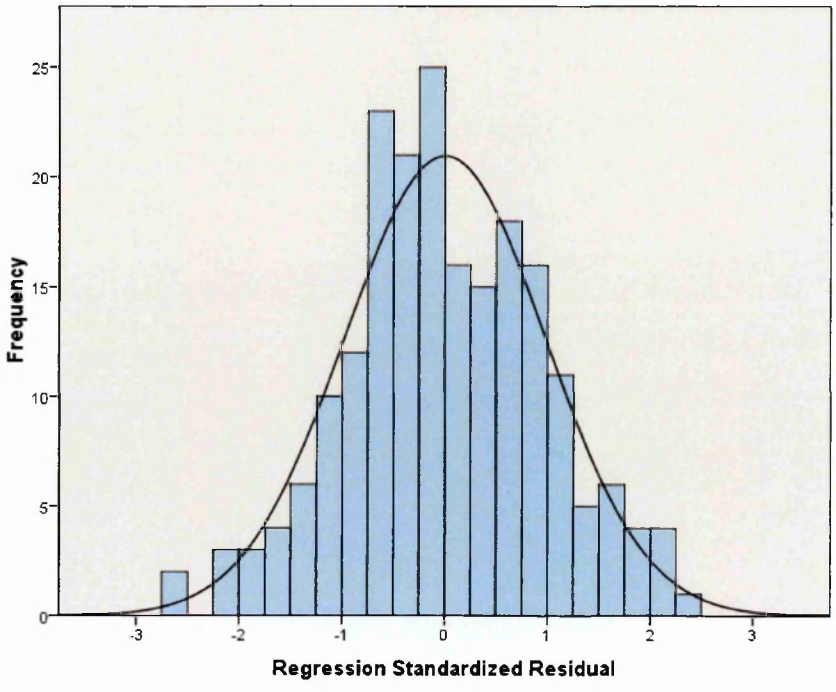


Figure 3-12 Histogram of residuals showing they follow a normal distribution in the male regression model.

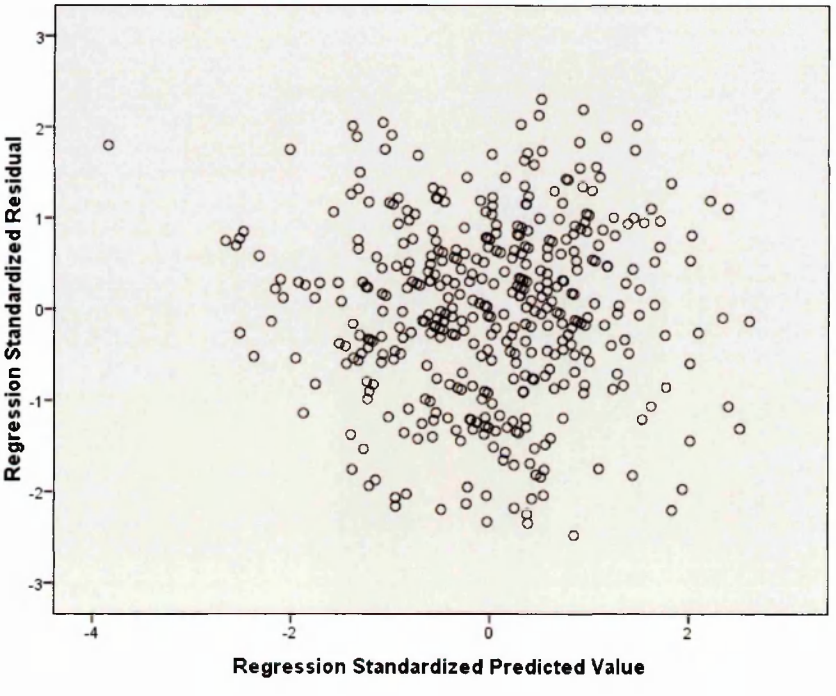


Figure 3-13 Scatterplot of standardised residual values against the standardised predicted values in the female regression model.

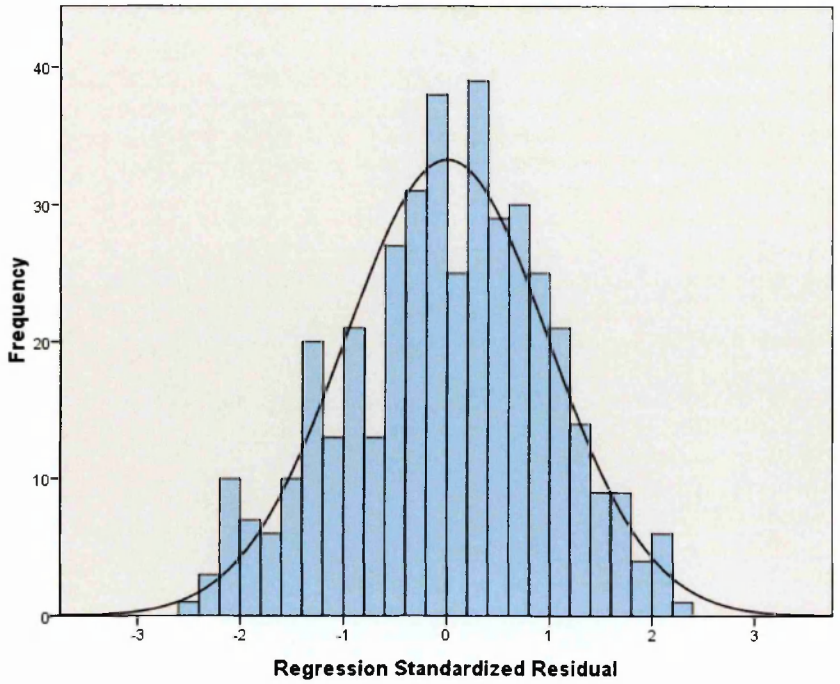


Figure 3-14 Histogram of residuals showing they follow a normal distribution in the female regression model.

In both models, shift work, depression, sleep disturbance and age were entered into the first block, impulsivity into the second block and neuroticism, extraversion, openness, agreeableness and conscientiousness into the final block. The results showed that in males, sleep disturbance was the largest predictor of diurnal preference (β = -0.24), followed by conscientiousness (β = 0.20) (Table 3-8). In females, conscientiousness was the largest predictor of diurnal preference (β = 0.26), followed by neuroticism (β = 0.19), age (β = 0.18), openness (β = -0.16), sleep disturbance (β = -0.14) and agreeableness (β = 0.13) (Table 3-9).

		B	*SE B*	*β*
Step 1				
	Shift work	-1.91	3.03	-0.04
	Depression	-0.02	0.23	-0.01
	Sleep disturbance	-1.06	0.29	-0.26***
	Age	1.30	0.79	0.11
Step 2				
	Shift work	-1.76	3.02	-0.04
	Depression	0.11	0.23	0.00
	Sleep disturbance	-0.99	0.29	-0.25***
	Age	1.24	0.79	0.11
	Impulsivity	-0.30	0.18	-0.12
Step 3				
	Shift work	-1.77	3.00	-0.04
	Depression	0.22	0.26	0.07
	Sleep disturbance	-0.94	0.30	-0.24**
	Age	1.40	0.81	0.12
	Impulsivity	-0.17	0.21	-0.07
	Neuroticism	0.14	0.12	0.12
	Extraversion	0.19	0.14	0.13
	Openness	0.02	0.12	0.01
	Agreeableness	0.05	0.11	0.03
	Conscientiousness	0.21	0.09	0.20*

($R = 0.39$, $R^2 = 0.15$, Adj $R^2 = 0.11$)

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Table 3-8 Hierarchical multiple regression analysis of personality traits as predictors of diurnal preference in males

		B	*SE B*	*β*
Step 1				
	Shift work	1.00	1.99	0.02
	Depression	-0.19	0.18	-0.05
	Sleep disturbance	-0.69	0.22	-0.16**
	Age	2.16	0.56	0.19***
Step 2				
	Shift work	1.37	1.98	0.03
	Depression	-0.14	0.18	-0.04
	Sleep disturbance	-0.58	0.22	-0.14**
	Age	1.98	0.56	0.17***
	Impulsivity	-0.38	0.14	-0.13**
Step 3				
	Shift work	1.14	1.87	0.03
	Depression	-0.25	0.21	-0.07
	Sleep disturbance	-0.59	0.21	-0.14**
	Age	2.02	0.55	0.18***
	Impulsivity	-0.07	0.15	-0.03
	Neuroticism	0.24	0.07	0.19**
	Extraversion	-0.03	0.09	-0.02
	Openness	-0.25	0.07	-0.16***
	Agreeableness	0.23	0.08	0.13**
	Conscientiousness	0.36	0.07	0.26***

($R = 0.44$, $R^2 = 0.20$, Adj $R^2 = 0.18$)

** $p <. 0.01$
*** $p < 0.001$

Table 3-9 Hierarchical multiple regression analysis of personality traits as predictors of diurnal preference in females.

3.4 Discussion

3.4.1 Associations between diurnal preference, demographic and personality variables

The results from the study agree with both hypotheses outlined in section 3.2. Firstly, the results were consistent with previous research that showed conscientiousness was associated with diurnal preference (Jackson and Gerard, 1996. Gray and Watson, 2002, Cavallera and Giampietro, 2007, DeYoung et al., 2007, Randler, 2008, Tonetti et al., 2009). Secondly, the relationship between eveningness and impulsivity, highlighted in a previous study (Caci et al., 2005), was confirmed, even after controlling for confounding factors. This is all the more interesting due to the fact that Caci and colleagues conducted their study within a French population and using a French version of the I_7 which further confirms the cross cultural validity of this measure.

When examining gender differences in associations between diurnal preference and the other variables; Sleep disturbance, conscientiousness and impulsivity were all significantly correlated with HÖ score in both sexes. Furthermore, in females, associations between HÖ score and agreeableness and openness were evident. Hierarchical multiple regressions indicated that sleep disturbance was the biggest predictor of HÖ score in men and conscientiousness in women. As the relationship between sleep disturbance and eveningness is known (Chung et al., 2009) and given that PSQI scores were significantly higher in males it was not unexpected for it to be a large predictor. The emergence of agreeableness and openness as correlates of HÖ score in females but not in males, on the other hand, is not consistent with other studies. Randler (2008) only found gender differences between diurnal

preference and the neuroticism dimension, where they were related only in females. Moreover, Tonetti et al (2009) found no differences between genders.

In the combined sample, conscientiousness, agreeableness and openness were both correlated with HÖ score, and were also found to be its predictors in addition to neuroticism. This partially supports previous research, all of which have identified conscientiousness as being associated with diurnal preference (Jackson and Gerard, 1996. Gray and Watson, 2002, Cavallera and Giampietro, 2007, DeYoung et al., 2007, Randler, 2008, Tonetti et al., 2009). However, despite general agreement on conscientiousness, research on the other dimensions of personality has not been as consistent. Some studies found associations between morningness and agreeableness (Deyoung et al., 2007; Randler, 2008), although in the latter, the results were not significant for the age range used in our current investigation. A relationship between eveningness and neuroticism has also been reported in several studies (Deyoung et al., 2007; Randler, 2008; Tonetti et al., 2009). Other research found an association with extraversion (Jackson and Gerard, 1996) and one study established an association between openness and eveningness (Cavellara & Giampietro, 2007).

The differences seen between genders, as well as those is the combined sample, are likely to be partly due to the different measures of personality used in each study. As discussed previously, measures of the five factor model have been developed according to the author's own interpretation of what this personality theory comprises. This means that not all the five factor measures correlate well with each other or measure exactly the same constructs (John and Srivasta, 1999). For example, Deyoung et al (2007) and Randler et al (2008) used different versions of the Big five inventory (see section 1.7.3) which does not include some items which appear under the openness dimension of the NEO-FFI (John and Srivastava, 1999) which may explain why openness emerged in this study and not in others. The one

dimension that has been found to consistently correlate with diurnal preference across the studies is conscientiousness and it may be that this dimension is the best conceptualised between measures.

Different measures of diurnal preference were also used between studies with only DeYoung and colleagues (2007) and Tonetti and coworkers (2009) using an intrinsic measure of diurnal preference that has been correlated with biological markers. It is unlikely though, that this is the cause of the disparity between results due to the high correlations between diurnal preference questionnaires (Randler et al, 2008).

Additionally, Deyoung et al only provided correlational statistics and without controlling for any confounding factors. The present study used regression analysis in addition to correlational statistics and thus provided evidence of causality of the relationships. Furthermore, none of the previous studies used both the HÖ questionnaire and the NEO-FFI or controlled for all the factors taken into account in this study, which may also explain the emergence of different personality variables.

The anomaly of neuroticism being a predictor in the multiple regression (see section 3.3.2.3), may be due to the inclusion of impulsivity and depression which are facets of neuroticism (Costa & McCrae, 1992), as well as sleep disturbance which it is known to correlate with. This meant that the variance shared between these factors led to the emergence of neuroticism in the final step of the model. Indeed, neuroticism was not correlated with HÖ score in either of the correlational analyses and only emerged in the multiple regression after controlling for a number of other variables. Previous research also indicates that if any relationship is evident between neuroticism and morningness it is likely to be a negative one, several studies having found links between eveningness and depression (high neuroticism) (Drennan et al., 1991,

Chelminski et al., 1999, Hidalgo et al., 2009) and other studies having directly linked neuroticism to eveningness (Deyoung et al, 2007; Randler, 2008; Tonetti et al, 2009).

Openness to experience emerged as a dimension associated with diurnal preference in only one other study (Cavellara & Giampietro, 2007). This may be due to cultural differences or because the current study was the only one to use the NEO-FFI. When exploring the characteristics of openness, there is evidence to support why this negative association with morningness may be plausible. Low openness corresponds with a person having a conventional and traditional outlook and behaviour as well as having narrow interests and being practical and down to earth. Conversely, high openness is characterised by an active imagination, aesthetic sensitivity, attentiveness to inner feelings, preference for variety and intellectual curiosity (Costa and McCrae, 1992). Openness is also the only big five trait positively associated with IQ (McCrae, 1993). By comparison, eveningness has been shown to correlate with creative thinking (Cavellara & Giampietro, 2007), higher verbal IQ in women (Killgore and Killgore, 2007) and higher intelligence score (Roberts and Kyllonen, 1999). Matthews et al (1988) also found that eveningness was associated with radical political opinions. Furthermore, evening types tend to act out in an independent and non conformist manner, resisting following traditional standards (Díaz-Morales, 2007). This strongly suggests a negative association between openness and morningness.

When investigating the multiple regression further, impulsivity was not a significant predictor of HÖ score in step three, which contradicts previous findings (Caci et al., 2005). Nevertheless, there was a correlation between impulsivity and morningness, as well as its emergence as a predictor of HÖ score in step two of the model. Impulsivity was no longer significant after the addition of the other personality variables in step three. This indicates that the variance accounted for prior to the variables being added is actually due to correlations

between impulsivity and one or more of the personality dimensions. In fact, it has been proposed that impulsivity is comprised of neuroticism, conscientiousness and extraversion (Whiteside and Lynam, 2001), which these results appear to give some support to, as the largest correlation was found to be with conscientiousness.

3.4.2 Gender differences

The results showed that women scored higher on neuroticism, agreeableness and conscientiousness dimensions of the five factor model, which is consistent with previous research (Feingold, 1994). A study of the Big five personality traits across 55 cultures also reported extraversion as being higher in women (Schmitt et al., 2008) but this was not seen in this study. The investigation of cultural differences with regard to personality and sex have produced some interesting results. Gender differences in personality traits are often larger in prosperous and democratic cultures where equal opportunities between the sexes are evident (Costa et al., 2001, Schmitt et al., 2008). This is thought to be due to the constraint of personality traits in men and women living in less developed countries whereas these personality traits are free to diverge in developed countries (Schmitt et al., 2008). This may also be interesting in relation to gender differences between personality and diurnal preference, but it would be necessary to expand research into other countries that are less developed than those where studies have been already been undertaken.

When assessing gender differences with regard to HÖ score, women obtained higher scores on the HÖ questionnaire which has also been reported previously (Moe et al., 1991). The results also showed that men were more impulsive than women as was reported by Caci et al. (2005). Additionally, scores on the PSQI were found to be higher in men, although means for both males and females were greater than the recommended cut off of 5, above which

suggests significant sleep disturbance. This is not in line with research that suggests women encounter more sleep problems (Maume et al., 2009) and have a higher incidence of insomnia (Morin et al., 1999). Studies that have utilised the PSQI have lent some support to this with higher scores found in middle aged and elderly women (Vitiello et al., 2004, Buysse et al., 2008), although others have found no gender difference (Buysse et al., 1991, Voderholzer et al., 2003). The results may be in part due to the fact that the reported sleep problems in women were attributed to the increase in work-family obligations (Maume et al., 2009). The present study mainly consisted of University students who may not have the same commitments as those reported in Maume and colleagues study. Furthermore, women monitor their health more closely than men (Verbrugge, 1989) and so may be more likely to seek medical advice and treatment. As participants who reported sleep problems and/or who took sleep medication were excluded from the present study, this may account for the lower incidence of sleep problems in women in this sample.

3.4.3 Age differences between the genders

No relationship was seen between sleep disturbance and age as would have been expected, where sleep disturbance is reportedly worse is older subjects (Buysse et al., 1991, Vitiello, 1997). This is most likely due to the age range used in the study which may have been too narrow to detect any relationship. Indeed, studies that have utilised the PSQI to investigate age related changes in sleep disturbance have compared elderly men and women with young people.

Age in both men and women was associated with depression and extraversion and this was also the case in the combined sample. Depression, as defined by the HADS, has been shown

in a number of studies to increase with age (Crawford et al., 2001, Stordal et al., 2001, Hinz et al., 2004).

The dimensions of the five factor model have also previously been shown to associate with age. Both neuroticism and extraversion reportedly decrease with age and agreeableness and conscientiousness increase (McCrae et al., 1999). In females, openness and conscientiousness were also associated with age, but rather than openness decreasing it increased. The same was seen in the combined sample and as previously suggested may be explained by the limited age range of the sample. Indeed, McCrae et al (2000) investigated this relationship in different populations and came to this conclusion through comparing five groups of participants, the lowest group of which included 14-17 year olds and the highest over 50 year olds. In the British sample, there was an increase in openness from the 14-17 year old age group to 22-29 year old age group after which there was a steady decrease. As the mean age of the combined sample was 25.2, this could explain the positive relationship. Lastly, HÖ score was associated with age in females only, indicating that morningness increases with age in these participants. This was also evident in the combined sample, and agrees with previous research (May et al., 1993, Caci et al., 2005).

3.4.4 Limitations of the study

Recruitment took place over a period of 8 months with levels dipping outside of term-time, and with the greatest response at the beginning of the academic year. If time had allowed, a longer period of recruitment would have resulted in a greater response, particularly as the 8 months included the summer break.

Recruitment was conducted through an email, local newspaper and poster campaign, and prospective participants were given the option of answering the questionnaires either online or by filling out hardcopies. Despite this, all the participants completed the questionnaires online with those requesting hard copies failing to return them. This meant that no comparison could be made between the two methods, although previous research suggests that they are not significantly different in the results they produce (Buchanan, 2003). Additionally, a large proportion of participants were students or staff at the University and so the sample was not representative of the entire population.

The study also used self report questionnaires which are susceptible to response bias. This is where a participant attempts to portray themselves in a favourable light and responds to the questions accordingly. In addition to the subjective nature of self report questionnaires, which imposes limitations on any study using them, the length of a scale can also have an effect on the data obtained. If a measure is too long, a participant may lose interest and either not read the questions properly, marking any answer, or fail to complete it. The online survey constructed in the present study incorporated six questionnaires, ranging from 14 to 60 questions. It was therefore lengthy and took approximately twenty minutes to complete, which may have been the cause of the large number of participants who failed to complete it (17.2%).

3.5 Conclusion

These results indicate that conscientiousness rather than impulsivity is the most important personality variable associated with diurnal preference, with openness and agreeableness associated to a lesser degree, and should, therefore, be considered when undertaking any

future research in this area. Furthermore, the relationship between openness and diurnal preference needs to be confirmed in future studies.

Chapter 4: Analysis of genetic data

4.1 Hypotheses

4.1.1 Hypothesis 1

In Chapter 3 conscientiousness was identified as the largest predictor of diurnal preference. As previous research has identified links between diurnal preference and several clock gene polymorphisms it is possible that by selecting for extremes of conscientiousness score, this dimension of the five factor model may directly associate with these clock gene polymorphisms. The first hypothesis therefore is:

- The allele frequency of clock gene polymorphisms previously known to associate with diurnal preference will differ between high and low conscientiousness groups.

