

# Circadian Adaptation in Offshore Shift Workers Returning to Day Life at Home

by

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Previous research has shown that subjects working 12h shift schedules (18.00-06.00h) offshore for 2 weeks adapt to the night shift. However, if adaptation occurs, shift workers will be out of synchrony when they return home to day life with consequent problems of poor night sleep and reduced daytime alertness.

A total of 17 subjects working two shift schedules were studied offshore; 18.00-06.00h and 19.00-07.00h 2-3 weeks night of shift. Differences were observed in circadian adaptation to the night shift, and this was reflected by differences in sleep duration and quality that was observed between the two groups. Sleep appeared to be worse in those working 19.00-07.00h compared to the 18.00-06.00h shift schedule. These differences between the two shift schedules may be due to differences in morning light exposure countering circadian adaptation to night shift work.

This project investigated the effect of light treatment to hasten circadian adaptation in offshore shift workers by determining circadian status (using the timing of the rhythm of urinary 6-sulphatoxymelatonin) and sleep (by actigraphy and sleep diaries). Light was administered with a portable light box (Litebook<sup>®</sup>). After completion of their night shift (day 1) subjects wore specialised sunglasses until 13.00h. On day 2 subjects wore sunglasses until 13.00h and then received light treatment for 1h. For the following 3 days the sunglasses and light treatment were scheduled an hour earlier each day. The light regimen was timed to phase advance the melatonin rhythm.

The findings effectively demonstrated the importance of appropriately timed bright light to hasten circadian adaptation upon returning home onshore. A significance difference ( $p < 0.05$ ) in actigraphic sleep duration onshore was observed when comparing the no light treatment leg against the light treatment leg. In conclusion, light treatment appropriately timed can be beneficial to aid circadian adaptation after working a night shift.



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AMI	Acute myocardial infarction
aMT6s	6-sulphatoxymelatonin
AVP	Arginine vasopressin
AWL	Actiwatch-L
BMI	Body mass index
CBT	Core body temperature
CHD	Coronary heart disease
CPM	Counts per minute
CI	Confidence interval
CIU	Clinical investigation unit
CSS	Charcoal stripped urine
CT	Circadian time
CVD	Coronary heart disease
DEC. H	Decimal hour
DGDW	Double glass-distilled water
DLMO	Dim light melatonin onset
DLMOff	Dim light melatonin offset
DSPS	Delayed sleep phase syndrome
EEG	Electroencephalogram
EMG	Electromyogram
GHT	Geniculohypothalamic tract
Glu	Glutamate
H	Hours
HDL	High density lipoprotein
HÖ	Horne Östberg
HSE	Health and Safety Executive
IGL	Intergeniculate leaflet
IR	Insulin receptor
KSS	Karolinska sleepiness scale
LD	Light-dark
LED	Light emitting diode
LDL	Low density lipoprotein
NAS	N-acetylserotonin



NMDA	N-methyl d-aspartate
NPY	Neuropeptide Y
NREM	Non-rapid eye movement
OIM	Offshore installation manager
OR	Odds ratio
PRC	Phase response curve
PTA	Pretectal area
QC	Quality control
REM	Rapid eye movement
RGC	Retinal ganglion cells
RHT	Retinal hypothalamic tract
RIA	Radioimmunoassay
SCN	Suprachiasmatic nuclei
SD	Standard deviation
SEM	Standard error of the mean
SPZ	Subparaventricular zone
TAG	Triacylglycerol
Tmin	Temperature minimum
Tau	Free-running period
VIP	Vasoactive intestinal polypeptide
5-HT	Serotonin
Yrs	Years

## 1. INTRODUCTION

### 1.1. Biological rhythms

The earth's rotation around its axis generates daily environmental rhythms. Life on earth has evolved mechanisms to allow our body's physiology to adjust to, and predict these environmental changes. Biological rhythms make up the essential components of these physiological homeostatic mechanisms. Simple organisms, such as *Neurospora*, through to complex organisms such as humans all show robust biological rhythms (review, Ronneberg et al., 2003). Biological clocks impose a structure that enables organisms to change behaviour priorities in relation to the time of day, month or year.