4.1.2 Hypothesis 2

As the personality variables openness, agreeableness and impulsivity were also associated with diurnal preference in Chapter 3 but to a lesser extent than conscientiousness it s possible that they may also associate with the clock gene polymorphisms described. The second hypothesis, therefore, is:

- Openness, agreeableness and impulsivity will associate with clock gene polymorphisms previously linked to diurnal preference but to a lesser extent than with conscientiousness.

4.2 Methods

For full details of methods see Chapter 2. Methodologies specific to this study are outlined below.

4.2.1 Study design

From the 671 participants who took part in the initial study (Chapter 3), 20% extremes (n = 201) of conscientiousness score were selected into high and low groups. An intermediate group of the central 20% was also selected (see section 2.2.3). Participants who fell into these groups were contacted and asked to donate a buccal swab for DNA analysis. Those who responded were genotyped for *PER1* T2434C, *PER2* C111G, *PER2* 10870, *CLOCK* C3111T, *CLOCK* rs11932595 and *PER3* VNTR polymorphisms (see section 2.3).

4.2.2 Statistical analysis

Statistical analysis was performed using SPSS version 11. Correlations used Pearson's *r* and differences between gender used an independent *t* test. ANOVAs were used to check for differences between conscientiousness groups and MANOVAs to investigate differences between genotypes on the dependent variables. Pearson χ^2 tests were performed to assess differences in genotype between conscientiousness groups and Fisher's exact test to examine allele frequencies. Finally, multinomial logistic regressions were performed in order to find which variables predicted specific genotypes. Explanations for each test can be found in section 2.4.

4.3 Results

Of the 20% extremes selected and intermediate group, 174 participants responded (117 females, 67.2%; mean age of 25.5 ± 5.4 years). The high, intermediate and low conscientious groups contained 65, 57 and 52 participants and had mean conscientiousness scores of 35.8 ± 5.4, 51.4 ± 1.1 and 64.0 ± 3.4, respectively (Table 4-1). Genotyping of polymorphisms *PER2* C111G, *PER2* 10870, *CLOCK* C3111T and *CLOCK* rs11932595 was successful for all participants. This was not the case for *PER1* T2434C where one genotype was unable to be determined and also for *PER3* VNTR where 9 participants could not be successfully genotyped. In both cases, no amplification products could be visualised after PCR for those participants.

Histograms of the scores for each questionnaire were plotted in order to check whether they still followed a normal distribution in the smaller sample. As the depression scale of the HADS (Fig. 4-1), the I_7 (Fig 4-2) and the extraversion (Fig. 4-3) and conscientiousness (Fig. 4-4) scales of the FFI had kurtotic distributions, the scales were converted to *z* scores or transformations were performed. As both the depression scale and the I_7 were positively skewed as well as breaking the assumption of homogeneity of variance (where variances in each group are equal), they were transformed using a log-10 transformation. This was calculated in SPSS by taking the log of each score plus one. Extraversion and conscientiousness also broke the assumption of homogeneity of variance but no transformation could rectify this, therefore they were converted to *z* scores so they at least followed a normal distribution.

	Low	Intermediate	High	Low vs High
Age	25.4 ± 6.0	24.8 ± 4.9	26.3 ± 5.3	$t(115) = -0.82$
Depression	5.4 ± 4.2	3.0 ± 2.7	2.6 ± 2.9	$t(115) = 4.82$*
Sleep disturbance	7.2 ± 2.9	5.3 ± 2.6	4.7 ± 2.6	$t(115) = 4.94$*
Neuroticism	43.9 ± 10.2	40.8 ± 8.6	37.3 ± 8.3	$t(115) = 3.88$*
Extraversion	44.8 ± 8.4	50.1 ± 6.0	52.2 ± 7.4	$t(115) = -5.05$*
Openness	52.7 ± 6.9	49.0 ± 6.5	48.1 ± 7.4	$t(115) = 3.44$*
Agreeableness	49.7 ± 6.5	52.0 ± 6.0	54.3 ± 6.5	$t(115) = -3.78$*
Conscientiousness	35.8 ± 5.4	51.4 ± 1.1	64.0 ± 3.4	$t(115) = -34.29$*
HÖ score	42.9 ± 11.0	47.3 ± 11.2	55.1 ± 12.4	$t(115) = -5.56$*
Impulsivity	9.7 ± 4.7	7.1 ± 3.9	5.0 ± 3.1	$t(115) = 5.64$*

Table 4-1 Descriptive statistics for conscientiousness groups. *t*, independent *t* test, * $p < 0.001$.

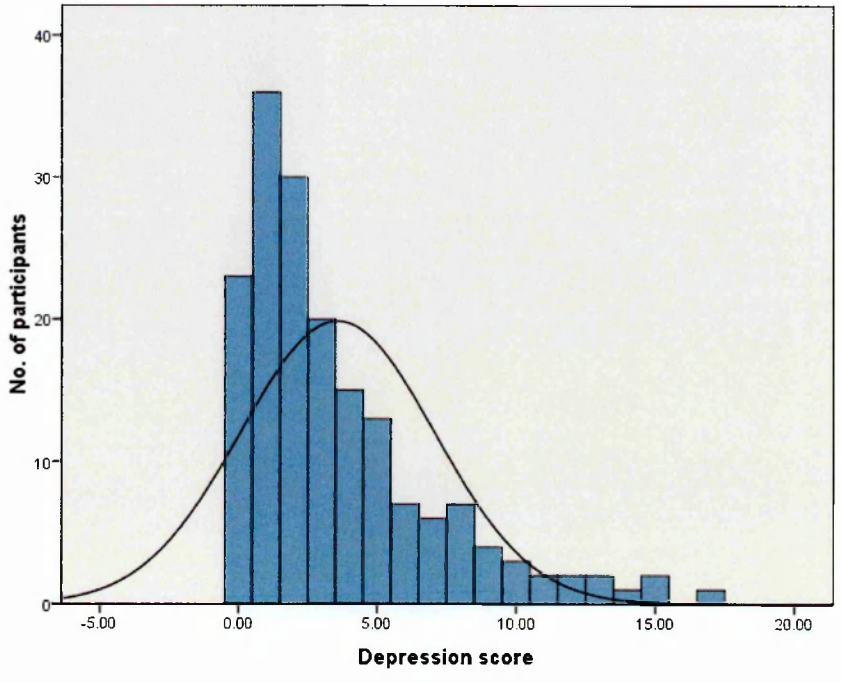


Figure 4-1 Histogram of the number of participants with a given depression score.

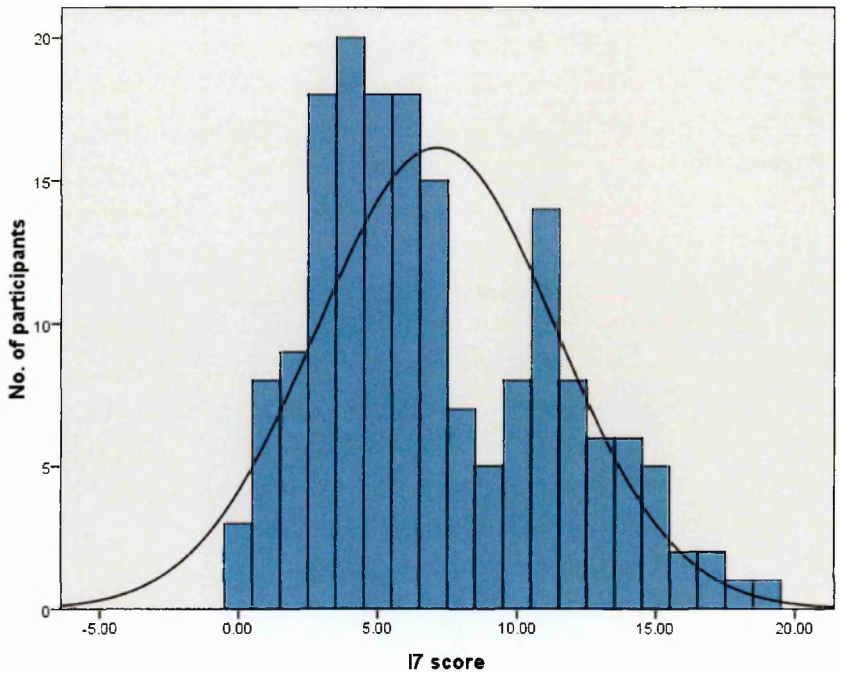


Figure 4-2 Histogram of the number of participants with a given I_7 score.

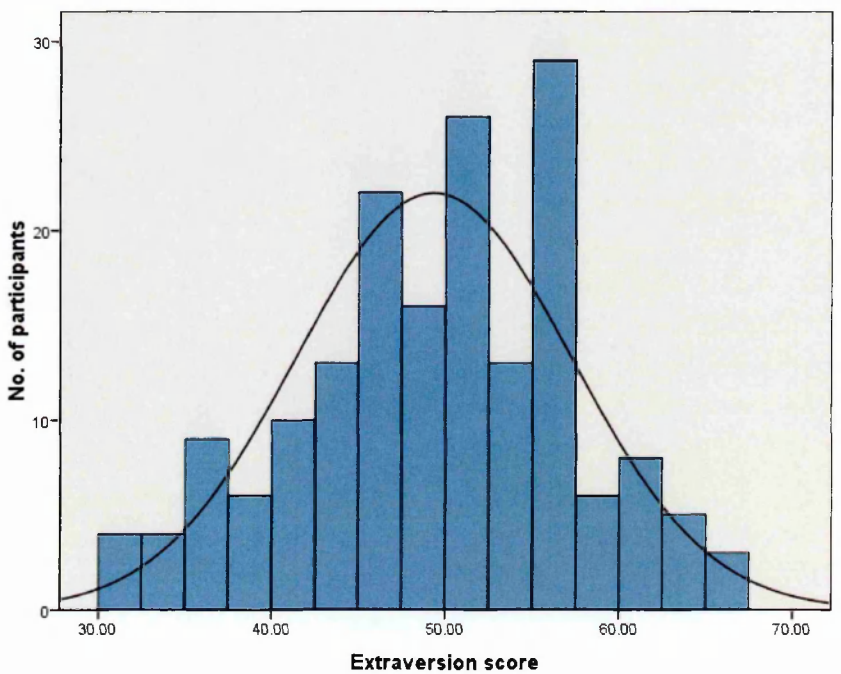


Figure 4-3 Histogram of the number of participants with a given extraversion score.

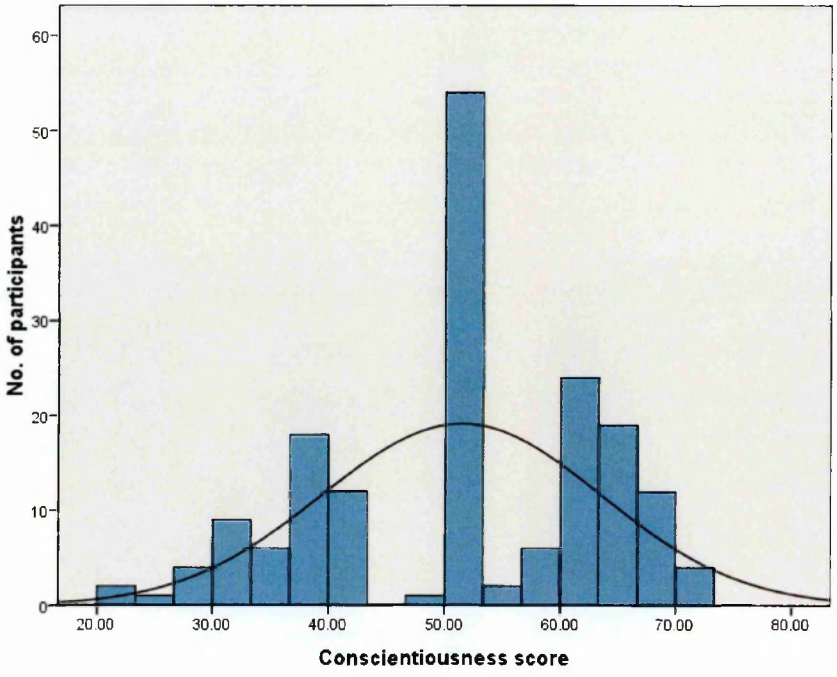


Figure 4-4 Histogram of the number of participants with a given conscientiousness score.

4.3.1 Associations within the smaller sample

Independent *t* tests were carried out for age, depression, sleep disturbance, impulsivity, HÖ score and the dimensions of the five factor model, to examine gender differences, as previously performed in Chapter 3. This was in order to determine whether the smaller sample retained the same relationships (Table 4-2) and revealed that only conscientiousness ($t[172]= - 3.13$, $p < .01$) remained significantly different between genders with females obtaining higher conscientiousness scores (males: 47.5 ± 12.9, females: 53.4 ± 11.2).

Correlational analyses found that age was only associated with openness ($r = 0.19, p < 0.05$) in the smaller sample, whereas HÖ was now also associated with extraversion ($r = 0.17, p < 0.05$) in addition to impulsivity ($r = -0.20, p < 0.01$), sleep disturbance ($r = -0.26, p < 0.01$), openness ($r = -0.18, p < 0.05$), agreeableness ($r = 0.26, p < 0.01$) conscientiousness ($r = 0.38,$

$p < 0.01$) and depression ($r = -0.27, p < 0.01$), but still not with neuroticism ($r = -0.12, p > 0.05$) or age ($r = 0.09, p > 0.05$) (Table 4-3).

Variable	Males	Females	Combined mean	Male vs female
Age	25.1 ± 5.4	25.7 ±5.4	25.5 ± 5.4	$t(172) = -0.75$
Depression	3.9 ± 4.0	3.4 ± 3.2	3.6 ± 3.5	$t(172) = 0.81$
Sleep disturbance	6.2 ± 2.8	5.4 ± 2.8	5.7 ± 2.8	$t(172) = 1.69$
Neuroticism	39.6 ± 9.9	40.8 ± 9.1	40.4 ± 9.3	$t(172) = -0.83$
Extraversion	49.9 ± 7.6	49.0 ± 8.0	49.3 ± 7.9	$t(172) = 0.65$
Openness	50.2 ± 7.3	49.5 ± 7.2	49.7 ± 7.2	$t(172) = 0.65$
Agreeableness	51.1 ± 6.3	52.7 ± 6.7	52.2 ± 6.6	$t(172) = -1.52$
Conscientiousness	47.5 ± 12.9	53.4 ± 11.2	51.5 ± 12.1	$t(172) = -3.13^{**}$
HÖ score	46.6 ± 11.5	50.0 ± 13.0	48.9 ± 12.6	$t(172) = -1.67$
Impulsivity	8.1 ± 4.5	6.6 ± 4.1	7.1 ± 4.3	$t(172) = 1.91$

Table 4-2: Descriptive statistics, t, independent t test, $^{*}p < 0.05$, $^{**}p < 0.01$.

Variables	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1. Age	—									
2. Depression	0.07	—								
3. HÖ score	0.09	-0.27**	—							
4. Impulsivity	-0.04	0.18*	-0.20**	—						
5. GPSQI	0.11	0.26**	-0.26**	0.25**	—					
6. Neuroticism	-0.10	0.49**	-0.12	0.11	0.30**	—				
7. Extraversion	-0.14	-0.47**	0.17*	0.10	-0.22**	-0.48**	—			
8. Openness	0.19*	0.06	0.18*	0.35**	0.20**	-0.02	0.15*	—		
9.Agreeableness	0.06	-0.32**	0.26**	-0.26**	-0.09	-0.28**	0.25**	-0.13	—	
10. Conscientiousness	0.08	-0.36**	0.38**	-0.42**	-0.34**	-0.28**	0.38**	-0.25**	0.31**	—

* $r < 0.05$ level, ** $r < 0.01$ level (2-tailed).

Table 4-3 Pearson correlations between demographic factors, control variables and personality variables.

4.3.2 Group differences

In order to establish whether there were differences between high and low conscientiousness group membership for each variable, independent *t* tests were performed (Table 4-1). The results indicated that all variables except age were significantly different between high and low conscientiousness groups with high scores on depression (t[115] = 4.82, low: 5.4 ± 4.2, high: 2.6 ± 2.9), sleep disturbance (t[115] = 4.94, low: 7.2 ± 2.9, high: 4.7 ± 2.6), neuroticism (t[115] = 3.88, low: 43.9 ± 10.2, high: 37.3 ± 8.3) , openness (t[115] = 3.44, low: 52.7 ± 6.9, high: 48.1 ± 7.4) and impulsivity (t[115] ± 5.64, low: 9.7 ± 4.7, high: 5.0 ± 3.1) indicative of low conscientiousness group membership and high scores on extraversion (t[115] = -5.05, low: 44.8 ± 8.4, high: 52.2 ± 7.4), agreeableness (t[115] = -3.78, low: 49.7 ± 6.5, high: 54.3 ± 6.5), conscientiousness (t[115] = -34.29, low: 35.8 ± 5.4, high: 64.0 ± 3.4) and HÖ score (t[115] = -5.56, low: 42.9 ± 11, high: 55.1 ± 12.4) indicative of high conscientiousness group membership.

As *t* tests only allow the comparison of two means, an ANOVA was conducted to confirm whether there was indeed a significant difference in conscientiousness scores between all three conscientiousness groups. As Levene's test indicated that variances were not equal between groups, Welch's *F*-ratio was used and showed a significant difference between the three groups (F [2, 85] = 636.96, $p < 0.001$). Planned contrasts revealed that both high and low conscientious groups were significantly different from the intermediate group (t[100] = -3.29, $p < 0.001$) and from each other (t[82] = -32.69, $p < 0.001$).

In order to see whether the rest of the variables differed between all three groups a MANOVA was conducted with age, sleep disturbance, neuroticism, openness, agreeableness, depression,

extraversion and impulsivity and HÖ score as dependent variables, and conscientiousness groups as the grouping factor. Levene's test indicated that extraversion ($F = 3.70$, $p < 0.05$) did not meet the assumption of homogeneity of variance. Box's test showed that covariances between groups were equal ($M = 121.5$, $p = 0.06$). The MANOVA was significant, suggesting a difference between groups on one or more variables (Pillai's trace:$F = 6.07$, $p < 0.001$).

A MANOVA assesses differences on dependent variables between groups by forming a linear combination of the dependent variables and groups are compared on that variable. This combination is created for each participant by multiplying the participants score on each variable by the weight (B), with the weights computed so that they maximise the differences between groups. The weights for this MANOVA can be seen in Table 4-4, and they showed that all variables except age contributed significantly towards discriminating the groups. Sleep disturbance (B = 2.49, $p < 0.001$), depression (B = 0.28, $p < 0.001$) and extraversion (B = -0.94, $p < 0.001$) all contributed to distinguishing the low group from the other two groups, with participants scoring higher on sleep disturbance and depression and lower on extraversion than those in other groups. HÖ score (B = -12.22, $p < 0.001$; B = -7.87, $p < 0.001$), impulsivity (B = 0.26, $p < 0.001$; B = 0.13, $p < 0.01$), neuroticism (B = 6.62, $p < 0.001$; B = 3.46, $p < 0.05$), and agreeableness (B = -4.58, $p < 0.001$; B = -2.34, $p < 0.05$) all contributed significantly to the discrimination of low and intermediate groups from each other as well as the high group. These results suggest that participants in the low and intermediate groups scored higher on impulsivity and neuroticism and lower on HÖ score and agreeableness than those in the high conscientiousness group.

In order to determine whether these relationships were due to associations between individual variables and conscientiousness rather than just a combination of variables, univariate ANOVA's were performed for each variable. This resulted in significant differences between

groups for all variables except age, even after a Bonferroni correction was applied ($p = 0.008$) (Table 4-5). As an ANOVA only indicates that a significant difference is present and not where the differences lie, it was necessary to perform *post hoc* analysis in order to distinguish which variables differed between which groups. These tests comprise pairwise comparisons that compare all the group combinations whilst controlling the Type I error rate. Hochberg's GT2 was chosen as the group sizes were unequal. The Games-Howell test was also used because extraversion did not meet the assumption of homogeneity of variance in the MANOVA and the test does not assume equal variances. Significant differences were revealed between high and low conscientious groups for sleep disturbance (both: $p < 0.001$) neuroticism (both: $p < 0.001$), extraversion (both: $p < 0.001$), openness ($p < 0.001, p < 0.01$), agreeableness (both: $p < 0.001$), depression (both: $p < 0.001$), impulsivity (both: $p < 0.001$) and HÖ score (both: $p < 0.001$). Differences were also seen between low and intermediate groups for sleep disturbance ($p < 0.001, p < 0.01$), extraversion (both: $p < 0.001$), openness (both: $p < 0.01$), depression (both: $p < 0.01$) and impulsivity (both: $p < 0.05$) and between intermediate and high groups for impulsivity ($p < 0.01, p < 0.05$) and HÖ score (both: $p < 0.001$) (Table 4-6). These results confirm the correlational statistics in which all variables except for age were correlated with conscientiousness.

Dependent variable		B
Sleep disturbance		
	Low	2.49***
	Intermediate	0.63
	High	0[a]
Depression		
	Low	0.28***
	Intermediate	0.08
	High	0[a]
HÖ Score		
	Low	-12.22***
	Intermediate	-7.87***
	High	0[a]
Impulsivity		
	Low	0.26***
	Intermediate	0.13**
	High	0[a]
Neuroticism		
	Low	6.62***
	Intermediate	3.46*
	High	0[a]
Extraversion		
	Low	-0.94***
	Intermediate	-0.26
	High	0[a]
Openness		
	Low	4.60***
	Intermediate	0.89
	High	0[a]
Agreeableness		
	Low	-4.58***
	Intermediate	-2.34*
	High	0[a]
Age		-0.16
	Low	-0.27
	Intermediate	0[a]
	High	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

[a] Parameter set to 0 as it is redundant.

Table 4-4 Weights of variables (B) which maximally distinguish conscientiousness groups.

Dependent variable	*F*
Age	1.15
Sleep disturbance	13.24**
Neuroticism	7.89**
Extraversion	15.33**
Openness	6.83**
Agreeableness	7.55**
Depression	12.31**
Impulsivity	16.07**
HÖ score	16.88**

** $p < 0.001$, $df = 2$.

Table 4-5 Univariate test statistics for between subject effects. Significant results indicate a difference in the dependent variable between conscientiousness groups.

Dependent variable	Groups (I)	(J)	Mean difference between groups (I - J)	Hochberg GT2 significance	Games-Howell significance
Age	Low	Intermediate	0.11	0.91	0.83
	Intermediate	High	-0.27	0.35	0.25
	High	Low	0.16	0.78	0.70
Sleep disturbance	Low	Intermediate	1.85	0.00**	0.00**
	Intermediate	High	0.63	0.47	0.37
	High	Low	-2.48	0.00**	0.00**
Neuroticism	Low	Intermediate	3.15	0.19	0.20
	Intermediate	High	3.46	0.10	0.07
	High	Low	-6.62	0.00**	0.00**
Extraversion	Low	Intermediate	-0.68	0.00**	0.00**
	Intermediate	High	-0.26	0.32	0.21
	High	Low	0.94	0.00**	0.00**
Openness	Low	Intermediate	3.71	0.02*	0.01**
	Intermediate	High	0.89	0.86	0.76
	High	Low	-4.60	0.00**	0.00**
Agreeableness	Low	Intermediate	-2.23	0.19	0.16
	Intermediate	High	-2.34	0.13	0.10
	High	Low	4.58	0.00**	0.00**
Depression	Low	Intermediate	0.20	0.00**	0.00**
	Intermediate	High	0.07	0.45	0.38
	High	Low	-0.28	0.00**	0.00**
Impulsivity	Low	Intermediate	0.13	0.02*	0.02*
	Intermediate	High	0.13	0.01**	0.01**
	High	Low	-0.26	0.00**	0.00**
HÖ score	Low	Intermediate	-4.35	0.15	0.11
	Intermediate	High	-7.87	0.00**	0.00**
	High	Low	12.22	0.00**	0.00**

*$p < 0.05$, ** $p < 0.01$

Table 4-6 Results of Hochberg and Games-Howell tests showing which groups differed for each dependent variable.

4.3.3 Group differences between genotypes

All polymorphisms were found to be in Hardy-Weinberg equilibrium by using an online calculator at http://www.oege.org/software/hardy-weinberg.shtml (Table 4-7). This principle states that allele and genotype frequencies in a population remain constant over time but not if certain factors are introduced such as inbreeding, mutation or selection. In the laboratory, these factors are not evident and therefore deviations from equilibrium may be indicative of genotyping error. Pearson's χ^2 tests were performed for *CLOCK* C3111T, *CLOCK* rs11932595 and PER3 VNTR (Table 4-8, a, b and c) and for *PER1* T2434C, *PER2* C111G and *PER2* 10870 polymorphisms (Table 4-9 a, b and c) in order to determine whether genotypes differed significantly between the three conscientiousness groups and between the high and low groups. For the *PER1* T2434C, *PER2* C111G and *PER2* 10870 polymorphisms, 33.3% of the expected cell values were less than 5 and for *PER2* C111G and *PER2* 10870 a number of the expected values were less than 1. This meant that the assumptions of the χ^2 test were violated in these instances and the results may not, therefore, be reliable. As an alternative, Fishers exact test was carried out using the allele frequencies of each of these polymorphisms (Table 4-10 a, b and c). Neither Pearson's χ^2 tests nor Fisher's exact tests showed any significant differences for any of the polymorphisms between groups.

Polymorphism	χ^2 statistic
PER1 T2434C	0.79
PER2 C111G	0.01
PER2 10870	0.00
CLOCK C3111T	1.60
CLOCK rs11932595	0.85
PER3 VNTR	2.86

Table 4-7 χ^2 statistic's for each polymorphism. A value less than 3.84 indicates no significant difference between observed and expected values for genotype counts and indicates that the polymorphism is in Hardy-Weinberg equilibrium.

(a)

	T/T	T/C	C/C
Low	28	18	6
Intermediate	30	21	6
High	29	28	8

(b)

	A/A	A/G	G/G
Low	24	18	10
Intermediate	27	22	8
High	22	35	8

(c)

	4/4	4/5	5/5
Low	22	20	6
Intermediate	19	26	10
High	29	21	12

Table 4-8: Contingency tables showing the number of participants in each group with a certain genotype of the following polymorphisms (a) *CLOCK* C3111T, all three groups: $\chi^2 = 1.29$ (4), $p = 0.86$, high vs low: $\chi^2 = 1.05$ (2) $p = 0.59$. (4), $p = 0.86$, (b) *CLOCK* rs11932595 all three groups: $\chi^2 = 1.29$ (4), $p = 0.24$, high vs low: $\chi^2 = 4.37$ (2), $p = 0.11$. (c) *PER3 VNTR*, $\chi^2 = 3.34$ (4), $p = 0.50$, high vs low : $\chi^2 = 1.22$ (2), $p = 0.54$.

(a)

	T/T	T/C	C/C
Low	30	19	2
Intermediate	37	18	2
High	47	18	0

(b)

	C/C	C/G	G/G
Low	47	4	1
Intermediate	47	10	0
High	56	9	0

(c)

	A/A	A/G	G/G
Low	35	15	2
Intermediate	47	10	0
High	50	14	1

Table 4-9: Contingency tables showing the number of participants in each group with a certain genotype of the following polymorphisms (a) *PER1* T2434C all three groups: $\chi^2 = 4.05$ (4), $p = 0.40$, high vs low: $\chi^2 = 4.15$ (2), $p = 0.13$. (b) *PER2* C111G all three groups: $\chi^2 = 4.57$ (4), $p = 0.33$, high vs low: $\chi^2 = 2.29$ (2), $p = 0.32$. (c) *PER2* 10870, $\chi^2 = 4.78$ (4), $p = 0.31$, high vs low: $\chi^2 = 1.59$(2), $p = 0.45$.

(a)

	T	C
Low	79	21
High	112	18

(b)

	C	G
Low	98	6
High	121	9

(c)

	A	G
Low	85	17
High	114	15

Table 4-10: Contingency tables showing the number of alleles per conscientiousness group for the following polymorphisms (a) *PER1* T2434C, Fishers exact test $p = 0.34$, (b) *PER2* C111G, Fishers exact test $p = 0.79$, (c) *PER2* 10870, Fishers exact test $p = 0.34$, all 2-sided.

Table 4-11 shows the allele frequencies for each polymorphism, these were calculated in order to assess the prevalence of each allele in the population and to compare them to previous studies. From this table, it is evident that the C allele of *PER1* T2434C, the G allele of *PER2* 10870 and the G allele *PER2* C111G in particular, are all of a very low frequency in comparison to the other polymorphisms. There is very little difference between allele frequencies of the two groups for any of the polymorphisms as expected when taking into account the previous insignificant results. In comparison to other studies the allele frequencies obtained are similar to those previously reported.

Bar charts were plotted to show the percentage of participants who had a particular genotype for each polymorphism within the three conscientiousness groups (Figure 4-5). Again, figures 4-5 a, b and c show the low percentage of participants who were homozygote for the rarest allele. Figures 4-5 d, e and f, show a more even distribution of genotypes for those particular polymorphisms.

	High		Intermediate		Low		Combined		Previous studies	
PER1 T2434C	T 0.86	C 0.14	T 0.81	C 0.19	T 0.79	C 0.24	T 0.82	C 0.18	T 0.83	C[1] 0.17
PER2 C111G	C 0.93	G 0.07	C 0.91	G 0.09	C 0.94	G 0.06	C 0.93	G 0.07	C 0.92	G[2] 0.08
PER2 10870	A 0.88	G 0.12	A 0.91	G 0.09	A 0.83	G 0.17	A 0.87	G 0.13	A 0.83	G[3] 0.17
CLOCK C3111T	T 0.66	C 0.34	T 0.71	C 0.29	T 0.71	C 0.29	T 0.69	C 0.31	T 0.73	C[4] 0.27
CLOCK rs11932595	A 0.61	G 0.39	A 0.67	G 0.33	A 0.63	G 0.37	A 0.64	G 0.36	A 0.64	G[5] 0.36
PER3 VNTR	4 0.65	5 0.35	4 0.58	5 0.42	4 0.67	5 0.33	4 0.63	5 0.37	4 0.68	5[6] 0.32

Table 4-11: Allele frequencies for each polymorphism in conscientiousness groups and the population average from previous studies. [1] Carpen et al (2006) [2] Carpen et al. (2005); [3] Englund et al (2009); [4] Katzenberg et al (1998); [5] Sookoian et al (2008); [6] Archer et al (2003).

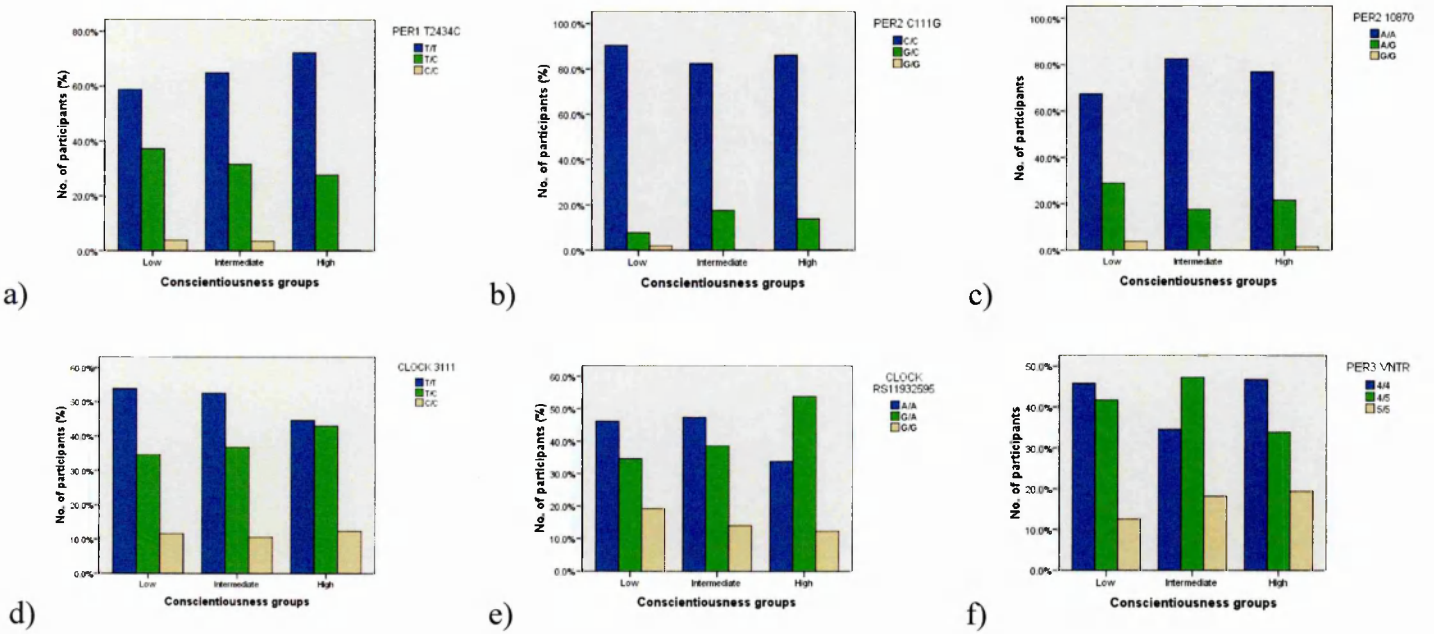


Figure 4-5: Bar charts of the percentage of participants with (a) *PER1* T2434 (b) *PER2* C111G (c) PER2 10890 (d) *CLOCK* C3111T (e) *CLOCK* rs11932595 (f) *PER3 VNTR*, genotypes within conscientiousness groups. Blue represents the homozygote with the most frequent allele, green, the heterozygote and brown, the homozygote with the rarest allele.

4.3.4 Relationships between genotype and personality and demographic variables

In order to investigate differences in personality, demographic and control variables between polymorphisms, separate MANOVAs were conducted for *PER1* T2434C, *PER2* C111G, *PER2* 10870, *CLOCK* rs11932595, *CLOCK* C3111T and *PER3* VNTR. Sleep disturbance, impulsivity, depression, HÖ score, neuroticism, extraversion, openness, agreeableness and conscientiousness were entered as dependent variables and each polymorphism as the grouping factor. A Bonferroni correction of p = 0.008 was applied to the results due to multiple testing, and a further correction of p = 0.006 was applied to the univariate ANOVA test results. The assumptions of the MANOVA were firstly checked for each analysis. Box's M was not significant for any MANOVA. Levene's test was not significant for any variable except for agreeableness ($F = 5.28$, $p < 0.01$) in the analysis using *CLOCK* rs11932595 as a grouping factor.

Following this, multinomial logistic regressions were conducted for each polymorphism in order to find which variables best predict genotype. A multinomial logistic regression was used as opposed to a multiple regression as the dependent variable can be categorical whereas in multiple regression it cannot. Furthermore, this regression was used in addition to the MANOVA as categorical variables can also be entered as predictor variables whereas only continuous variables can be entered into a MANOVA. This allowed the addition of night shift work and gender as extra variables. The heterozygote was used as the reference category and gender, sleep disturbance, shift work, depression, HÖ score, impulsivity, and each dimension of the five factor model as predictors. χ^2 tests were performed for each model to assess whether the addition of any of the variables would significantly affect the predictive power of the model. Significant results were obtained for *PER2* C111G and *CLOCK* rs11932595 before

a Bonferroni correction was applied, indicating that the predictive value of the model in these cases may be increased above chance (Table 4-12). Additionally, during the analysis of *PER1* T2434C, *PER2* C111G, *PER2* 10870 and *CLOCK* C3111T, SPSS produced a warning that suggested the model for each of these variables was unreliable.

	χ^2	Significance
PER1 T2434C	18.92	0.650
PER2 C111G	35.42	0.035*
PER2 10870	30.42	0.109
CLOCK C3111T	26.36	0.236
CLOCK rs11932595	38.58	0.016*
PER3 VNTR	17.40	0.741

Table 4-12: χ^2 values for each model.

4.3.4.1 *PER1* T2434C

The MANOVA conducted for *PER1* T2434C was not significant, indicating that there were no significant differences between genotypes on the dependent variables (Pillai's trace: $F = 0.55$, $p = 0.93$). As the multinomial logistic regression for this polymorphism was not significant, a binary logistic regression was performed between the T/T and C/C genotypes as this comparison was not made using the multinomial logistic regression. This too did not provide a significant result ($\chi^2 = 11.66, p = 0.39$).

4.3.4.2 *PER2* C111G

The MANOVA conducted on *PER2* C111G was significant before a correction for multiple testing (Pillai's trace: $F = 1.77$, $p < 0.05$) suggesting there may be differences between genotype on one or more dependent variables. Examination of the coefficients for the linear combinations distinguishing genotypes indicated that sleep disturbance contributed the most to differentiating the genotypes (Table 4-14). Specifically, sleep disturbance contributed significantly to discriminating the C/C (B = -7.44, $p < 0.05$) and C/G (B = -7.04, $p < 0.05$) genotypes from each other as well as the G/G genotype. Furthermore, participants with these genotypes scored lower on this variable than those with a G/G genotype.

Univariate ANOVAs showed a difference in sleep disturbance between genotype ($F = 3.67$, $p < 0.05$) (Table 4-13) but this became insignificant after a Bonferroni correction was applied. *Post hoc* analysis could not be performed due to only one participant having the G/G genotype.

Dependent variable	*PER2* C111G
Sleep disturbance	3.67*
Depression	0.72
HÖ Score	2.84
Impulsivity	0.58
Neuroticism	1.98
Extraversion	1.57
Openness	1.14
Agreeableness	0.87
Conscientiousness	1.00

Table 4-13 Univariate ANOVA *F* statistics for between subject effects. The values indicate differences between *PER2* C111G genotype on the dependent variables.

Dependent variable		B
Sleep disturbance		
	C/C	-7.44*
	C/G	-7.04*
	G/G	0[a]
Depression		
	C/C	-0.25
	C/G	-0.18
	G/G	0[a]
HÖ Score		
	C/C	9.79
	C/G	3.44
	G/G	0[a]
Impulsivity		
	C/C	0.00
	C/G	-0.06
	G/G	0[a]
Neuroticism		
	C/C	-17.85
	C/G	-16.61
	G/G	0[a]
Extraversion		
	C/C	1.41
	C/G	1.64
	G/G	0[a]
Openness		
	C/C	-1.59
	C/G	0.83
	G/G	0[a]
Agreeableness		
	C/C	-6.05
	C/G	-6.41
	G/G	0[a]
Conscientiousness		
	C/C	0.93
	C/G	1.16
	G/G	0[a]

* $p < 0.05$

[a] Parameter set to 0 as it is redundant.

Table 4-14 Weights of variables (B) which maximally distinguish genotypes in *PER2* C111G

In the multinomial logistic regression using *PER2* C111G as the outcome variable, only the C/C compared to the C/G genotypes could be calculated as the frequency of the G/G genotype was too low. Significant differences were seen between C/C and C/G genotypes for impulsivity (Wald = 4.10, $p < 0.05$) and HÖ score (Wald = 6.78, $p < 0.01$) (Table 4-15). Furthermore, the odds ratios indicated that the higher the impulsivity (Exp (B) = 12.79) and HÖ score (Exp (B) = 1.06) the greater likelihood of being a C/C genotype as opposed to a C/G. In order to compare the *PER2* C111G genotypes C/C and G/G a binary logistic regression was performed. The predictive value of the model was not increased above chance ($\chi^2 = 12.04$, $p = 0.36$) according to a χ^2 test, and so again no reliable conclusions could be drawn.

	C/C			
	B (*SE*)	Wald	Exp (*b*)	95% CI
Gender	-0.31 (0.55)	0.32	0.73	0.25 - 2.16
Sleep disturbance	-0.02 (0.10)	0.04	0.98	0.81 - 1.19
Shift work	-17.42 (0.00)	-	-	-
Depression	-1.01 (1.01)	0.99	0.37	0.05 - 2.66
HÖ Score	0.06 (0.02)	6.78**	1.06	1.02 - 1.11
Impulsivity	2.55 (1.26)	4.10*	12.79	1.09 - 150.69
Neuroticism	-0.05 (0.03)	2.16	0.95	0.89 - 1.02
Extraversion	-0.53 (0.37)	2.14	0.59	0.29 - 1.20
Openness	-0.07 (0.04)	2.94	0.93	0.86 - 1.01
Agreeableness	-0.06 (0.04)	2.16	0.94	0.87 - 1.02
Conscientiousness	0.30 (0.38)	0.61	0.74	0.35 - 1.56

$R^2 = 0.18$ (Cox and Snell), 0.32 (Nagelkerke), 0.24 (McFadden). Model $\chi^2 = 35.42$, $p < 0.05$. Reference catagory C/G. * $p < 0.05$, ** $p < 0.01$

Tables 4-15 Multinomial logistic regression with *PER2* C111G genotype as the outcome variable.