Biological rhythms are classified by their periodicity, which is often related to a particular environmental cycle. Examples of biological rhythms are ultradian rhythms, which are short cycles, such as the rapid eye movement (REM) cycle in sleep; they have a period of less than 24 hours. Infradian rhythms are biological rhythms with a period longer than 24 hours. Examples include the oestrous cycle and the human menstrual cycle. Circannual rhythms are cycles occurring approximately once a year e.g. bird migration and hibernation. Circadian rhythms are those that occur over a 24 hour period. The term "circadian" comes from the Latin *circa*, "around", and *diem*, "day", meaning literally "about a day".

### 1.2. Circadian rhythms

In most organisms the possession of a circadian clock allows the adjustment of physiological mechanisms to the varying demands of the solar cycle. Circadian rhythms are endogenous, that is they are internally generated, but they can also be affected exogenously (by external factors) e.g. light. The environmental factors that entrain or synchronise a rhythm to a given periodicity are referred to as zeitgebers, German for 'time-giver' (review,

Arendt, 1998). The most potent entraining agent of the circadian system for all organisms is the light-dark environment at dawn and dusk (review, Ronneberg and Foster, 1997). Under constant conditions, and in the absence of time cues, the periodicity of the circadian clock and its output circadian rhythms is slightly longer than 24h, approximately 24.2-24.3h in sighted individuals (Middleton and Stone, 1996; Czeisler et al., 1999; Wright et al., 2001). The period is genetically determined and referred to as *tau* ( $\tau$ ). Examples of different  $\tau$ 's include ~23.25h for patients with the advanced phase syndrome (Jones et al., 1999) and longer  $\tau$ 's seen in the totally blind subjects (Lockley et al., 1997).

### **1.2.1. Circadian entrainment**

Entrainment of the circadian timing system to light-dark cycles is known as photoentrainment (Freedman et al., 1999). Human circadian rhythms are synchronised to the 24h day mostly by light dark cycles (Czeisler, 1995) but to a lesser extent by other time cues e.g. social cues, sleep time and exercise (Mistlberger and Skene, 2005).

If the endogenous period of circadian rhythms is longer than 24h, synchronisation requires a phase advance of the clock by this amount; if less than 24h, the clock must be delayed each day to remain entrained. In blindness, in continuous darkness or very dim light conditions below a particular light intensity (<10 lux) and in the absence of other major time cues, human circadian rhythms free run (Broadway and Arendt, 1986; Kennaway and Van Dorp, 1991; Middleton and Stone, 1996; Lockley et al., 1997; Arendt, 1998b; Hack et al., 2003; Wright et al., 2005). The length of *tau* therefore relates to the amount that is required to phase shift each day.



### 1.2.2. Circadian rhythm characteristics

The amplitude, period, duration and acrophase are important characteristics of a circadian rhythm, illustrated in Figure 1-1.