4.3.4.3 *PER2* 10870

No significant differences were seen in the MANOVA conducted on *PER2* 10870 (Pillai's trace: $F = 0.11$, $p = 0.48$). Furthermore, a binary logistic regression comparing A/A and G/G genotypes also produced no significant results ($\chi^2 = 18.10$, $p = 0.08$)

4.3.4.4 *CLOCK* C3111T

Similarly, the MANOVA conducted on *CLOCK* C3111T was not significant ($F = 1.29$, $p = 0.19$), with a binary logistic regression between T/T and C/C genotypes also insignificant ($\chi^2 = 8.47$, $p = 0.67$).

4.3.4.5 *CLOCK* rs11932595

Analysis of *CLOCK* rs11932595 showed significant results before a correction for multiple tests (Pillai's trace: $F = 1.90$, $p < 0.05$). The coefficients in Table 4-16, revealed that conscientiousness contributed the most to distinguishing the genotypes. Moreover, it significantly discriminated the A/G genotype from the other two genotypes ($B = 0.46$, $p < 0.05$) so that participants with this genotype scored higher on conscientiousness than participants with the other two genotypes.

Further investigation through univariate ANOVAs revealed differences in genotype on impulsivity score ($F = 3.66$, $p < 0.05$) (Table 4-17) but this became insignificant after the application of a Bonferroni correction. *Post hoc* analysis suggested differences between A/A and A/G genotypes (Hochberg's GT2: $p < 0.05$; Games-Howell: $p < 0.05$) but not G/G and

A/G (Hochberg's GT2: $p = 0.35$; Games-Howell: $p = 0.27$) or A/A and G/G (Hochberg's GT2: $p = 0.97$; Games-Howell: $p = 0.90$) (Table 4-18).

Dependent variable		B
Sleep disturbance		
	A/A	0.31
	A/G	-0.23
	G/G	0[a]
Depression		
	A/A	-0.02
	A/G	-0.08
	G/G	0[a]
HÖ Score		
	A/A	-0.51
	A/G	0.69
	G/G	0[a]
Impulsivity		
	A/A	0.02
	A/G	-0.09
	G/G	0[a]
Neuroticism		
	A/A	-3.16
	A/G	-2.38
	G/G	0[a]
Extraversion		
	A/A	-0.08
	A/G	0.03
	G/G	0[a]
Openness		
	A/A	-0.96
	A/G	1.76
	G/G	0[a]
Agreeableness		
	A/A	0.66
	A/G	2.62
	G/G	0[a]
Conscientiousness		
	A/A	0.66
	A/G	0.46*
	G/G	0[a]

* $p < 0.05$, [a] Parameter set to 0 as it is redundant.

Table 4-16 Weights of variables (B) which maximally distinguish genotypes in *CLOCK* rs11932595

Dependent variable	*CLOCK* rs11932595
Sleep disturbance	0.65
Depression	0.76
HÖ Score	0.17
Impulsivity	3.66*
Neuroticism	1.10
Extraversion	0.24
Openness	2.74
Agreeableness	2.37
Conscientiousness	2.72

*$p < 0.05$

Table 4-17 Univariate ANOVA *F* statistics for between subject effects. The values indicate differences between *CLOCK* rs11932595 genotype on the dependent variables.

Dependent variable	Genotype (I)	(J)	Mean difference (I - J)	Hochberg's GT2 significance	Games-Howell significance
Impulsivity	A/A	A/G	0.11	0.027*	0.027*
	G/G	A/G	0.09	0.354	0.266
	A/A	G/G	0.02	0.968	0.902

*$p < 0.05$

Table 4-18 Results of Hochberg GT2 and Games-Howell tests for *CLOCK* rs11932595 showing which genotypes differed on impulsivity.

The results of the multinomial logistic regression for *CLOCK* rs11932595 indicated that there was a significant difference between A/A and A/G genotypes for neuroticism (Wald = 5.00, $p < 0.05$), impulsivity (Wald = 7.95, $p < 0.01$) and openness (Wald = 13.55, $p < 0.001$) (Table 4-19). The odds ratios (Exp (b)'s) in the comparison between A/A and A/G genotypes indicated that if a subject increased their neuroticism or openness score by one unit then the relative risk of being an A/A genotype as opposed to a A/G genotype would decrease by 0.94 and 0.89 respectively, suggesting that a participant would be more likely to have a A/G genotype. The opposite is true for impulsivity score, where a one unit increase in impulsivity score would result in an increase of 11.86 in the relative risk of being an A/A genotype as opposed to a A/G genotype, given the other variables in the model are held constant. This means that a participant would more likely be an A/A genotype the higher the impulsivity score.

In the comparison between G/G and A/G genotypes, only a significant difference for openness was evident (Wald = 5.04, $p < 0.05$), with the odds ratio indicating that a one unit increase in openness score would result in the relative risk of being a G/G genotype as opposed to a A/G genotype decreasing by 0.92. Therefore, the higher the openness score the greater the likelihood of being a A/G genotype.

A binary logistic regression was performed in order to compare the *CLOCK* rs11932595 genotypes A/A and G/G. A χ^2 test indicated that the predictive value of the model was not increased above chance ($\chi^2 = 7.97$, $p = 0.72$) and therefore no reliable conclusions could be drawn.

	A/A				G/G			
	B (*SE*)	Wald	Exp (*b*)	95% CI	B (*SE*)	Wald	Exp (*b*)	95% CI
Gender	-0.05 (0.41)	0.02	0.95	0.43 - 2.11	0.11 (0.53)	0.04	1.11	0.4 0- 3.13
Sleep disturbance	0.09 (0.07)	1.42	1.09	0.94 - 1.26	0.01 (0.10)	0.01	1.01	0.83 - 1.22
Shift work	-2.20 (1.36)	2.62	0.11	0.01 - 1.59	-1.22 (1.55)	0.62	0.30	0.01 - 6.14
Depression	0.44 (0.71)	0.38	1.55	0.38 - 6.28	0.48 (0.95)	0.26	1.62	0.25 - 10.39
HÖ Score	0.01 (0.02)	0.13	1.01	0.97 - 1.04	0.01 (0.02)	0.36	1.01	0.97 - 1.06
Impulsivity	2.47 (0.88)	7.95**	11.86	2.13 - 66.18	0.90 (1.15)	0.61	2.46	0.26 - 23.53
Neuroticism	-0.06 (0.03)	5.00*	0.94	0.90 - 0.99	0.01 (0.03)	0.06	1.01	0.95 - 1.07
Extraversion	-0.11 (0.26)	0.20	0.89	0.54 - 1.48	0.41 (0.34)	1.46	1.51	0.77 - 2.93
Openness	-0.11(0.03)	13.55***	0.89	0.84 - 0.95	-0.09 (0.04)	5.04*	0.92	0.85 - 0.99
Agreeableness	-0.05 (0.03)	2.49	0.95	0.89 - 1.01	-0.05 (0.04)	1.62	0.95	0.88 - 1.03
Conscientious	-0.17 (0.25)	0.45	0.50	0.52 - 1.38	-0.55 (0.33)	2.83	0.58	0.30 - 1.10

$R^2 = 0.20$ (Cox and Snell), 0.23 (Nagelkerke), 0.11 (McFadden). Model $\chi^2 = 38.58$, $p < 0.05$. Reference category A/G.

$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 4-19. Multinomial logistic regression with *CLOCK* rs11932595 genotype as the outcome variable.

4.3.4.6 *PER3* VNTR

The MANOVA performed on *PER3 VNTR* was not significant (Pillai's trace: $F = 0.60$, $p = 0.90$) and a binary logistic regression comparing 4/4 and 5/5 genotypes also produced insignificant results ($\chi^2 = 8.80$, $p = 0.64$).

4.4 Discussion

4.4.1 Associations within the smaller sample

The smaller sample size only retained one gender association, that of conscientiousness significantly higher in females. This relationship was also weaker than that in the larger group even though the proportion of males and females in the sample was the same. The correlations between variables were also different to those in the larger sample, with age only associated with openness and HÖ score additionally associated with extraversion. These differences are due, not only to the difficulty in detecting relationships in the smaller sample but also because the sample was selected for by extreme conscientiousness scores which would cause different associations to emerge.

4.4.2 Conscientiousness group differences

Investigation of group differences through a MANOVA revealed that sleep disturbance, impulsivity, depression, HÖ score, neuroticism, openness, agreeableness and extraversion all differed between conscientiousness groups. Furthermore, all of the variables were able to distinguish at least one group from the other two, with sleep disturbance, depression, extraversion and openness contributing to the discrimination of the low group from the other two and HÖ score, impulsivity, neuroticism and agreeableness able to distinguish both low and intermediate groups from each other and from the high group. Univariate analysis revealed differences in all variables except age between the high and low groups, with only differences in sleep disturbance, impulsivity, extraversion, openness and depression evident between low and intermediate groups and differences in impulsivity and HÖ score evident between high and intermediate groups.

As conscientiousness was shown to correlate with all these variables, both in the larger sample (see Chapter 3) and in the smaller sample, it would reasonable to expect there to be a difference between them in the high and low groups.

4.4.3 Conscientiousness group differences between genotype

No associations were found between the clock gene polymorphisms studied and conscientiousness group membership and therefore Hypothesis 1 was rejected. This may be due to a number of reasons. Firstly, conscientiousness is a broad dimension of personality making it difficult to attribute to a polymorphism, therefore if broken down it is possible that clock gene polymorphisms may associate with one or more of its facets. Secondly, although conscientiousness is correlated with diurnal preference, the variance accounted for in this association may be different to that between diurnal preference and the clock gene polymorphisms in which relationships have previously been found. Indeed, Chapter 3 showed that there was a large proportion of variance (84%) that was unaccounted for in the multiple regression model, of which genetic factors may have had some bearing. Lastly, it is possible that more than one gene accounts for the individual differences in conscientiousness and that the contribution of any one gene may be small and not sufficient to determine phenotype (Ebstein et al., 2000). Personality, therefore, may be influenced by interactions between a combination of genes (known as epistasis) rather than a single polymorphism.

4.4.4 Associations between genotype and personality variables

Comparisons between genotypes on the dependent variables produced significant results, before a Bonferroni correction, for both *PER2 C111G* and *CLOCK* rs11932595, partially

supporting Hypothesis 2. In the multinomial logistic regression, three variables were identified that predicted *CLOCK* rs11932595 genotype before a correction for multiple tests. Participants with high neuroticism and openness scores were more likely to be an A/G genotype and those with high impulsivity scores an A/A genotype. However, the MANOVA indicated a significant difference between genotype on conscientiousness with participants with the A/G genotype scoring higher than those with the other genotypes. Follow-up univariate tests showed impulsivity as significantly different between genotypes, before a Bonferroni correction, and *post hoc* tests indicated a significant difference on impulsivity between A/A and A/G genotypes. Different results may have been obtained between the MANOVA, univariate analysis and regression because a MANOVA uses linear combinations of the variables in order to see which best discriminates the groups, whereas univariate analysis assesses each variable separately and multiple regression enters all variables into the same model. The results, therefore, indicate that as well as the genotypes differing along the individual variables, they may also have differed along a combination of variables. Differences in neuroticism and openness may only have been detected in the regression and not the univariate tests because it corrects for correlations between variables as previously discussed.

These results should be viewed cautiously as a binary logistic regression should have confirmed these differences in a comparison between the two homozygotes, but failed to do so. It would be expected that if a difference in a variable occurred between two genotypes the largest difference would be seen between homozygotes, but this was not the case. Furthermore, as no differences were seen in genotypes between conscientiousness groups it is unlikely that concientiousness alone was able to distinguish genotypes. It may be that a combination of several variables including conscientiousness contributed to the significant MANOVA.

The MANOVA conducted using *PER2* C111G as the grouping variable revealed that sleep disturbance was a significant contributor in the discrimination of C/C and C/G genotypes from each other and the G/G genotype, before a Bonferroni correction. Univariate tests also indicated that sleep disturbance was significantly different between genotypes but *post hoc* tests could not be performed due to the low frequency of the G/G genotype. The results from the multinomial logistic regression differed from those of the MANOVA and univariate tests. HÖ score and impulsivity differed significantly between C/C and C/G genotypes with participants who scored highly in both variables more likely to be a C/C genotype. This result is contradictory, as previous research (Caci et al., 2005) and the results from Chapter 3 indicate a negative relationship between HÖ score and impulsivity, therefore a high scorer on impulsivity is likely to be a low scorer on the HÖ questionnaire. Again, these results should be treated extremely cautiously due to the low frequency of the G/G genotype, which may have skewed the results.

Few studies have been conducted on *CLOCK* rs11932595, but of those that have, Sookoian et al (2007) found an association between this polymorphism and non-alcoholic fatty liver disease. Further research suggested that *CLOCK* variants may be associated with agreeableness (Terracciano et al., 2008). Evidence has also recently been emerged for an association between *PER2* 10870 and increased alcohol consumption (Spanagel et al., 2005). This suggests that these genes have a role outside of the circadian clock and that the genotypes of the polymorphisms studied may influence the levels of certain traits. In order to confirm whether these associations exist within the general population, the findings in this study need to be replicated in a sample that has not selected for extremes of a specific trait.

4.4.5 Limitations of the study

4.4.5.1 Sample size

The initial recruitment target calculated from power analysis was 1650 (see Chapter 2), with 165 participants in each group. As only 651 participants were recruited, 20 % extremes were selected instead and new power calculations performed requiring 45 per group. All groups subsequently had numbers above this, indicating there was adequate power to detect differences in allele frequencies between the groups. This was not the case after the failure of genotyping nine participants for the *PER3* VNTR polymorphism (see section 2.3) which brought the low conscientiousness group down to 44 participants when studying this polymorphism, just under the calculated group size necessary for adequate power for the study. The decision to expand the extreme groups to 20% could mean that associations that may have been present in a larger population using 10% extremes could have been missed. Indeed, many studies that investigated extreme diurnal preference used groups composed of either the 5% (Carpen et al., 2006, Jones et al., 2007) and 7% (Archer et al., 2003, Carpen et al., 2005) extremes of their data. Conversely, others have found associations within the entire population without selecting extreme groups (Katzenberg et al., 1998).

The results of the relationships between genotypes and personality variables should also be treated cautiously as these results may be skewed due to the selection of the sample by conscientiousness score.

4.4.5.2 Genetic analysis

Genotyping varied in success among the seven polymorphisms. The polymorphism *CLOCK* rs12648271 was particularly difficult and failed completely using both a Taqman genotyping assay and pyrosequencing. Due to the position of the polymorphism in the gene a different primer could not be designed and no further analysis could be undertaken. The *PER3* VNTR also proved difficult to genotype as no result was achieved for nine participants, even after DNA purification and using a hot start enzyme. Whole genome amplification using Genomiphi still did not result in successful genotyping. These failures could be the result of using saliva samples rather than blood as they contain more contaminants which may interfere with the PCR process limiting the amount of DNA amplified.

Another possibility could be that the PCR primers or conditions may not have been as effective as those used for the other polymorphisms, and therefore may have needed more or purer DNA to amplify successfully. Indeed, buccal swabs were sent with instructions to those unwilling to complete the behavioural test, it cannot be guaranteed that those instructions were followed correctly, resulting in less DNA. This seems to be the case for one participant whos genotype could not be established for either *PER1* or the *PER3* VNTR, although they were successfully genotyped for the other three polymorphisms. No other studies have reported issues genotyping *PER3* VNTR using the same extraction and PCR methods (Archer et al., 2003; Viola et al., 2007) so it is reasonable to conclude that the samples themselves may have contained too little DNA for this primer set to be effective or that they contained contaminants that prevented the PCR from working effectively under these conditions.

The use of ARMs PCR also had its limitations, as genotype was determined by whether or not a DNA fragment was visible. This could have led to the incorrect assigning of an allele

because a PCR reaction may have failed rather than the allele not being present. In order to combat this, a proportion of samples that had been genotyped using ARMs were also genotyped using pyrosequencing. Of course, this could still lead to mis-assigning of some alleles but gave confidence in the results obtained.

In order to confirm results using other methods, a number of samples were retested. Again, this does not avoid mis-designation of some samples but adds support to the accuracy of the method. Ideally, all samples would have been tested twice but time and cost constraints did not make this viable.

4.4.5.3 Statistical analysis

When examining differences in variables between conscientiousness groups, Levene's test revealed that extraversion did not meet the assumption of homogeneity necessary when conducting a MANOVA. Log, reciprocal and square root transformations were performed but still the groups were heterogeneous for this variable. Extraversion scores were therefore converted to *z* scores in order to comply with the assumption of normality. In order to overcome the heterogeneity issue, the Games-Howell *post hoc* test was selected as it does not assume equal variances.

When performing MANOVAs on each polymorphism, group variances were not equal for agreeableness on *CLOCK* rs11932595. Again, Games-Howell *posthoc* analyses were performed, and the results from the univariate tests were similar to the regression analysis. The MANOVA and regression was not significant after a Bonferroni correction, but the number of tests performed meant that this correction was very conservative and the confirmatory results from the regression suggest this may be the case. Similarly, the results

from the *PER2* C111G univariate tests were not significant after a Bonferroni correction but in addition to this no *posthoc* analysis could be performed and only a C/C and C/G comparison could be carried out in the regression. This was due to only one participant being a G/G genotype and means that the results from this analysis should be viewed cautiously.

4.5 Conclusion

The present study failed to confirm the hypothesis 1 set out in section 4.2, that associations would be found between clock gene polymorphisms previously linked with diurnal preference and conscientiousness group membership. Hypothesis 2 was partially supported by a relationship identified between *CLOCK* rs11932595 and openness before a Bonferroni correction. Further relationships were identified, also before a Bonferroni correction, between *CLOCK* rs11932595 and impulsivity and neuroticism as well as between *PER2* C111G and sleep disturbance. Due to the selection of the sample and low frequency of one *PER2* C111G genotype this can only be considered a tentative association. Therefore, *CLOCK* rs11932595 should be considered a possible candidate for future studies that seek to explore further the genetic basis of personality.

Chapter 5 Analysis of behavioural data

5.1 Hypotheses

5.1.1 Hypothesis 1

Chapters 3 and 4 utilised self-report questionnaires as a way of assessing personality, diurnal preference and control variables. As these measures do not reflect state dependent fluctuations, a comparison with a behavioural measure would further strengthen the relationships previously found. The GoStop impulsivity paradigm, a variant of the stop signal task, is a measure of response inhibition (see section 1.8.2) which has previously been associated with ADHD. Patients with ADHD are characteristically low in conscientiousness and high in impulsivity and during the stop signal paradigm display a longer mean stop latency. This same parameter of the GoStop task has also been linked to high impulsivity in non-pathological populations (Logan et al., 1997), although others have found no link (Lijffijt et al., 2004). Additionally, an association has previously been found between conscientiousness and the GoStop (Edmonds et al., 2009) suggesting that it may be a useful tool in determining state dependent fluctuations within a sample selected for by conscientiousness score.

As Chapter 4 showed that the low conscientiousness group was associated with high impulsivity, it is possible that this group may also associate with the GoStop impulsivity paradigm. Therefore, the first hypothesis is:

- That the low conscientiousness group will have a longer mean stop latency on the

GoStop task, compared to the high conscientiousness group.

5.1.2 Hypothesis 2

As stated previously, the stop signal paradigm has consistently been linked to high impulsivity in pathological populations, but results in non-pathological populations have been less consistent (Lijffijt et al., 2004). A comparison of this task with the results of the I_7 questionnaire would, therefore, further elucidate any relationship. As the groups have been selected for via conscientiousness score and not I_7 score this must be taken into account, although impulsivity is thought to be comprised of conscientiousness (in addition to neuroticism and extraversion) and so any association may still be seen. Hence, the second hypothesis is:

- That impulsivity, as defined by the I_7 questionnaire, will associate with mean stop latency as measured by the GoStop task. Specifically, impulsivity will positively correlate with a longer mean stop latency.

5.1.3 Hypothesis 3

In Chapter 4, the clock gene polymorphism *CLOCK* rs11932595 was linked to impulsivity, as measured by the I_7 questionnaire, before a Bonferroni correction. This indicates that this polymorphism may also associate with behavioural impulsivity as measured by the GoStop. No other polymorphisms in Chapter 4 were linked to impulsivity, but as the GoStop measures behavioural impulsivity and due to the fact that diurnal preference correlates with impulsivity, and in the case of *PER2* 10870 that increased alcohol consumption is associated with impulsivity, it is possible that the other polymorphisms previously discussed may also

associate with the GoStop task. Therefore, the third hypothesis is:

- That the clock gene polymorphisms analysed in Chapter 4 will associate with the GoStop impulsivity paradigm.

5.2 Methods

For full details of methods see Chapter 2. Methodologies specific to this study are outlined below.