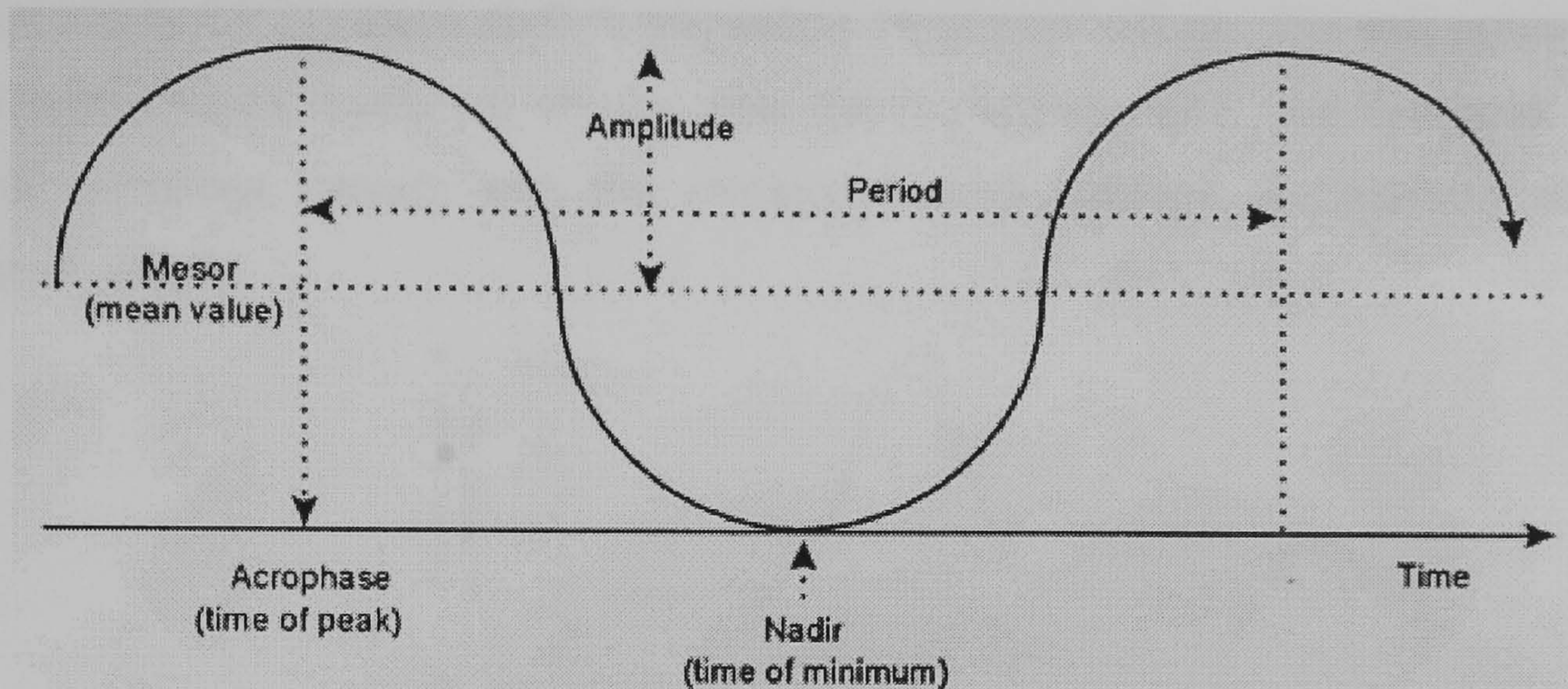


Figure 1-1: Schematic diagram of the characteristics of a circadian rhythm

The mesor is the average value around which the variable oscillates. The amplitude is the difference between the peak (or trough) and the mean value of the wave. The period is the time elapsed for one complete oscillation or cycle. The acrophase is the time at which the peak of a rhythm occurs and the nadir is the lowest value of an oscillatory function. These are all important characteristics of a circadian rhythm (Shanahan et al., 1997).

Circadian rhythms, illustrated diagrammatically in Figure 1-2, of plasma melatonin, core body temperature (CBT), subjective alertness, task performance (reaction time in secs) and triacylglycerol (TAG) are from humans being held in constant routine conditions (i.e. controlled light, temperature, posture, activity, meals, fluid intake, awake). The essence of the constant routine is therefore to reduce or eliminate all periodic changes in behaviour, in addition to maintaining a constant environment (review, Duffy and Dijk, 2002). Lighting must be controlled due to studies showing that light exposure to levels of 100 lux or more will substantially phase shift circadian rhythms (Zeitzer et al., 2000). Secondly posture and activity should be



controlled as this may shift the human circadian clock (Buxton et al., 1997) as well as modifying rhythm amplitudes and/or profiles.

The peak in the melatonin rhythm, shown by the dotted line, and the low-point of the temperature rhythm are within 1h of each other. The low point of the temperature rhythm is shortly after the melatonin peak, and the peak in TAG coincides closely with the melatonin peak (review, Rajaratnam and Arendt, 2001).

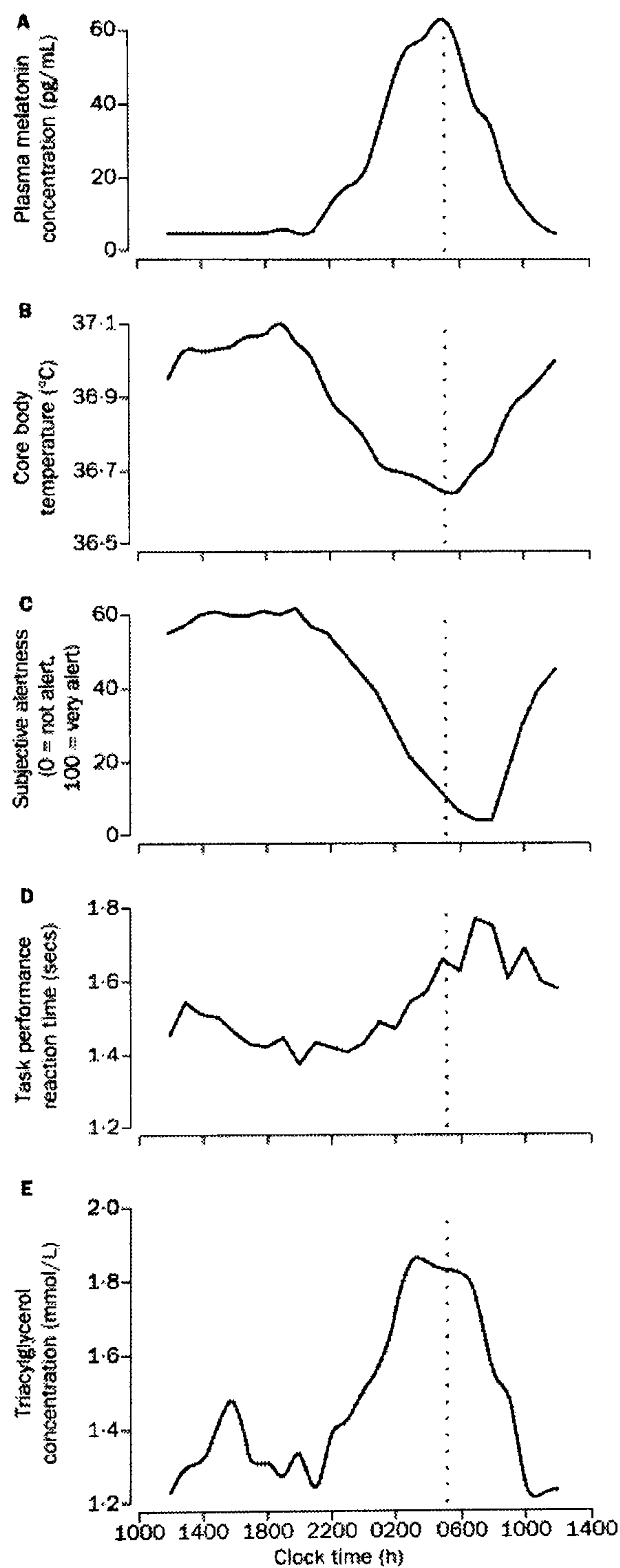


Figure 1-2: Examples of circadian rhythms (review, Rajaratnam and Arendt, 2001).



### 1.3. Circadian physiology

The fundamental components of the human circadian timing system consist of the eye, providing photoreceptors for light entrainment, the suprachiasmatic nuclei (SCN), the central pacemaker and output parameters, of which one is melatonin production from the pineal gland. These are schematically represented in Figure 1-3.