5.2.1 Participants

Participants within the high, low and intermediate conscientiousness groups defined in Chapter 4 were contacted and asked to take part in the GoStop impulsivity task (see section 1.8.2 and 2.1.3). They were also asked whether they still complied with the exclusion criteria (see section 2.2.1), and those that did not took no further part in the study.

5.2.2 Study design

Participants who agreed to take part underwent an 11-minute behavioural test (see section 2.1.3) either at their home or at the University. They were given a £5 gift voucher as compensation for their time and inconvenience.

5.2.3 Statistical analysis

Statistical analysis was performed using SPSS version 11. Correlations used Pearson's *r* and differences between gender whereas differences between high and low groups and some genotypes used an independent *t* test. ANOVAs were also used to check for differences between genotypes where all three remained. Pearson χ^2 tests were performed to assess differences in genotype between conscientiousness groups and Fisher's exact test to examine allele frequencies. Finally, multinomial logistic regressions were performed in order to find whether mean stop latency predicted specific genotypes. Explanations for each test can be found in section 2.4.

5.3 Results

A total of 105 participants completed the behavioural test (mean age 25.5 ± 5.8 yrs [standard deviation], 71 females, 67.6%). Stop latency, latency in the no stop trials and inhibition in the stop, no stop trials and novel trials were calculated for each individual at each stop signal delay by the program. This data was then entered into SPSS and the means for each variable for each individual were calculated.

The primary measure of the GoStop task was mean stop latency, other variables were calculated in order to exclude participants who did not fully understand the task as well as to ensure the task performed as expected. Mean latency in the no stop trials and mean inhibition in the novel trials were measures of attention, where differences between groups on these variables would indicate a difference in maintaining attention during the task. Mean inhibition in the stop trials was calculated in order to confirm that the adjusting stop signal delay

tracking algorithm was successful and mean inhibition in the no stop trials was calculated as a control measure which would indicate whether or not a participant understood the task.

Before the data were analysed, mean stop latency, mean inhibition in the stop, no-stop and novel trials were all examined. Participants were excluded if in any of the blocks they inhibited on all or none of the stop-signal trials, if overall in the no stop trials they had fewer than 66% correct responses, or a stop latency that was less than 50 ms. Failure of the first two criteria led to exclusion of participants because it could provide an inaccurate measure of stop latency (Schachar et al., 2000). Participants were excluded on the third criteria because stop latencies of this speed are unrealistically fast, and so unreliable. One participant was excluded because they inhibited all the stop trials in a block, a second participant had less than 66% accuracy in the no stop trials, and a further four participants had stop latencies of less than 50ms. Two participants were excluded as they represented outliers in the data, one had a long mean stop latency and mean latency in the no stop trial and a final participant had a novel trial inhibition of 5%.

This left the sample with 97 participants [mean age 25.5 ± 5.9 yrs (standard deviation), 65 females 67.0%]. Low, intermediate and high conscientiousness groups consisted of 26 [mean age 25.6 ± 6.9 yrs (standard deviation), 8 females, 30.8%], 31 [mean age 24.4 ± 4.9 yrs (standard deviation), 22 females, 71.0%] and 40 [mean age 26.3 ± 5.9 yrs (standard deviation), 35 females, 87.5%] participants, respectively.

A histogram was plotted for each variable to ensure it followed a normal distribution. Age, as well as scores on the I_7, the depression scale of the HADS, the global score of the PSQI, neuroticism and conscientiousness scores of the FFI and the behavioural variables mean inhibition, mean inhibition in the novel trials, mean stop latency and mean latency in the no

stop trials did not follow a normal distribution (Fig. 5-1 a-d and Fig. 5-2 a-e). As impulsivity, depression and conscientiousness did not conform with assumption of homogeneity of variance they were converted using a log-10 transformation, with impulsivity and depression converted using the formula log(score + 1) as a number of participants scored zero for these variables. The remaining variables were converted to *z* scores so they conformed with the assumption of normality.

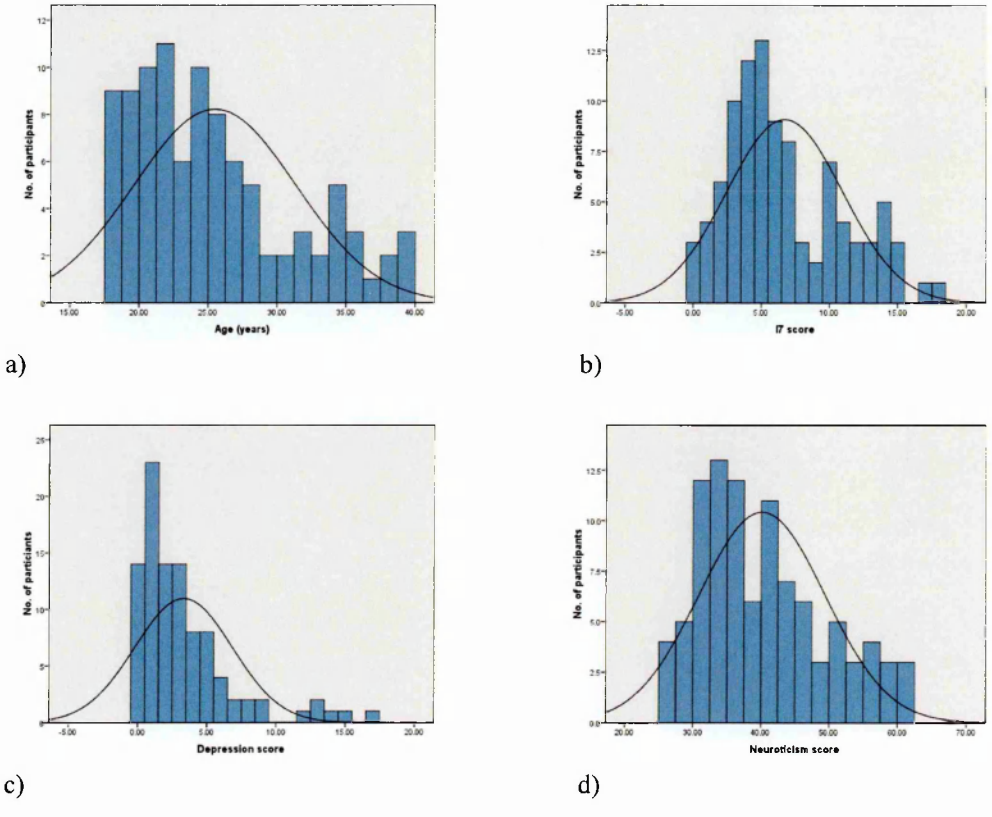
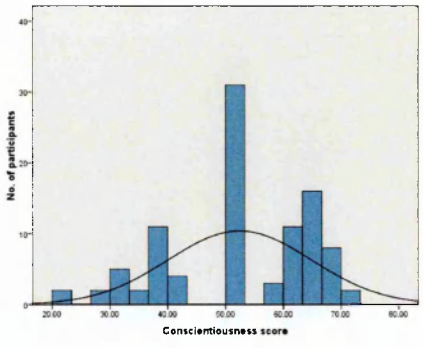
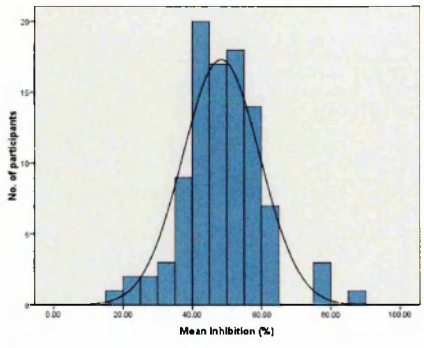


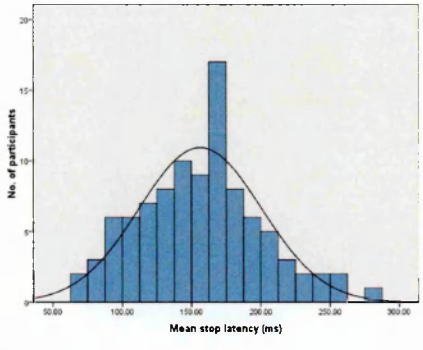
Figure 5-1 Histograms to show deviation from normality for a) age b) I_7 score c) depression score d) Neuroticism score.



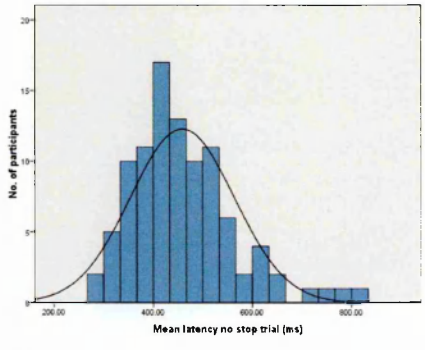
a)



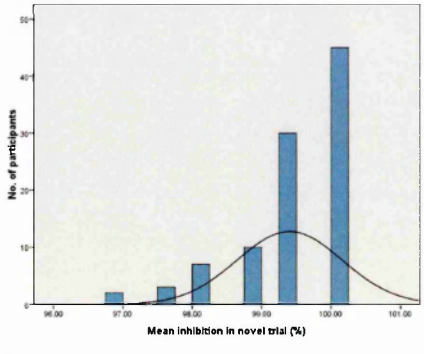
b)



c)



d)



e)

Figure 5-2 Histograms to show deviation from normality for a) Conscientiousness score b) Mean inhibition c) Mean stop latency d) Mean latency in the no stop trials e) Mean inhibition in the novel trial.

5.3.1 Associations within the smaller sample

Independent *t* tests were used to assess whether the same gender differences were apparent in age, depression, HÖ score, sleep disturbance, impulsivity and the dimensions of the five factor model within the smaller sample (Table 5-1). Conscientiousness [$t(43) = -4.84$, $p < 0.001$; Males: 43.8 ± 12.6; Females: 56.7 ± 9.9] remained significantly different between genders with women having higher scores, as was shown in Chapter 4 . Additionally, sleep disturbance [$t(95) = 2.90$. $p < 0.01$; Males: 6.6 ± 2.7; Females: 4.9 ± 2.6] and impulsivity [$t(95) = 2.99$, $p < 0.01$; Males: 8.8 ± 4.8; Females: 5.7 ± 3.6] also differed between genders, with scores in both these variables higher in men, as was seen in the larger sample in Chapter 3. None of the other variables were significantly different between genders.

Correlational analysis revealed that age was only associated with HÖ score ($r = 0.20$, $p < 0.05$) in the smaller sample, where morningness increased with age (Table 5-3). Furthermore, eveningness was correlated with depression ($r = -0.30$, $p < 0.01$) and impulsivity ($r = -0.25$, $p < 0.01$) and morningness with agreeableness ($r = 0.23$, $p < 0.05$) and conscientiousness ($r = 0.26$, $p < 0.01$), having lost its associations with extraversion, sleep disturbance and openness that were evident in Chapter 4.

When investigating differences between groups in the smaller sample, all variables except for age ($t[64] = -0.44$, $p > 0.05$) and agreeableness ($t[64] = -1.58$, $p > 0.05$) were significantly different between high and low groups (Table 5-2). Participants in the low conscientiousness group had higher scores on depression ($t[64] = 4.11$, $p < 0.001$), sleep disturbance ($t[64] = 4.21$, $p < 0.001$), impulsivity ($t[64] = 4.26$, $p < 0.001$), neuroticism ($t[64] = 2.62$, $p < 0.05$), extraversion ($t[64] = 3.00$, $p < 0.01$) and openness ($t[64] = 2.53$, $p < 0.05$), whereas those in

the high group were more morning orientated ($t[64] = -2.82, p < 0.001$) and, understandably, higher in conscientiousness ($t[27] = -16.42, p < 0.001$).

Variable	Male	Female	Combined	Male vs Female
Age	25.1 ± 5.9	25.7 ± 5.9	25.5 ± 5.9	t(95) = -0.41
Depression	4.3 ± 4.2	2.8 ± 3.1	3.3 ± 3.5	t(95) = 1.98
HÖ score	49.8 ± 10.7	56.7 ± 9.9	49.9 ± 12.4	t(95) = -0.06
Sleep disturbance	6.6 ± 2.7	4.9 ± 2.6	4.9 ± 2.6	t(95) = 2.90**
Impulsivity	8.8 ± 4.8	5.7 ± 3.6	5.5 ± 2.7	t(95) = 2.99**
Neuroticism	41.3 ± 10.0	39.6 ± 8.9	40.1 ± 9.3	t(95) = 0.88
Extraversion	48.3 ± 8.1	49.7 ± 8.6	49.2 ± 8.4	t(95) = -0.76
Openness	49.7 ± 7.7	49.0 ± 7.3	49.2 ± 7.4	t(95) = 0.43
Agreeableness	51.4 ± 6.0	53.0 ± 6.9	52.5 ± 6.6	t(95) = -1.09
Conscientiousness	43.8 ± 12.6	56.7 ± 9.9	52.5 ± 12.4	t(43) = -4.84***

Table 5-1 Descriptive statistics for combined sample and genders, *t*, independent *t* test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Variable	Low	Intermediate	High	Low vs High
Age	25.6 ± 6.9	24.4 ± 4.9	26.3 ± 5.9	*t*(64) = -0.44
Depression	5.7 ± 4.6	2.4 ± 2.2	2.5 ± 2.8	*t*(64) = 4.11***
HÖ score	46.3 ± 11.0	46.6 ± 11.5	54.8 ± 12.5	*t*(64) = -2.82***
Sleep disturbance	7.1 ± 2.9	5.4 ± 2.6	4.5 ± 2.2	*t*(64) = 4.21***
Impulsivity	9.3 ± 4.6	7.1 ± 4.4	4.7 ± 2.7	*t*(64) = 4.26***
Neuroticism	44.2 ± 10.9	39.3 ± 9.1	38.2 ± 7.5	*t*(64) = 2.62*
Extraversion	44.4 ± 9.0	51.0 ± 6.4	51.0 ± 8.4	*t*(64) = 3.00**
Openness	52.2 ± 7.2	49.1 ± 6.2	47.4 ± 7.9	*t*(64) = 2.53*
Agreeableness	50.7 ± 7.1	52.8 ± 5.8	53.4 ± 6.9	*t*(64) = -1.58
Conscientiousness	35.2 ± 5.8	51.5 ± 1.0	64.4 ± 3.2	*t*(27) = -16.42***

Table 5-2 Descriptive statistics for conscientiousness groups, t, independent t test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Variables	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1. Age	—									
2. Depression	0.08	—								
3. HÖ score	0.20*	-0.30**	—							
4. Impulsivity	-0.06	0.30**	-0.25**	—						
5. GPSQI	0.17	0.32***	-0.17	0.26*	—					
6. Neuroticism	-0.06	0.40***	-0.15	0.09	0.34***	—				
7. Extraversion	-0.18	-0.41***	0.05	0.12	-0.30**	-0.55***	—			
8. Openness	0.05	0.07	0.14	0.40***	0.16	-0.01	0.19	—		
9.Agreeableness	0.04	-0.36***	0.23*	-0.30**	-0.01	-0.32**	0.26**	-0.11	—	
10. Conscientiousness	0.09	-0.41***	0.26**	-0.41***	-0.35***	-0.27*	0.32***	-0.24*	0.19	—

*$p < 0.05$, **$p < 0.01$, *** $p < 0.001$

Table 5-3 Pearson correlations between demographic factors, control variables and personality variables.

5.3.2 Differences in genetic data in the smaller sample

Due to some genotypes of the polymorphisms investigated being quite rare it was necessary to check whether the smaller sample resulted in loss of some genotypes and/ or a difference in allele frequencies. The contingency tables (Table 5-4 a and c) show the loss of all *PER1* T2434C C/C genotypes and *PER2* 10870 G/G genotypes in the smaller sample. As in Chapter 4, χ^2 tests were performed to determine whether there were differences in genotypes for each polymorphism between conscientiousness groups. Consistent with the previous results, no relationships were seen for any of the polymorphisms between all three groups and between high and low groups (Table 5-4 a-c, 5-5 a-c). Due to the low number of participants with the G/G genotypes for *PER2* C111G and the loss of C/C and G/G genotypes for *PER1* T2434C, *PER2* 10870, respectively, the assumptions of the χ^2 were broken and so fishers exact tests were performed for these polymorphisms, comparing allele frequencies between high and low groups (Table 5-6 a-c). Once more, the results were consistent with those from Chapter 4, showing no significant differences between groups on any of the polymorphisms.

Fishers exact tests were also performed on the allele frequencies in the combined sample comparing results from the larger sample in Chapter 4 with those in the current chapter (Table 5-7). The allele frequencies did not differ between the smaller and larger sample and so subsequent analyses can be deemed reliable.

(a)

	T/T	T/C	C/C
Low	14	12	0
Intermediate	23	8	0
High	29	11	0

(b)

	C/C	C/G	G/G
Low	23	2	1
Intermediate	24	7	0
High	34	6	0

(c)

	A/A	A/G	G/G
Low	16	10	0
Intermediate	26	5	0
High	33	7	0

Table 5-4: Contingency tables showing the number of participants in each group with a certain genotype of the following polymorphisms (a) *PER1* T2434C all three groups: $\chi^2 = 3.32$ (2), $p = 0.19$, high vs low: $\chi^2 = 2.42$ (1), $p = 0.12$. (b) *PER2* C111G all three groups: $\chi^2 = 5.00$ (4), $p = 0.29$, high vs low: $\chi^2 = 2.26$ (2), $p = 0.32$. (c) *PER2* 10870, $\chi^2 = 5.06$ (2), $p = 0.08$, high vs low: $\chi^2 = 3.62$ (1), $p = 0.06$.

(a)

	T/T	T/C	C/C
Low	12	11	3
Intermediate	16	11	4
High	16	19	5

(b)

	A/A	A/G	G/G
Low	10	10	6
Intermediate	16	11	4
High	11	24	5

(c)

	4/4	4/5	5/5
Low	12	9	5
Intermediate	8	14	9
High	18	13	9

Table 5-5: Contingency tables showing the number of participants in each group with a certain genotype of the following polymorphisms (a) *CLOCK* C3111T, all three groups: $\chi^2 = 1.14$ (4), $p = 0.89$, high vs low: $\chi^2 = 0.25$ (2) $p = 0.88$. (b) *CLOCK* rs11932595 all three groups: $\chi^2 = 6.74$ (4), $p = 0.15$, high vs low: $\chi^2 = 3.07$ (2), $p = 0.22$. (c) *PER3 VNTR*, $\chi^2 = 3.51$ (4), $p = 0.48$, high vs low : $\chi^2 = 0.11$ (2), $p = 0.95$.

(a)

	T	C
Low	40	12
High	69	11

(b)

	C	G
Low	48	4
High	74	6

(c)

	A	G
Low	42	10
High	73	7

Table 5-6: Contingency tables showing the number of alleles per conscientiousness group for the following polymorphisms (a) *PER1* T2434C, Fishers exact test $p = 0.24$, (b) *PER2* C111G, Fishers exact test $p = 1.00$, (c) *PER2* 10870, Fishers exact test $p = 0.11$, all 2-sided.

	Chapter 4		Chapter 5		Significance
PER1 T2434C	T 0.82	C 0.18	T 0.84	C 0.16	0.85
PER2 C111G	C 0.93	G 0.07	C 0.91	G 0.09	0.80
PER2 10870	A 0.87	G 0.13	A 0.89	G 0.11	0.83
CLOCK C3111T	T 0.69	C 0.31	T 0.66	C 0.34	0.76
CLOCK rs11932595	A 0.64	G 0.36	A 0.61	G 0.39	0.77
PER3 VNTR	4 0.63	5 0.37	4 0.58	5 0.42	0.56

Table 5-7 Comparison between allele frequencies from Chapters 4 and 5 and significance values of Fishers exact tests.

5.3.3 Behavioural data

Independent t tests were used to compare genders on mean stop latency ($t[95] = 1.39$, $p > 0.05$) , mean inhibition in the stop ($t[95] = -0.40$, $p > 0.05$) and novel trials ($t[95] = -0.94$, $p > 0.05$) and mean latency in the no stop trials ($t[95] = 0.33$, $p > 0.05$) (Table 5-8) and no differences were seen on any of the variables. In the combined sample, mean stop latency was 156.3 ± 44.2 ms and mean latency in the no stop trials was 458.7 ± 104.9 ms, which is in line with previous studies (Lijffijt et al., 2004). The GoStop task was set to use adjusting stop signal delays where a correct response would result in an increased stop delay (making the test harder) and an incorrect response resulted in a decreased stop signal delay (making the test easier). This was so that the test would converge on a stop signal delay that allowed participants to inhibit half of the time. This proved successful as mean inhibition across the trials was 48.4 ± 11.2 %. The difficulty associated with the length of stop signal delays was confirmed where there was a decrease in mean inhibition as the stop signal delay increased (Figure 5-3). Mean inhibition in the novel trial, where a non-matching number was introduced which required the participants to inhibit a response, was 99.4 ± 0.8 %, indicating that the participants were able to maintain attention during the task.