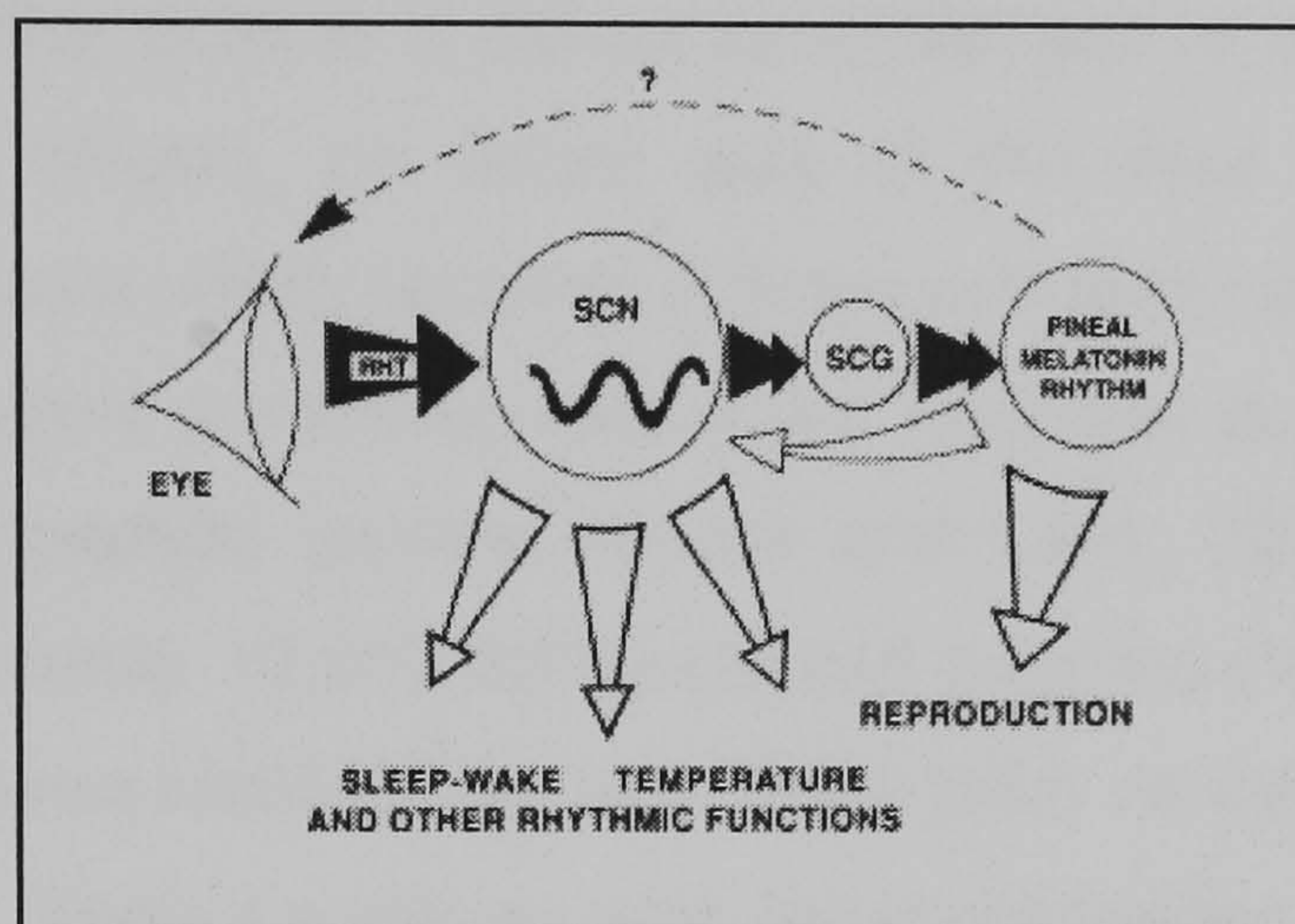


Figure 1-3: Output pathways of the SCN, (review, Arendt, 1995).

#### 1.3.1. Location of the clock

By careful lesioning in the frontal part of the hypothalamus Stephan and Zucker (1972) identified a small cluster of cells known as the SCN. Loss of the SCN abolished the oestrous cycle and circadian rhythms in behaviour such as drinking and locomotion (Stephan and Zucker, 1972).

Many strategies have been used to study the function of the clock, one of these includes ablation of the SCN, which has been shown to abolish rhythms *in vivo* (Ralph and Menaker, 1988; Tousson and Meissl, 2004). The host SCN is ablated, which results in loss of rhythmicity. Some rhythms can then be restored by the implantation of a donor clock (Ralph and Lehman, 1991). Transplantation of the SCN, enables some disrupted rhythms to be restored (Ralph and Menaker, 1988). For example, rhythmicity of a hamster with a lesioned SCN can be restored with an SCN graft, but the period of the restored rhythm is determined by the donor SCN tissue, so that the recipient



hamster exhibits the  $\tau$  of the donor hamster, at least in locomotor activity (Ralph and Menaker, 1988).

### **1.3.2. Suprachiasmatic nuclei**

The central pacemaker, or 'clock', is located in the SCN of the hypothalamus and this generates circadian rhythms (Moore and Eichler, 1972; Stephan and Zucker, 1972). The SCN is a paired structure and is situated immediately above the optic chiasm, on either side of the third ventricle. It has 2 subdivisions, the core which receives primary and secondary visual afferents, and contains neurons producing vasoactive intestinal polypeptide (VIP) and gastrin releasing peptide (review, Moore and Leak, 2002). Each nucleus contains approximately 10,000 tightly packed neurons (Van Esseveldt et al., 2000). VIP has been identified as one of the main neurotransmitters of SCN neurons (review, Reghunandanan and Reghunandanan, 2006). The shell largely surrounds the core, and receives input from non-visual sources and contains neurones producing arginine vasopressin (AVP) and calretinin (Leak and Moore, 2001).