Variable	Male	Female	Combined	Male vs Female
Mean inhibition (stop)	47.8 ± 11.5	48.7 ± 11.1	48.4 ± 11.2	$t(95) = -0.40$
Mean inhibition (novel)	99.3 ± 0.9	99.5 ± 0.7	99.4 ± 0.8	$t(95) = -0.94$
Mean stop latency	165.2 ± 50.5	152.0 ± 40.5	156.3 ± 44.2	$t(95) = 1.39$
Mean latency (no stop)	463.8 ± 112.3	456.3 ± 101.9	458.7 ± 104.9	$t(95) = 0.33$

Table 5-8 Descriptive statistics of behavioural variables for each gender and combined. *t*, independent *t* test.

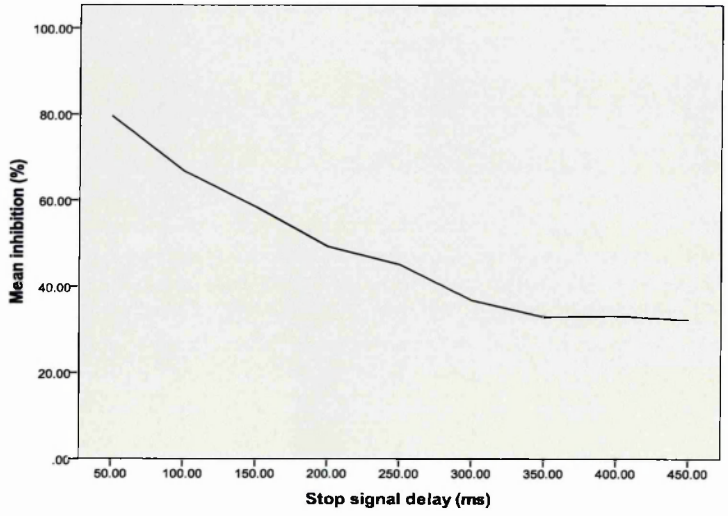


Figure 5-3. Percentage inhibition at different stop signal delay times.

5.3.4 Associations between behavioural and self report impulsivity

In order to investigate whether self-report and behavioural measures of impulsivity were associated and therefore measured the same or similar construct, a partial correlation was performed between I_7 score and mean stop latency whilst controlling for time of day and season of the test. No relationship was evident between these two methods of measuring impulsivity (r = 0.02, p > 0.05). This was further confirmed when mean stop latency, was plotted against I_7 score and showed no linear relationship (Figure 5-4).

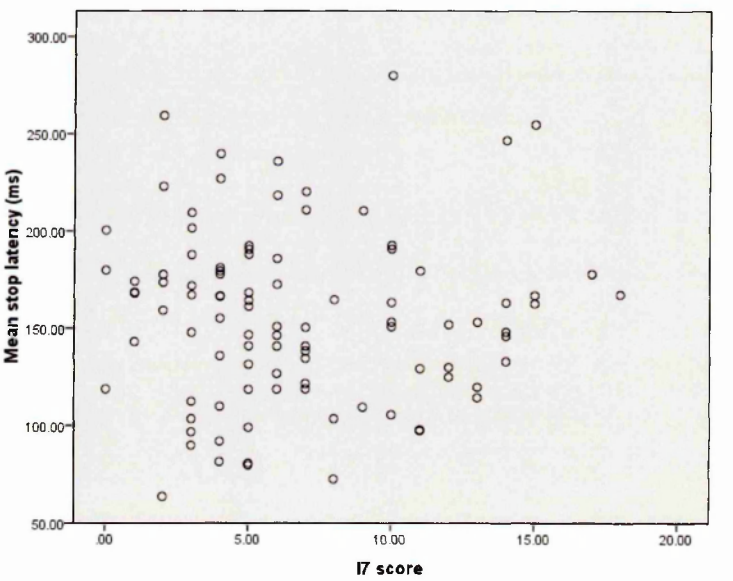


Figure 5-4 Bivariate scatterplot of I_7 score against mean stop latency.

5.3.5 Differences in behavioural variables between conscientiousness groups

Independent *t* tests were conducted for the behavioural variables mean inhibition in the stop and novel trials, mean stop latency and mean latency in the no stop trials, with a comparison between high and low groups (Table 5-9). No differences were seen on any of the variables. As variation in the variables mean latency in the no stop trials ($t[64] = -0.19$, $p > 0.05$) and mean inhibition in novel trials ($t[64] = -1.55$, $p > 0.05$) signify attentional deficits, the results showed that both high and low conscientiousness groups were able to maintain attention equally well though out the task. Mean inhibition in the stop trial did also not differ significantly between groups ($t[64] = 0.20$, $p > 0.05$) again suggesting that the adjusting stop signals tracking system was successful. Furthermore, no differences were seen between groups for mean stop latency, the primary measure of the Gostop task ($t[64] = 0.61$, $p > 0.05$), indicating that individuals low in conscientiousness do not have longer mean stop latencies. This was confirmed by plotting mean stop latency against conscientiousness score, where no relationship was identifiable (Figure 5-5).

Variable	Low	Intermediate	High	Low vs High
Mean inhibition	49.0 ± 13.4	47.9 ± 9.9	48.4 ± 10.8	t(64) = 0.20
Mean inhibition (novel)	99.2 ± 0.8	99.4 ± 0.8	99.5 ± 0.7	t(64) = -1.55
Mean stop latency	165.9 ± 46.3	145.2 ± 37.2	158.7 ± 47.0	t(64) = 0.61
Mean latency (no stop)	459.4 ± 130.1	450.2 ± 79.9	464.9 ± 106.1	t(64) = -0.19

Table 5-9 Descriptive statistics of behavioural variables for conscientiousness groups. *t*, independent *t* test.

As independent *t* tests indicate the direction of a relationship but not whether a specific variable is a predictor of group membership a binary logistic regression was performed, comparing high and low conscientiousness groups. In block one, season and time of test were entered as these were variables that may have affected performance on the test. As mean stop latency is the primary measure of the GoStop, this was then entered into the second block.

A χ^2 test (χ^2 = 9.54, p = 0.09) indicated that the predictive value of the model was not increased above chance and therefore that mean stop latency was not significant in predicting conscientiousness group membership.

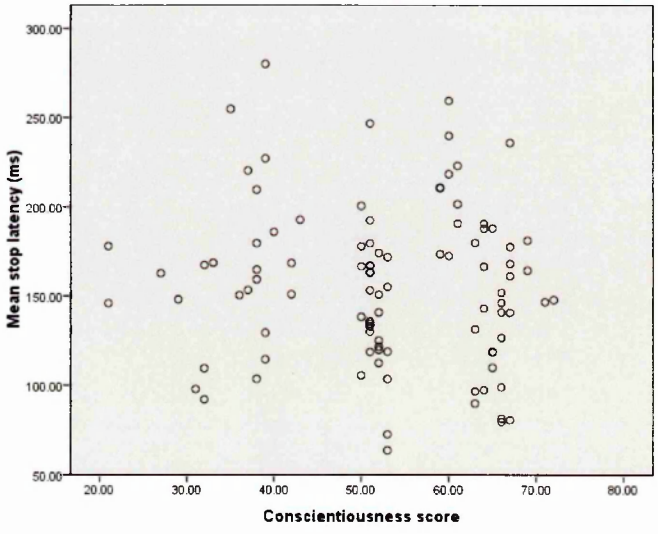


Figure 5-5 Bivariate scatterplot of mean stop latency against conscientiousness score.

5.3.6 Associations between behavioural test and genetic data

An ANOVA was performed for each polymorphism to examine differences in genotype on mean stop latency. Following this, regression analysis was performed to find whether this variable predicted any of the polymorphisms genotypes. A Bonferroni correction for multiple testing was applied to each analysis (p = 0.008). The results of each analysis are detailed separately.

5.3.6.1 *PER1* T2434C

An independent t test was conducted to compare differences in mean stop latency between T/T and T/C genotypes due to the smaller sample size resulting in no C/C genotypes. There were no significant differences in mean stop latency between these genotypes (t[95] = -0.39, p > 0.05).

In order to establish whether mean stop latency was a predictor of genotype a hierarchical binary logistic regression was undertaken comparing T/T and T/C genotypes. Time and season of test were entered into the first block and mean stop latency into the second. The predictive value of the model was not increased above chance (χ^2 = 2.36, p = 0.80) and, therefore, mean stop latency was not a predictor of *PER1* T2434C genotype.

5.3.6.2 *PER2* C111G

An ANOVA comparing the mean stop latency for each *PER2* C111G produced an insignificant result ($F = 0.48$, $p > 0.05$). A multinomial logistic regression with *PER2* C111G as the outcome variable and time and season of test and mean stop latency as predictor variables produced a similar result. C/G genotype was used as the reference category and both homozygotes compared against it. The predictive value of the model was not increased above chance ($\chi^2 = 10.19$, $p > 0.05$), and thus, mean stop latency did not predict *PER2* C111G genotype. A binary logistic regression comparing G/G and C/C genotypes could not be undertaken because only one participant had a G/G genotype.

5.3.6.3 *PER2* 10870

Due to there only being participants with A/A and A/G genotypes in the smaller sample an independent *t* test was conducted to compare mean stop latency between genotypes. There was no significant difference between genotypes on this variable ($t[95] = 0.51$, $p > 0.05$). Following this, a binary logistic regression was performed between A/A and A/G genotypes with time and season of test entered into block one and mean stop latency into block two. This was to establish whether mean stop latency predicted either of these genotypes but a χ^2 test was not significant ($\chi^2 = 3.73$, $p > 0.05$) suggesting that the probability of this variable predicting genotype was not increased above chance.

5.3.6.4 *CLOCK* C3111T

An ANOVA was performed in order to compare mean stop latency between *CLOCK* C3111T genotypes. Levene's test was significant, indicating that the assumption of homogeneity of variance was broken for this variable. The ANOVA only just failed to reach significance before a Bonferroni correction ($F = 2.92$, $p = 0.06$). A multinomial logistic regression was performed which compared T/T and C/C genotypes to the T/C genotype. Time and season of test as well as mean stop latency were entered as predictor variables but a χ^2 test was not significant, suggesting these variables did not predict *CLOCK* C3111T genotype ($\chi^2 = 16.31$, $p = 0.09$). Furthermore, a comparison of T/T and C/C through a binary logistic regression, with time and season of test entered into block one and mean stop latency into block two, also proved insignificant ($\chi^2 = 7.46, p = 0.19$).

5.3.6.5 *CLOCK* rs11932595

The ANOVA comparing mean stop latency between *CLOCK* rs11932595 genotypes was also insignificant ($F = 1.91, p = 0.15$), as well as Levene's test. A multinomial logistic regression that compared A/A and G/G genotypes to the A/G genotype was significant ($\chi^2 = 18.51, p < 0.05$) but the categorisations were unreliable and so confidence could not be placed in the results. A binary logistic regression was then performed comparing A/A and A/G genotypes and with time and season of test in block one and mean stop latency in block two, but the result was not significant ($\chi^2 = 2.94, p = 0.71$)

5.3.6.6 *PER3* VNTR

In order to compare the means of mean stop latency between *PER3* VNTR genotypes, an ANOVA was performed. Levene's test was not significant indicating that the assumption of homogeneity of variance was met. The ANOVA was significant before a Bonferroni correction ($F = 3.48$, $p < 0.05$) and the Hochberg GT2 *post hoc* test, chosen because of the unequal group sizes, revealed a significant difference between 5/5 and 4/5 genotypes ($p = 0.03$) (Table 5-10). With the 5/5 genotype having a significantly higher mean stop latency than the 4/5 genotype.

Dependent variable	Genotype (I)	(J)	Mean difference (I - J)	Hochberg's GT2 significance
Mean stop latency	4/4	4/5	0.14	0.90
	4/5	5/5	-0.67	0.03*
	5/5	4/4	-0.53	0.12

*$p < 0.05$

Table 5-10 Results of Hochberg GT2 and Games-Howell tests for *PER3* VNTR showing which genotypes differed on mean stop latency.

To find whether mean stop latency was a predictor of *PER3* VNTR genotype, a multinomial logistic regression was performed comparing 4/4 and 5/5 genotypes with the 4/5 genotypes and with time and season of test also included as predictors. The result was not significant ($\chi^2 = 16.81$, $p = 0.08$) suggesting that the predictive value of the model was not increased above chance.

In order to compare 4/4 and 5/5 genotypes, a binary logistic regression was performed, with time and season of test entered into block one and mean stop latency into block two but again the result was not significant ($\chi^2 = 8.07, p = 0.09$)

5.4 Discussion

The present study sought to establish whether mean stop latency, a measure of the GoStop task, would differ between conscientiousness groups. Furthermore, a comparison of this behavioural measure of impulsivity with a trait-dependent measure of impulsivity, the I_7, was conducted as well as an investigation into possible genetic markers associated with the GoStop task.

5.4.1 Associations within the smaller sample

Rather than losing relationships by making the sample smaller through the excluded participants who did not take part in the behavioural test, some of the gender associations evident in Chapter 3 re-emerged. Conscientiousness, sleep disturbance and impulsivity were all significantly different between genders, whereas only conscientiousness was significant in Chapter 4. By eliminating some participants this altered the means of each variable producing a greater difference between the two and, therefore, a significant result. The same was not true for correlational analysis, where age was now associated with HÖ score as opposed to openness, which was evident in Chapter 4. HÖ score was also associated with depression, impulsivity, agreeableness and conscientiousness, but not extraversion, sleep disturbance and openness, as was seen in Chapter 4. These differences are due, firstly, to the difficulty of detecting associations in a smaller sample size and secondly, the selection of conscientiousness groups, which were further diminished by only a proportion of participants completing the behavioural test.

When assessing differences in genotype and allele frequencies in the smaller sample two, both *PER1* T2434C and *PER2* 10870 no longer had any individuals with the rarest homozygote genotype. Despite this, allele frequencies did not differ significantly in the smaller sample and so the subsequent analyses carried out could be considered reliable.

5.4.2 Behavioural data

When investigating differences in mean stop latency between high and low conscientiousness groups no significant results were obtained and consequently, Hypothesis 1 (section 5.1.1) was rejected.

Although little research has been undertaken to compare the stop signal task with the dimensions of the five factor model of personality, one study did establish a link between conscientiousness and percentage inhibition in the GoStop task (Edmonds et al., 2009). They found that participants who inhibited more on the stop task also scored highly on conscientiousness. As someone scoring highly in conscientiousness is less spontaneous and controls their impulses more than a low scorer, an association between the two variables would not be unexpected. Additionally, variants of the stop signal task have been linked to ADHD, where children with this disorder inhibited less frequently than controls. As low conscientiousness is a main factor in the personality profile of ADHD (Nigg et al., 2002) this gives further support to a possible association between the two.

Edmonds et al did not select specifically for conscientiousness score and so any relationship would be expected to be more pronounced in the present study. Their results may have differed from those of the current study as they did not use adjusting stop signals to achieve a mean stop latency and instead percentage inhibition was their primary measure. Furthermore,

a different method of assessing conscientiousness was used and so both these points together could have resulted in a different association emerging.

5.4.2 Associations between self-report and behavioural impulsivity

The primary measure of the GoStop, mean stop latency, was not correlated with impulsivity, as measured by the I_7 questionnaire. Consequently, Hypothesis 2 (see section 5.1.2) was rejected as these measures of impulsivity were not associated. This may be due, first and foremost, to the fact that the sample was selected for by conscientiousness score, rather than by I_7 score. Although, as the GoStop has previously been found to be associated with conscientiousness (Edmonds et al., 2009) and the I_7 was associated with conscientiousness score in Chapter 3, it would not have been unreasonable to hypothesise that a direct association may have been evident.

Other research that has attempted to establish a link between self-report and behavioural measures of impulsivity have either selected extremes of impulsivity using a self-report questionnaire or used the entire sample. The results of these have been inconsistent, particularly in non-pathological populations. Several studies have proposed associations between variants of the stop signal task and self-reported impulsivity (Logan et al., 1997, Avila and Parcet, 2001, Marsh et al., 2002, Keilp et al., 2005). Marsh et al (2002) found a relationship between the I_7 and the GoStop, both of which were used in the current study. Their results may have been different, however (in addition to the selection process), because, as well as recruiting from the general public, they also recruited from probation and parole offices in order to boost the number of impulsive individuals. Also, they used fixed stop signals instead of adjusting, which meant they could only assess the percentage of inhibited responses at certain stop signal delays rather than stop latency. The other three studies used

different self-report questionnaires to the present study which may account for the difference in results. The mean stop latency of the combined sample in the current study was consistent with previous studies using this parameter as the primary measure (Lijffijt et al., 2004), and so it is unlikely that any differences were caused by discrepancies in the behavioural test.

There have also been numerous studies that have failed to find associations(Rodriguez-Fornells et al., 2002, Cheung et al., 2004, Lijffijt et al., 2004, Lansbergen et al., 2007, Edmonds et al., 2009). It may be that the I_7 questionnaire does not measure the same construct of impulsivity as the GoStop and that other measures may relate better. In fact, Lijffijt et al (2004) also used the I_7 and reported similar findings but suggested that impulsivity may not be as severe in a non-pathological population and therefore less likely to associate with behavioural impulsivity. As many behavioural tests have been developed to assess pathological behaviour it is not surprising that their associations with self report measures are lacking. Further work needs to be undertaken to close this gap in research and improve associations between trait dependent and behavioural measures.

5.4.4 Associations between behavioural impulsivity and clock gene polymorphisms

When examining associations between the behavioural impulsivity variables and clock gene polymorphisms few relationships emerged. An ANOVA comparing *PER3* VNTR genotypes was significant before a Bonferroni correction and *post hoc* analyses identified a difference in mean stop latency between 4/5 and 5/5 genotypes, with the 5/5 genotype having a longer mean stop latency. Follow-up multinomial logistic and binary logistic regressions were marginally insignificant. As there was no significant difference between 5/5 and 4/4 genotypes it is unlikely that a relationship actually exists but a further study in a larger sample

would confirm this. Furthermore, the relationship is in the opposite direction to what would be expected.

Several studies have shown that mean stop latencies are longer in participants with high scores on impulsivity (Logan et al., 1997) and the *PER3* VNTR 5/5 genotype has previously be shown to be linked with extreme morningness (Archer et al., 2003). Morningness itself is associated with low impulsivity (Caci et al., 2005) and high conscientiousness (Jackson & Gerard, 1996, Gray & Watson, 2002, Cavellara & Giampietro, 2007, Deyoung et al., 2007, Randler, 2008, Tonetti et al, 2009), whereas eveningness has been linked to ADHD (Caci et al., 2009a). From this it could be concluded that individuals with the *PER3* VNTR 5/5 genotype would have shorter mean stop latencies than those with a 4/5 or 4/4 genotypes, rather than a longer one reported here.

Both *PER1* T2434C and *PER2* 10870 unfortunately lost their rarest homozygotes due to the smaller sample size in this study. This meant that comparisons between the homozygotes of these polymorphisms could not be carried out and this is where a relationship, if any, would have been evident.

In Chapter 4, an association was reported between self-report impulsivity and *CLOCK* rs11932595, before a Bonferroni correction. From this it was hypothesised that this polymorphism may also be associated to behavioural impulsivity but no such results were obtained. When taking into account the other results from this Chapter it is not surprising, as no relationship was found between the self-report and behavioural measures of impulsivity. It could be that this polymorphism is associated with a different construct of impulsivity than that measured by the GoStop task, or that the smaller sample size has resulted in a loss of any relationship.

Hypothesis 3 is partly confirmed due to the tentative association between mean stop latency and *PER3* VNTR, but further work is needed to identify whether this is a true association.

5.4.5 Limitations of the study

5.4.5.1 Use of the GoStop task

As self-report measures do not account for state dependent fluctuations it was felt necessary to include a behavioural task in the study to provide a comparison. As there are no conscientiousness behavioural tests and as the GoStop had provided a measure of impulsivity which is itself associated with conscientiousness, it was selected. Adding weight to this selection was a study that reported an association between the GoStop and conscientiousness (Edmonds et al., 2009). Furthermore, as the I_7 had been used to measure impulsivity it was felt that this behavioural test would provide a good comparison of self-report and behavioural measures. Despite this, it must be taken into account that the GoStop is not a direct measure of conscientiousness and is therefore a limitation to this study.

Additionally, due to time constraints, only one behavioural task was used. As behavioural tests measure more narrowly defined components of impulsive behaviour than trait-dependent measures such as the I_7, the selection of one test meant that only one construct could be examined, in this case response inhibition. This could be a reason for the lack of association between the two measures, as the I_7 measures broader constructs that may not directly relate to behavioural impulsivity. Indeed, Reynolds et al (2006) suggested that disparity in the two methods of assessing impulsivity may be due to differences in the scope of the definition of the dimension being measured as well as differences in the objectivity of measurement

approach. Behavioural measures are less susceptible to self perception bias and so provide a more objective measure than self-report questionnaires, and thus may lead to different results.

5.4.5.2 Use of a non-pathological population

The lack of associations seen between trait-dependent impulsivity and behavioural impulsivity in the present study may also be due to the use of a non-pathological population. Indeed, many of the authors of the behavioural paradigms available, including the GoStop, reported associations between trait-dependent and behavioural measures but within populations with clinical disorders such as ADHD and used this as evidence for construct validity of the measure (Dougherty et al., 2005). Additionally, all but one of the studies that found no significant associations (outlined in section 5.3.2) used the I_7 to assess trait-dependent impulsivity. This suggests that high trait impulsivity (as defined by the I_7) in the general population may not be extreme enough to reveal differences in inhibitory motor control (Lijffijt et al., 2004). The lack of associations may also be due to the participant selection process, as participants were selected for conscientiousness and not impulsivity this may have skewed the results, and not given a true indication of the relationships present. If an evaluation of these two measures were to be repeated in future, selecting for extremes of impulsivity provide a better comparison to previous results.

5.4.5.3 Statistical analysis

As the sample size reduced further from what it was in Chapter 4, many of the rare genotypes that already had a low frequency were reduced even further and in the case of *PER1* T2434C and *PER2* 10870, lost completely. This meant that comparisons of homozygotes were not possible and so relationships that may have been present could not be seen.

5.5 Conclusion

Hypotheses 1 and 2 outlined in section 5.1 were rejected as conscientiousness group membership nor impulsivity, as defined by the I_7, were associated with behavioural impulsivity. These results indicate that behavioural impulsivity as measured by the GoStop may represent a different construct to that of trait dependent impulsivity or impulsivity may not be severe enough to highlight any differences in response inhibition within the general population as opposed to a pathological one. This would also explain the fact that there was no association between behavioural impulsivity and conscientiousness but that in Chapter 3 there was a relationship between self-report impulsivity and conscientiousness. Furthermore, a tentative association between the *PER3* VNTR and mean stop latency was found before a Bonferroni correction leading to partial confirmation of hypothesis 3 but further work is needed in a larger sample to replicate these findings.

Chapter 6 Discussion

6.1 Introduction

Since the posthumous publication of data collected by Blake (1967), researchers have proposed links between diurnal preference and personality variables. Recent research suggests that morningness is negatively correlated with the personality variable impulsivity (Caci et al., 2005), which it is now generally agreed to be composed of several higher order dimensions: Neuroticism, conscientiousness and extraversion (Whiteside and Lynam, 2001). Other studies presented evidence of a relationship between conscientiousness and morningness (Jackson and Gerard, 1996, Gray and Watson, 2002, Cavallera and Giampietro, 2007, DeYoung et al., 2007, Randler, 2008; Tonetti et al., 2009), indicating that it may be this dimension of personality that is responsible for the reported association between impulsivity and morningness.

Several researchers have proposed links between clock gene polymorphisms and diurnal preference (see Chapter 1), but few have investigated possible associations of variability in these genes with personality parameters. Evidence has emerged in various studies that clock genes may be pleiotropic in nature (see Chapter 1), thus having roles outside of the circadian clock. The aims of this study were to investigate this possibility further by examining whether clock gene polymorphisms that had been shown to associate with diurnal preference also associated with personality traits. Additionally, relationships between diurnal preference and personality were also investigated. This chapter will discuss the results in relation to previous research and theories of personality as well as suggesting possible future research directions.

6.2 The challenges of personality research

Personality is a vast subject area of which various theories exist and numerous instruments have been developed to measure different aspects of these theories. This has led to a number of difficulties and challenges associated with personality research, which have been highlighted by this thesis.

From a trait-dependent perspective, a large number of self report questionnaires are available (see section 1.7.3 and 1.8.1) that enable researchers to measure various facets of personality within any given theory. There is still a debate over which theory of personality to adhere to, but with a general consensus that there are likely to be five factors. However, disagreements over what each factor represents are still ongoing. This has led to the emergence of various instruments developed from different ideas of what the five factors consist of. For example, Goldberg developed the IPIP while proposing the fifth factor as intellect, whereas Costa and McCrae developed the NEO-PIR with their fifth factor designated openness. These differences have led to difficulties in the comparison of results from different studies, as although the measures generally correlate with each other, they are not identical and researchers have had to take this into account when reporting their findings.

The results from Chapter 3 highlight this problem, as although conscientiousness was found to be correlated with diurnal preference in a number of studies (Jackson and Gerard, 1996, Gray and Watson, 2002, Cavallera and Giampietro, 2007, DeYoung et al., 2007, Randler, 2008; Tonetti et al., 2009), correlations with other dimensions of the five factor model differed. As each study used a different measure of the five factor model of personality it is

likely that this caused the disparity between the results. Moreover, different measures of diurnal preference were also used, adding a further confounding factor.

Prior to the emergence of the five factor model, Eysenck's theory of personality and its related instruments were used to investigate the relationship between diurnal preference and personality. A review by Tankova et al (1994) which examined individual differences in circadian rhythms over a 30 year period found that extraversion was most commonly associated with eveningness, with neuroticism and psychoticism found to be associated in a number of other studies. These findings were questioned because of Eysenck's expansion of his theory to include psychoticism, which also resulted in the development of the EPQ that followed on from the EPI. Most of the studies that found an association with extraversion used the EPI, and Tankova et al concluded that this relationship may be due to an association between impulsivity and eveningness, the facets of which were moved from the EPI extraversion dimension to the EPQ psychoticism dimension on revision of Eysenck's theory.

This is a prime example of the difficulties involved in personality research, particularly when determining which measure to use. The present study sought to limit confounding factors imposed by these problems by selecting measures that were widely used and psychometrically sound. Furthermore, by selecting two measures of personality, the I_7 and the NEO-FFI, it enabled a comparison to be made, although future studies should consider expanding their selections to utilise more than one method of assessing the five factor model.

Similar, if not greater difficulties come with the measurement of state-dependent or behavioural personality traits. The results from Chapter 5 showed no link between state and trait-dependent measures of impulsivity, or between state-dependent impulsivity and conscientiousness, suggesting that these instruments measure different constructs. Results

from previous studies have also been inconsistent (Lijiffit et al., 2004), suggesting that there is still a great necessity for future research to identify what exactly each instrument measures and how they relate to each other.

When taking these points into perspective it is easy to see why in Chapter 4 there were few relationships between clock gene polymorphism and personality. Due to the complexity of personality it is likely that variations in many genes contribute to a trait rather than just one polymorphism. And whilst an association was discovered, before a Bonferroni correction, between impulsivity, neuroticism and openness and *CLOCK* rs11932595, it is likely that this is one of a number of genetic variations that influence these personality traits.

Overall the results from this thesis have emphasized the challenges associated with research into personality and have provided a basis for further research by highlighting areas which are in need of clarification and which should be addressed by future studies.

6.3 Eysenck and the five factor model of personality

In Chapter 1, both Eysenck's model of personality, the five factor model and the apparent overlaps between the two were discussed. The use of the I_7 questionnaire in Chapter 3 revealed that impulsivity was most highly correlated with the agreeableness, conscientiousness and neuroticism dimensions of the five factor model of personality, with agreeableness and conscientiousness negatively correlated and neuroticism positively. This is consistent with Eysenck's theory, as before constructing his PEN model, impulsiveness was placed under the extraversion dimension but was moved to the psychoticism dimension after he identified four facets of impulsivity described in Chapter 1. He found that the facet of narrow impulsiveness was correlated with psychoticism and neuroticism, with psychoticism

having been shown to associate negatively with the agreeableness and conscientiousness dimensions of the five factor model (Costa and McCrae, 1985). The other three facets were incorporated into venturesomeness and sensation seeking and placed under the extraversion dimension.

Whereas Eysenck described his higher order traits as orthogonal, the dimensions of the five factor model have been consistently shown to be intercorrelated. This was confirmed in Chapter 3 where all of the dimensions were correlated with one or more of the other dimensions. Digman et al (1997) speculated that when neuroticism is reverse scored as emotional stability that the positive intercorrelations may be due to socially agreeable responding as high scores are more desirable than low scores. As neuroticism was not reverse scored in this study but intercorrelations were evident, the results appear to support the theory that the big five may be simplified further into more basic underlying traits, such as the higher order factors, alpha and beta, suggested by Digman et al (1997) which were later replicated by DeYoung et al (2002) and termed stability (conscientiousness, agreeableness, neuroticism reversed) and plasticity (openness and extraversion). Indeed, the intercorrelations seen in the current study are similar to those reported by DeYoung et al (2002), the only differences being that conscientiousness and not agreeableness was correlated to openness, and this may be explained by their use of the more comprehensive NEO-PIR to measure the five factor model.

6.4 Biological links between diurnal preference and personality

In Chapter 3, morningness was shown to be positively correlated with conscientiousness and agreeableness, and negatively with openness, respectively. Similarly, a recent study found an association between the metatrait stability, but not plasticity, and morningness (DeYoung et

al., 2007), which implies that neuroticism reversed is also linked to morningness. This was not seen in the present study and associations between neuroticism and morningness in other research have been inconsistent (DeYoung et al., 2007). DeYoung et al (2007) suggested that these sporadic results may be due to only the variance that neuroticism shares with agreeableness and conscientiousness being associated with morningness and therefore the metatrait level may be the best way of exploring the relationship between personality and diurnal preference. However, this does not take into account the association between eveningness and openness reported in this and one other study (Cavallera and Giampietro, 2007).

A biological theory based on the metatraits, where stability is associated with variability in serotonergic function (DeYoung et al., 2002) and plasticity in dopaminergic function (DeYoung et al., 2005) has also been proposed. Several studies have shown evidence to support this theory. Manuck et al (1998) found a negative correlation between serotonin and neuroticism in men but not women, as well as a positive correlation with conscientiousness in men. Brummet et al (2008) found conflicting results where higher levels of serotonin associated with lower levels of neuroticism in females, finding the opposite in males. The authors suggested that the differences may be due to the method of assessment of prolactin response (a measure of central nervous system serotonergic activity) where the former study used peak prolactin response to fenfluramine and the latter, tryptophan.

This theory becomes more interesting in light of the interaction of these systems with the circadian clock. Serotonin modulates the entrainment of circadian rhythms to light in both Drosophila (Yuan et al., 2005) and mammals (Edgar et al., 1993, Miller et al., 1996) and therefore probably in humans as well. When taking into account the association between stability and morningness, this could lead us to believe, therefore, that individual differences

in serotonin function could be reflected in individual differences in circadian rhythm (Deyoung et al., 2007).

On a biological level, a case can also be made for a relationship between openness and eveningness. Openness has been linked to interactions between the dorsolateral prefrontal cortex of the brain and the dopaminergic system (Deyoung et al., 2005). A role for dopamine has also been proposed in the circadian clock. Hampp et al (2008) based their theory on previous findings that the monoamine oxidase A (*Maoa*) promoter was regulated by the clock components BMAL1, NPAS2, and PER2 in mice, and that a mutation in *Per2* led to reduced expression and activity of MAOA, resulting in increased levels of dopamine and altered behaviour. They proposed, therefore, that in humans, phosphorylation of PER2 by glycogen synthase kinase 3β (GSK-3β) favours accumulation of PER2 in the nucleus. There, it enhances the NPAS2/BMAL1 mediated transcription of *Maoa* and more enzyme is generated, ending up in the inner mitochondrial membrane where MAOA then degrades dopamine. The authors suggest that increased PER2 may lead to lower dopamine levels and therefore a more depressed mood state.

Wolfenstein and Trull (1997) investigated associations, in a non-pathological population, between scores on two depression inventories and the NEO-PIR and found that high scorers on depression also had elevated levels of openness to experience. This result was not confirmed in the current study, but this may be due to differences in the measures used. In clinical populations, SAD has been linked to high scores on the openness dimension in comparison to major depression (Bagby et al., 1996) and bipolar disorder (Jain et al., 1999). Moreover, a link between SAD and diurnal preference has also been reported (Johansson et al., 2003) as well as between depression and eveningness (Drennan et al., 1991, Hidalgo et al., 2009), further supporting a possible link between openness and eveningness.

6.5 Effects of clock gene polymorphisms outside of the circadian clock

Chapter 1 discussed the pleiotropic nature of clock genes and the results from Chapters 4 and 5 go some way towards supporting this. A large number of studies have found associations between the genes examined in this study and a variety of psychological and physical illnesses. In fact, one third to one half of variation in personality is due to genetic factors (Ebstein et al., 2000). In Chapter 4, associations were seen before a Bonferroni correction between *CLOCK* rs11932595 and neuroticism, openness and impulsivity. In Chapter 5, an association was seen between the *PER3* VNTR 5/5 genotype and mean stop latency on the GoStop task, although this was also not significant after a Bonferroni correction.

The finding that openness was the largest predictor of *CLOCK* rs11932595 genotype is all the more interesting when taking into account research in mice that suggests that the *Clock* gene regulates dopamine function (McClung et al., 2005). Mice lacking a functional *Clock* gene displayed an increase in cocaine reward and in the excitability of dopamine neurones in the midbrain ventral tegmental area (VTA). It also resulted in an increased expression of tyrosine hydroxylase (TH) (a rate limiting enzyme in the dopamine system) as well as changes in several genes known to regulate activity in the VTA. Although the mechanism underlying the regulation of VTA dopamine system by *Clock* is not known, the authors suggested that because mRNA expression in the VTA exhibits a circadian rhythm (Weber et al., 2004) and an enhancer element containing an E-box lies upstream of the TH gene (Yoon and Chikaraishi, 1994), that *Clock* may act as a transcriptional repressor directly regulating TH transcription.

As few studies have researched *CLOCK* rs11932595, little is known about the functional implications of the polymorphism but it is possible that different genotypes affect the structure and function of the resulting protein so that when it binds to E-box elements it may result in the differential expression of the TH gene.

Impulsivity (Crockett et al., 2008) and neuroticism (Takano et al., 2007) conversely, have been linked to the serotonin system, which may also be modulated by the circadian clock as previously discussed. There have been no reports on links between the *CLOCK* gene and serotonin, but these results suggest that it may also interact with this system.

The relationship between *PER3* VNTR genotype and mean stop latency on the GoStop is also worth discussing because of the conservative nature of the Bonferroni correction. Chapter 1 described a number of studies that have shown that the *PER* VNTR plays a role outside of the circadian clock. A comparison of 4/4 and 5/5 *PER3* VNTR genotypes showed differences in executive functioning in the early morning following sleep deprivation but not during wakefulness (Viola et al., 2007). The current study also did not find differences between 4/4 and 5/5 genotype but did between 5/5 and 4/5 genotypes, where 5/5 genotypes had longer mean stop latencies. A comparison of 4/4 and 5/5 genotypes produced a result close to significance before a Bonferroni correction and this may be apparent in a larger sample, although if any relationship is evident it would be expected to show most strongly between homozygotes. Additionally, the relationship was not in the direction that would be expected as one study has shown that longer mean stop latencies are linked to high impulsivity (Logan et al., 1997) although this has not been confirmed by subsequent research (Lijffit et al., 2004). High impulsivity is in turn associated with eveningness (Caci et al., 2005). The 5/5 polymorphism has been linked to morningness (Archer et al., 2003) and so in theory individuals with this genotype would have shorter mean stop latencies if any.

These results could mean that variation in the *PER3* VNTR could lead to differences in response inhibition between individuals. Another possibility, is that the association has occurred by chance due to multiple testing or through selection by conscientiousness score, therefore it is necessary to repeat the experiment in another population not selected in such a way.

Despite many studies reporting associations between clock genes and systems outside of the circadian clock, relatively little is known about how they exert these effects. In relation to the serotonin and dopamine systems, some of the modulation seems to occur through connections between the SCN and other brain regions (McClung, 2007). An example of this is the circadian rhythm in noradrenergic neuronal activity which appears to be regulated by an indirect projection from the SCN to the locus coeruleus (Aston-Jones et al., 2001). Moreover, circadian gene expression outside of the SCN, in these areas, may also contribute to either involvement in local oscillators or to involvement in non-rhythmic processes. In order to elucidate the exact mechanisms that underlie these biological processes, more research is necessary.

6.6 Limitations of the study

There are several limitations of this study that may have some effect on the results and therefore need to be taken into account. The most important ones are listed below:

6.6.1 Recruitment

For this study, an internet-based survey was selected as the main method of recruitment because it is cost effective, less error prone, and allows for greater automation and control

over the data. In comparison with other methods of recruitment, such as telephone and paper based surveys, several criticisms of internet-based measures have arisen. Firstly, they have lower completion rates than alternate methods (Williams et al., 2000). This was seen in the current study, where many participants failed to complete the entire survey so that their data had to be omitted from the final sample. This may therefore have created a bias against certain personality traits. Additionally, sampling biases pose a problem as discussed previously (see section 3.4.4), as well as the quality of the data (Kraut et al., 2004). The anonymous nature of the internet means that subjects can participate with malicious intent; inputting random information or completing a survey under the guise of someone else. Attempts were made to combat this in the present study by removing data where obviously fabricated contact details had been entered and by allowing only one submission per IP address to stop duplicate entries. This, however, did not stop multiple entries from different computers, and so inspection of the data was necessary to remove duplicate entries where the same contact details were apparent.

6.6.2 Self-report questionnaires

The measures used in the present study were found to be both reliable and valid (see Chapter 3), but issues still surround the use of psychological scales in research. Response biases are one such problem, with social desirability receiving most attention in the personality literature. This type of response bias is believed to fall into two dimensions; Self-deception where a participant believes their answer to be true and impression management, where a participant consciously attempts to portray themself in a positive way. This can distort the data, leaving accurate analysis difficult. To combat this, Eysenck and Eysenck added items to their questionnaire that enabled them to determine whether the participant was telling the truth, they termed this a 'lie scale'. None of the measures used in the current study

incorporated such items but one theory known as the candour hypothesis, suggests that participants who complete questionnaires online relative to paper and pencil administration are less likely to respond in a socially desirable fashion (Buchanan, 2000). This is thought to be due to the impersonal nature of the internet and reduced contact with the experimenter which decreases 'experimenter effects'. Research findings though have been mixed, with evidence found for and against this hypothesis (Joinson, 1999, Risko et al., 2006).

In another form of response bias, a participant has the tendency to either choose one position on a particular scale or to agree with every statement put to them. Researchers have tried to avoid this by adding a mixture of positive and negative statements and reverse scoring certain items as is the case with the present study, where all questionnaires used this approach.

6.6.3 Behavioural test

Only one behavioural test was selected to use as part of the study, the GoStop, mainly due to time constraints. This meant that only the response inhibition component of impulsivity was tested, rather than the whole construct, which may have led to the lack of association found between self-report and behavioural measures (see section 5.4.3). It was necessary to use a behavioural test as they provide a more objective, performance-based form of assessment than the self-report questionnaires previously described. They are also more sensitive to state-dependent fluctuations (Dougherty et al., 2003).

When conducting a behavioural test, it is necessary to try and eliminate or keep constant extraneous variables in order to avoid them confounding the results. For this reason, the same experimenter was used for each test, with the same instructions read to each participant. Also, ideally the tests should have been carried out in the same room at the same time of day for

each participant but due to availability of both the rooms and the participants this was not possible. For this reason time of test was added as a control variable. Additionally, the same computer was not used for all tests due to equipment failure.

Limitations of behavioural tests also include a participant's understanding of the task. If the task is not complex, it is less likely that IQ would influence the results. The GoStop is such a test, with clear instructions read out to each participant as well as flash cards being displayed. Despite this, there may still have been a small proportion of subjects who did not understand the task fully. but the criteria imposed when examining the data should have removed any outliers. Furthermore, participants were excluded under specific conditions (see Chapter 2) but were not screened for alcohol or stimulant use, which may have also affected their performance.

6.6.4 Association studies

The ability of association studies to detect and characterise genes that contribute to common traits is controversial (Hattersley and McCarthy, 2005). Small studies rarely find the correct result (Ioannidis, 2003) and initial positive findings are not confirmed (Ioannidis et al., 2001, Lohmueller et al., 2003). Conversely, large studies increase the danger of false positives as they are more sensitive to small bias effects such as population stratification (Marchini et al., 2004).