The SCN of the hypothalamus is the dominant circadian pacemaker in the mammalian brain, controlling the rest-activity cycle and virtually all other circadian functions (Stephan and Zucker, 1972), e.g. melatonin and cortisol rhythms, to provide a foundation for the successful elaboration of adaptive sleep and waking behaviour (Moore et al., 2002). Independent circadian oscillators exist in most cells and are coordinated by the SCN (review Kalsbeek et al., 2006).

### **1.3.3. Clock genes**

The molecular clock of mammals is composed of three period genes and two cryptochrome genes and these are thought to form the primary components of the auto regulatory negative feedback loop that constitutes the molecular clock of mammals (Albrecht and Eichele, 2003).



This negative feedback mechanism is illustrated in Figure 1-4. CLOCK (yellow) and BMAL1 (pink) are proteins of the positive limb and drive the expression of *Per*, *Cry* and *Rev-erba* genes in the nucleus. PER (red) and CRY (light green) proteins in the nucleus inhibit CLOCK/BMAL1 action by a yet unknown mechanism and thereby down-regulate their own expression and that of *Rev-erba* (dark green). When REV-ERB $\alpha$  protein is absent, *Bmal1* (and possibly also *Clock*) genes are derepressed and hence transcribed to produce new CLOCK/BMAL1 transcription factors that reinitiate a new circadian cycle. Clock proteins are post translationally modified; casein kinase I $\epsilon$  (CKI $\epsilon$ ), for example, phosphorylates PER2. Hyperphosphorylation of PER2 decreases its stability and thus promotes its degradation.