A major problem with association studies are genotyping error rates, as these not only decrease the statistical power of the study, but also increase the chance of an incorrect association being reported. This, coupled with publication bias in favour of positive results (Ioannidis et al., 1998), means that studies affected by genotyping error are likely to be

disproportionately represented in published work (Hattersley & McCarthy, 2005). The steps taken to limit genotyping error in the current study can be seen in section 4.5.3.2.

Lack of replication of positive results may also occur due to a number of other factors such as the fact that biases may vary between studies and also low power due to the efficiency of the sampling strategy (i.e sampling the entire distribution instead of the tail ends) (Colhoun et al., 2003). The emergence of false positive results may be due to multiple testing where only the positive results are reported.

6.6.5 Prevalence of sleep disturbance

The mean sleep disturbance value for all three studies was higher than the proposed cut off of 5, with males higher in each. This means that a large number of participants reported sleep disturbance and this in turn may have affected the results, although where possible this factor was controlled for. Previous research in the United States reported that 71% of University students are poor sleepers (Hicks et al., 2001), whereas another study in Hong Kong revealed that 57.5% of University students had increased sleep disturbance (Suen et al., 2008). It has been suggested that these problems may be due to stress, in addition to poor sleep hygiene (or habits), such as shorter sleep durations due to later bed times. Where shorter sleep duration is more likely to be caused by social activities rather than academic demands (Suen et al., 2008).

6.7 Future directions

The study reported here is inevitably limited by the financial and time limitations of a PhD studentship. However, the preliminary data presented here could be used to identify possible areas for future study.

6.7.1 Improvements to the present study

Through examining the limitations of the study it is possible that improvements could be made if the research was repeated in a modified format informed by the experience and results of the current study.

6.7.1.1 Diurnal preference and personality

A number of changes could be made to the first study to improve both the design and outcome. If time and finances had allowed, a greater cross-section of the general public could have been recruited rather than the largely university-based sample that was collected. This would enable the results to be generalised more easily to the population as a whole. Furthermore, a greater proportion of participants could complete the paper-based questionnaire, allowing a comparison between the two methods and possibly greater completion rates. Additionally, if a longer period of time was set aside for recruitment and finances allowed greater advertising a larger sample size could have been achieved, increasing the power of the study. Due to the size of the sample in this study, exclusion of participants based on PSQI scores would have led to a dramatic decrease in the number of participants. In a larger population, and in one more reflective of the general public, it would have been possible to exclude these participants without it having a decrease in the sample size.

6.7.1.2 Analysis of genetic data

Sample size was also an issue in the second study as the original selection of 10% extremes had to be expanded to 20% extremes due to the low number of respondents. The extremes in a

larger sample size would not have had to be expanded and associations may have been detected that were not in the smaller sample. Therefore, as in section 6.6.1.1, with more time and sufficient funding, a larger sample size would have been achieved. Moreover, in order to control for genotyping errors, a proportion of the samples could be sequenced to confirm their designations.

6.7.1.3 Analysis of behavioural data

In order to test whether associations existed between the behavioural and self-report measures of impulsivity, it would be necessary to select participants for impulsivity rather than conscientiousness. Also, the study could be improved by increasing the number of self report and behavioural measures used. The links between conscientiousness and behavioural impulsivity could be tested further also by increasing the number of behavioural tests used as conscientiousness may be related to a different facet of impulsivity than the one measured by the GoStop.

6.7.2 Future research

A number of interesting results have been obtained in this study which may justify and inform future work in this area.

6.7.2.1 Associations between openness, diurnal preference and *CLOCK* rs11932595

As the link between diurnal preference and openness has not been well established in previous research, but was evident in the current study, it would be interesting to select for 10% extremes of this trait. Genotyping of *CLOCK* rs11932595 could then be undertaken to

examine whether there were differences in high and low scoring groups, as was indicated in the current study. *PER2* polymorphisms that may lead to altered levels of PER2 protein in the nucleus, could also be identified and genotyped. Because the *PER* genes are expressed widely including in peripheral leukocytes (Archer et al., 2008), this could be investigated non-invasively in human volunteers. Furthermore, in order to elucidate the biological mechanism underlying any relationship found, central dopaminergic function (due to the established link between openness and the dopamine system) could be measured either via the non-invasive method of spontaneous eye blink rate (Karsen, 1983) or through collection of blood samples and measurement of dopamine beta-hydroxylase activity (Nagatsu and Udenfriend, 1972). This would help clarify whether there were differences in dopamine levels between high and low groups as well as between different genotypes.

6.7.2.2 The PER3 VNTR and response inhibition

The tentative association between mean stop latency on the GoStop and the PER VNTR 5/5 genotype could be investigated further within a population selected for by PER3 genotype. A comparison of PER VNTR 5/5 and 4/4 genotypes could be undertaken by both groups completing the GoStop task at their preferred time of day in order to confirm whether an association exists.

6.8 Conclusions

The research undertaken in this thesis contributes to the understanding of personality in relation to diurnal preference as well as suggesting candidate genes which may act as markers

to personality traits. Future research, as outlined above, will be needed to further clarify associations between the circadian clock and personality.

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8.0 Appendices

8.1 Appendix A Recruitment material

Information sheet for participants: Diurnal preference, clock gene polymorphisms, and personality

Different people have different daily rhythms, the sleep-wake cycle being a perfect example. Some people are able to rise early in the morning whereas others find it difficult. The length of sleep needed can also vary between individuals. Whether you have a morning or evening preference has been linked to genes which are involved in regulating peoples' body clocks (clock genes) in previous studies. Another study has shown that morning or evening preference is linked to certain personality traits. The aim of this study is to investigate whether there is a direct link between human behaviour and clock genes.

The University of Surrey Ethics Committee has reviewed the protocol for this study and granted ethical approval In order to take part you would need to complete a questionnaire to determine whether you have a morning or evening preference and provide a mouth swab. Depending on the results of the questionnaire you may then be invited to complete additional questionnaires and participate in behavioural tests at the University. We will then use your sample to look selectively at the genes that are involved in generating the biological clock, determining how they differ between you and other subjects and then relating them to the questionnaires you have completed. Any such results will be treated as highly confidential and you will not be identified at any time. Your privacy will be fully protected at all times. We will not use the sample for any other purpose without your full consent. You are able to withdraw from the study at any time.

If you require further information in relation to this study please contact me:

Alex Hogben 01483 683341

Aletrnatively, you may contact:

Dr. Malcolm von Schantz (supervisor) 01483 686468
Dr. Jason Ellis (supervisor) 01483 686936
Dr. Simon Archer (supervisor) 01483 686408

Consent form

Volunteers consent form: Diurnal preference, clock gene polymorphisms, and personality

I, the undersigned, voluntarily agree to take part in the study.

I have been given a full explanation by the scientific investigators of the nature and purpose of the study and understand that my contribution will be to leave a buccal swab and complete the relevant questionnaires.

I have been given the opportunity to question the investigators on all aspects of the study, and have understood the advice and information given as a result.

All documentation held on a volunteer is in the strictest confidence and complies with the data protection act (1998). I agree that I will not seek to restrict the use to which the result of the study may be put.

I confirm that I have read and understood the above and freely consent to participating in this study. I have been given adequate time to consider my participation and agree to comply with the instructions and restrictions of the study.

This form will be provided on computer disc or tape if required.

.....
Signature

.....
Name

.....
Date

Email

Healthy volunteers aged 18-39 required

My name is Alex Hogben and I am a PhD student in the School of Biomedical and Molecular Sciences. I am currently looking for volunteers to take part in a study which hopes to find a link between morning or evening preference, human behaviour and genetics.

It will involve filling out some online or paper based questionnaires. If selected, you will be invited to complete some online behavioural tests and donate mouth swab and saliva sample.. You will be contacted at a later date if you have been selected to take part in the behavioural tests. Compensation will be given for your time and inconvenience

If you are willing to take part please go to

www.surrey.ac.uk/SBMS/research/personality

Feel free to contact me at a.hogben@surrey.ac.uk for more information.

Poster

Healthy volunteers aged 18-39 required

We are currently looking for volunteers to take part in a study, which hopes to find a link between morning or evening preference, clock genes and human behaviour.

All you need to do is go to :

www.surrey.ac.uk/SBMS/research/personality

and complete some questionnaires

Then, if you are lucky enough to be selected you will be invited to complete some short online behavioural tests and donate a mouth swab and saliva sample. Compensation will be offered for your time and inconvenience.

Please contact Alex Hogben by email at a.hogben@surrey.ac.uk for more information.

a.hogben@surrey.ac.uk
www.surrey.ac.uk/SBMS/research/personality

a.hogben@surrey.ac.uk
www.surrey.ac.uk/SBMS/research/personality

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www.surrey.ac.uk/SBMS/research/personality

Advert

GENES AND BEHAVIOUR

Healthy volunteers aged 18-39 required

We are looking for volunteers to take part in a study of the link between morning or evening preference, human behaviour and genetic differences. Initially this will only involve filling in some online questionnaires.

For more information and to take part go to:
http://www.surrey.ac.uk/SBMS/research/personality/
Or for paper based copies please contact Alex Hogben at Email: a.hogben@surrey.ac.uk Tel: 01483683341

Address: SBMS, University of Surrey, Guildford GU2 7XH
This PhD is funded by the Surrey Sleep Research Centre

HORNE-OSTBERG QUESTIONNAIRE

SUBJECT CODE:_____ **DATE:**_____

INSTRUCTIONS

a) Please read each question very carefully before answering.

b) Answer all questions.

c) Answer questions in numerical order.

d) Each question should be answered independently of others. Do **NOT** go back and check your answers.

e) For some questions, you are required to respond by placing a cross alongside your answer. In such cases, select **ONE** answer only.

f) Please answer each question as honestly as possible. Both your answers and results will be kept in strict confidence.

QUESTION 1

Considering your own feelings, at what time would you get up if you were entirely free to plan your day?

Time: .....

QUESTION 2

Considering only your own feelings, at what time would you go to bed if you were entirely free to plan your day?

Time: .....

QUESTION 3

If there is a specific time you have to get up in the morning, to what extent are you dependent on being woken up by an alarm clock?

a. Not at all dependent [ ]

b. Slightly dependent [ ]

c. Fairly dependent [ ]

d. Very dependent [ ]

QUESTION 4

Assuming adequate environmental conditions, how easy do you find getting up in the morning?

a. Not at all easy [ ]

b. Slightly easy [ ]

c. Fairly easy [ ]

d. Very easy [ ]

QUESTION 5

How alert do you feel during the first half hour after having woken in the morning?

a. Not at all alert [ ]

b. Slightly alert [ ]

c. Fairly alert [ ]

d. Very alert [ ]

QUESTION 6

How is your appetite during the first half hour after having woken in the morning?

a. Not at all good [ ]

b. Slightly good [ ]

c. Fairly good [ ]

d. Very good [ ]

QUESTION 7

During the first half hour after having woken in the morning, how tired do you feel?

a. Very tired [ ]

b. Slightly tired [ ]

c. Fairly refreshed [ ]

d. Very refreshed [ ]

QUESTION 8

When you have no commitments the next day, at what time do you go to bed compared to your usual bedtime?

a. Seldom or never later [ ]

b. Less than one hour later [ ]

c. 1-2 hours later [ ]

d. More than 2 hours later [ ]

QUESTION 9

You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him is between 0700 and 0800h. Bearing in mind nothing else but your own inclinations, how do you think you would perform?

a. Would be on good form [ ]

b. Would be on reasonable form [ ]

c. Would find it difficult [ ]

d. Would find it very difficult [ ]

QUESTION 10

At what time in the evening do you feel tired and in need of sleep?

Time: .....

QUESTION 11

You wish to be at your peak for a test which you know is going to be mentally exhausting and lasting for two hours. You are entirely free to plan your day, when would you do this task?

a. 0800 – 1000 [ ]

b. 1100 – 1300 [ ]

c. 1500 – 1700 [ ]

d. 1900 – 2100 [ ]

QUESTION 12

If you went to bed at 2300h at what level of tiredness would you be?

a. Not at all tired [ ]

b. A little tired [ ]

c. Fairly tired [ ]

d. Very tired [ ]

QUESTION 13

For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Will you:

a. Wake up at the usual time and not go back to sleep [ ]

b. Wake up at the usual time and doze [ ]

c. Wake up at the usual time and go back to sleep [ ]

d. Wake up later than usual [ ]

QUESTION 14

One night you have to remain awake between 0400 and 0600h. You have no commitments the next day. Which suits you best:

a. Not to go to bed until 0600h [ ]

b. Nap before 0400h and sleep after 0600h [ ]

c. Sleep before 0400h and nap after 0600h [ ]

d. Sleep before 0400h and remain awake after 0600h [ ]

QUESTION 15

You have to do hours physical work. Which hours would you prefer to do it between:

a. 0800 – 1000 [ ]

b. 1100 – 1300 [ ]

c. 1500 – 1700 [ ]

d. 1900 – 2100 [ ]

QUESTION 16

You have decided to engage in some physical exercise. A friend suggests that you do this between 2200 and 2300h twice a week. How do you think you would perform:

a. Would be on good form [ ]

b. Would be on reasonable form [ ]

c. Would find it difficult [ ]

d. Would find it very difficult [ ]

QUESTION 17

Suppose that you can choose your own work hours, but had to work five hours in the day. Which five consecutive hours would you choose:

Hours: .....

QUESTION 18

At what time of day do you feel your best?

Time: .....

QUESTION 19

One hears of "morning" and "evening" types. Which do you consider yourself to be?

a. Morning type [ ]

b. More morning than evening [ ]

c. More evening than morning [ ]

d. Evening type [ ]

The Pittsburgh Sleep Quality Index (PSQI)

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

During the past month,

1. When have you usually gone to bed? _____

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

3. When have you usually gotten up in the morning? _____

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

5. During the past month, how often have you had trouble sleeping because you...	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
a. Cannot get to sleep within 30 minutes				
b. Wake up in the middle of the night or early morning				
c. Have to get up to use the bathroom				
d. Cannot breathe comfortably				
e. Cough or snore loudly				
f. Feel too cold				
g. Feel too hot				
h. Have bad dreams				
i. Have pain				
j. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason(s):				
6. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
9. During the past month, how would you rate your sleep quality overall?	Very good (0)	Fairly good (1)	Fairly bad (2)	Very bad (3)

The depression scale of the Hospital anxiety and depression scale.

The next questions are about how you feel. Read each item and tick the box next to the reply that comes closest to how you have been feeling in the past weeks. Don't take too long over your replies, your immediate reaction will probably be more accurate than a long thought-out response.

DI still enjoy the things I used to enjoy:	
Definitely as much	0
Not quite so much	1
Only a little	2
Hardly at all	3

DI can laugh and see the funny side of things:	
As much as I always could	0
Not quite so much now	1
Definitely not so much now	2
Not at all	3

DI feel cheerful:	
Not at all	3
Not often	2
Sometimes	1
Most of the time	0

D I feel as if I am slowed down:	
Nearly all the time	3
Very often	2
Sometimes	1
Not at all	0

D I have lost interest in my appearance:	
Definitely	3
I don't take as much care as I should	2
I may not take quite as much care	1
I take just as much care as ever	0

D I look forward with enjoyment to things:	
As much as I ever did	0
Rather less than I used to	1
Definitely less than I used to	2
Hardly at all	3

D I can enjoy a good book or radio or TV program:	
Often	0
Sometimes	1
Not often	2
Very seldom	3

Impulsiveness scale of the Impulsiveness, venturesomeness, empathy-7 questionnaire

Please answer YES (Y) or NO (N) to each question

1	Do you often buy things on impulse?	( )
2	Do you generally do and say things without stopping to think?	( )
3	Do you often get into a jam because you do things without thinking?	( )
4	Are you an impulsive person?	( )
5	Do you usually think carefully before doing anything	( )
6	Do you often do things on the spur of the moment?	( )
7	Do you mostly speak before thinking things out?	( )
8	Do you often get involved in things you later wish you could get out of?	( )
9	Do you get so `carried away' by new and exciting ideas, that you never think of possible snags?	( )
10	Do you need to use a lot of self-control to keep out of trouble?	( )

11	Would you agree that almost everything enjoyable is illegal or immoral?	( )
12	Are you often surprised at people's reactions to what you do or say?	( )
13	Do you think an evening out is more successful if it is unplanned or arranged at the last moment?	( )
14	Do you usually work quickly, without bothering to check?	( )
15	Do you often change your interests?	( )
16	Before making up your mind, do you consider all the advantages and disadvantages?	( )
17	Do you prefer to `sleep on it' before making decisions?	( )
18	When people shout at you, do you shout back?	( )
19	Do you usually make up your mind quickly?	( )

PLEASE CHECK THAT YOU HAVE ANSWERED ALL THE QUESTIONS

NEO-FFI

Below are a number of characteristics which may or may not apply to you. Please indicate your degree of agreement with each one according to the following scale.

Disagree strongly	**Disagree**	**Disagree more than agree**	**Agree more than disagree**	**Agree**	**Agree strongly**
1	**2**	**3**	**4**	**5**	**6**

1	I am not a worrier.	N-
2	I like to have a lot of people around me.	E
3	I don't like to waste my time daydreaming.	O-
4	I try to be courteous to everyone I meet.	A
5	I keep my belongings clean and tidy.	C
6	I often feel inferior to others.	N
7	I laugh easily.	E
8	Once I find the right way to do something, I stick to it.	O-
9	I often get into arguments with my family and co-workers.	A-
10	I'm pretty good at pacing myself so as to get things done on time	C
11	When I'm under a great deal of stress, sometimes I feel like I'm going to pieces	N
12	I don't consider myself to be especially "light-hearted".	E-
13	I am intrigued by the patterns I find in nature and art.	O
14	Some people think I'm selfish and egotistical.	A-

15	I am not a very methodical person.	C-
16	I rarely feel lonely or blue.	N-
17	I really enjoy talking to people .	E
18	I believe letting students hear controversial speakers can only confuse and mislead them.	O-
19	I would rather cooperate with others than compete with them.	A
20	I try to perform all the tasks assigned to me conscientiously.	C
21	I often feel tense and jittery.	N
22	I like to be where the action is.	E
23	Poetry has little or no effect on me.	O-
24	I tend to be cynical and sceptical of others' intentions.	A-
25	I have a clear set of goals and work towards them in an orderly fashion.	C
26	Sometimes I feel completely worthless.	N
27	I usually prefer to do things alone.	E-
28	I often try new and foreign foods.	O
29	I believe that most people will take advantage of you if you let them.	A-
30	I waste a lot of time before settling down to work.	C-
31	I rarely feel fearful or anxious.	N-
32	I often feel as if I'm bursting with energy.	E
33	I seldom notice the moods or feelings that different environments produce.	O-
34	Most people I know like me	A
35	I work hard to accomplish my goals.	C
36	I often get angry at the way people treat me.	N

37	I am a cheerful, high-spirited person...	E
38	I believe we should look to our religious authorities for decisions on moral issues	O-
39	Some people think of me as cold and calculating.	A-
40	When I make a commitment, I can always be counted on to follow through	C
41	Too often, when things go wrong, I get discouraged and feel like giving up.	N
42	I am not a cheerful optimist.	E-
43	Sometimes when I am reading poetry or looking at a work of art, I feel a chill or wave of excitement.	O
44	I'm hard-headed and tough-minded in my attitudes.	A-
45	Sometimes I'm not as dependable or reliable as I should be.	C-
46	I am seldom sad or depressed.	N-
47	My life is fast-paced.	E
48	I have little interest in speculating on the nature of the universe or the human condition.	O-
49	I generally try to be thoughtful and considerate.	A
50	I am a productive person who always gets the job done.	C
51	I often feel helpless and want someone else to solve my problems	N
52	I am a very active person.	E
53	I have a lot of intellectual curiosity.	O
54	If I don't like people, I let them know.	A-
55	I never seem to be able to get organised.	C-
56	At times I have been so ashamed I just wanted to hide.	N

57	I would rather go my own way than be a leader of others.	E-
58	I often enjoy playing with theories or abstract ideas.	O
59	If necessary, I am willing to manipulate people to get what I want.	A-
60	I strive for excellence in everything I do.	C

Self-report questionnaire

1) Name:_____

2) Address:_____

3) Telephone Number:_____

4) Date of birth:_____

5) Gender:_____

6) Email address:_____

6) Are you currently taking any medication? If yes, please specify:_____

_____

7) Do you do night shift work?_____

8) Have you been diagnosed with a sleep disorder?_____

9) Do you suffer from a chronic illness?_____

8.3 Appendix C Published manuscript