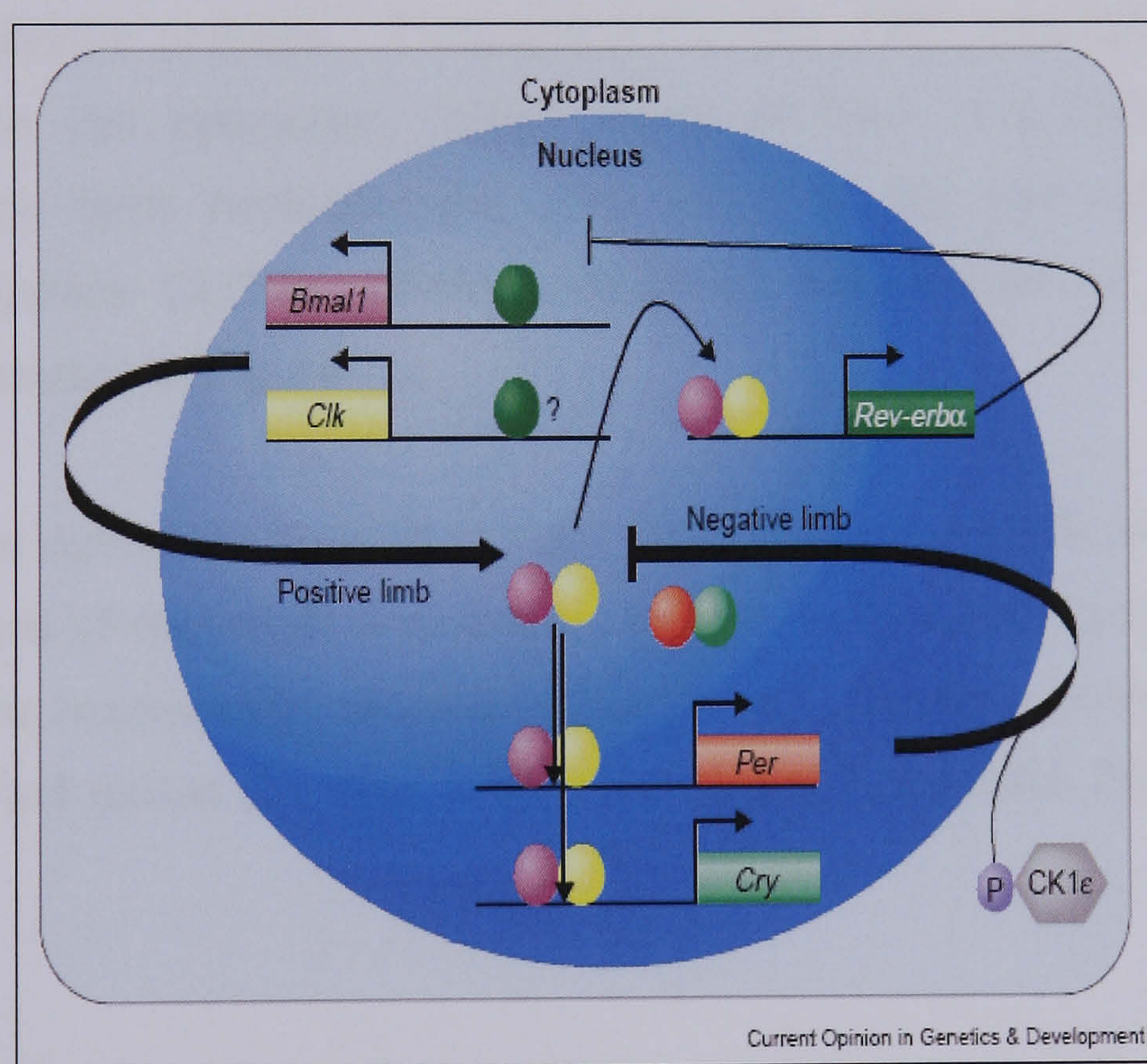


Figure 1-4: Molecular clock pathways (Albrecht and Eichele, 2003)



A typical circadian cycle would begin with activation of *Per* (and *Cry*) transcription by CLOCK/BMAL1 in the early morning. Transcript levels peak around noon and protein levels in the cytoplasm reach a peak approximately 2 hours later. PER shuttles between the cytoplasm and the nucleus. In the cytoplasm, it is degraded following hyperphosphorylation and in the nucleus it complexes with CRY and thereby blocks CLOCK/BMAL1 function resulting in termination of *Per* and *Cry* transcription. At some point when too much PER is degraded in the cytoplasm, PER concentration in the nucleus is too low to keep up negative feedback and the cycle reinitiates (Albrecht and Eichele, 2003).

The process begins when CLOCK and BMAL1 proteins dimerize to drive the transcription of the *Per* (*Per1*, *Per2*, and *Per3*) and *Cry* (*Cry1* and *Cry2*) genes. In turn, *Per* and *Cry* are translocated to the cytoplasm and translated into their respective proteins. Throughout the day, PER and CRY proteins rise within the cell cytoplasm. When levels of PER and CRY reach a threshold, they form heterodimers, feed back to the cell nucleus and negatively regulate CLOCK: BMAL1 mediated transcription of their own genes, as illustrated in Figure 1-4.

Light exposure during the subjective night produces phase shifts of locomotor activity rhythms (Pittendrigh and Daan, 1976). It is widely accepted that resetting of the mammalian circadian clock by light involves acute induction of *Per1* and *Per2* genes (Dunlap, 1999; Lowrey and Takahashi, 2000).

#### **1.4. Circadian photoreception**

Light enters the eye, and this photic information is relayed to the SCN via neural pathways.



### 1.4.1. The eye

Light enters the eye via through the cornea; this is an external transparent surface. Once the light has traveled through the cornea, it passes through the aqueous humour before reaching the back of the retina. As illustrated in Figure 1-5. The retina is a highly differentiated neuroectodermal tissue. Macroscopic examinations show that the retina consists of two distinct regions (the macular area, which is specialized for vision) and the peripheral retina. Microscopic examinations show that the macular area can be further sub-divided into the fovea and the foveola (review, Bonnel et al., 2003). Light is then focused onto the retina, the light sensitive layer of the eye, which contains light detecting cells called photoreceptors.

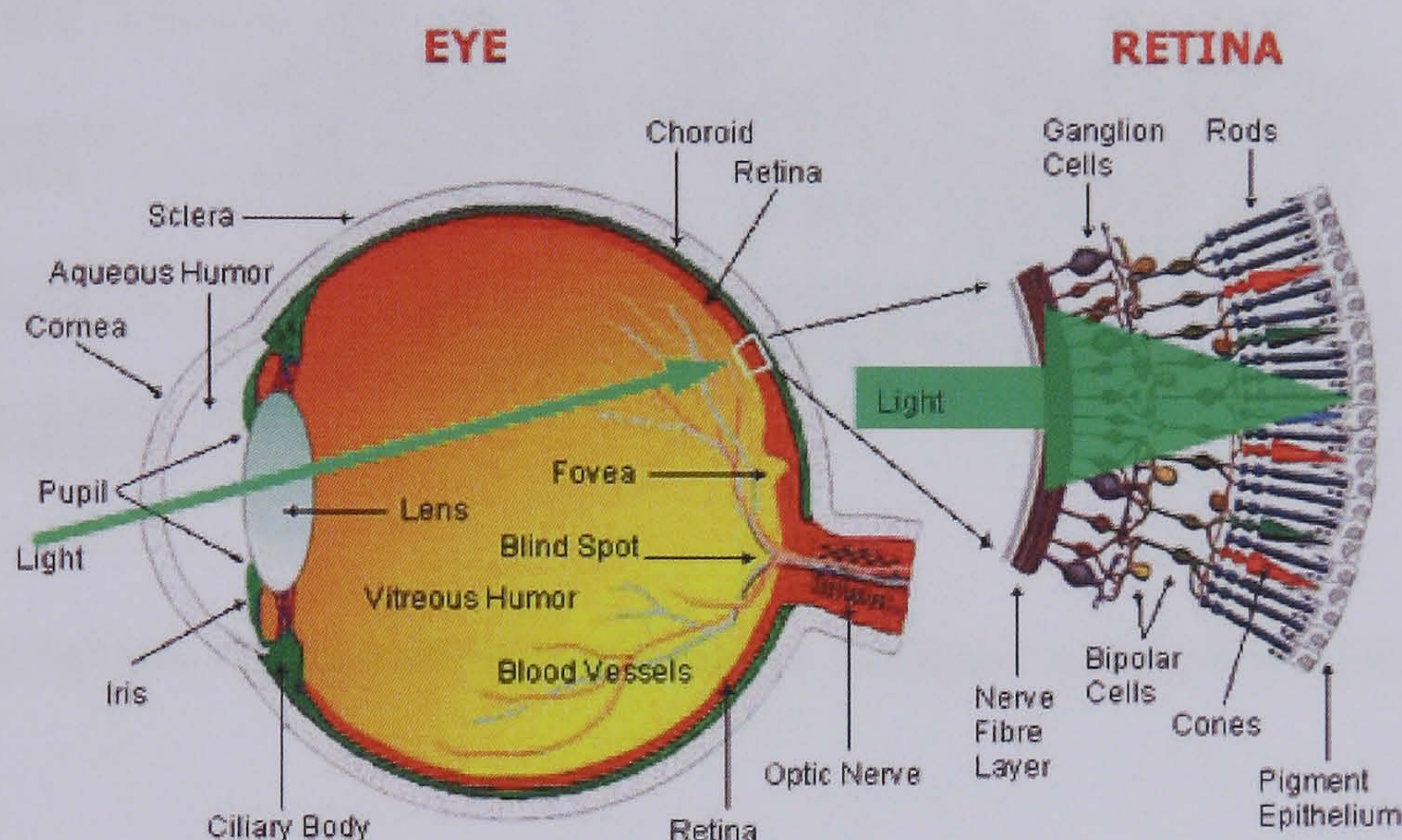


Figure 1-5: Anatomy of the eye ([www.astrosurf.com/re/eye\\_anatomy.jpg](http://www.astrosurf.com/re/eye_anatomy.jpg)).

### 1.4.2. Visual photoreception

The mammalian retina contains two types of photoreceptors, which are responsible for image formation, and these are known as rods and cones (Figure 1-6).

Cones are adapted to photopic (daytime) conditions. Blue cones are short wavelength absorbers (S-cones), green cones are medium wavelength absorbers (M-cones) and red cones are long wavelength absorbers (L-



cones). The difference in their absorption properties allow us to perceive colour. Rods are sensitive to dim light, allowing night vision. Rods are the major component of the peripheral retina, with a maximal density around the perimacular region (Curcio et al., 1990).

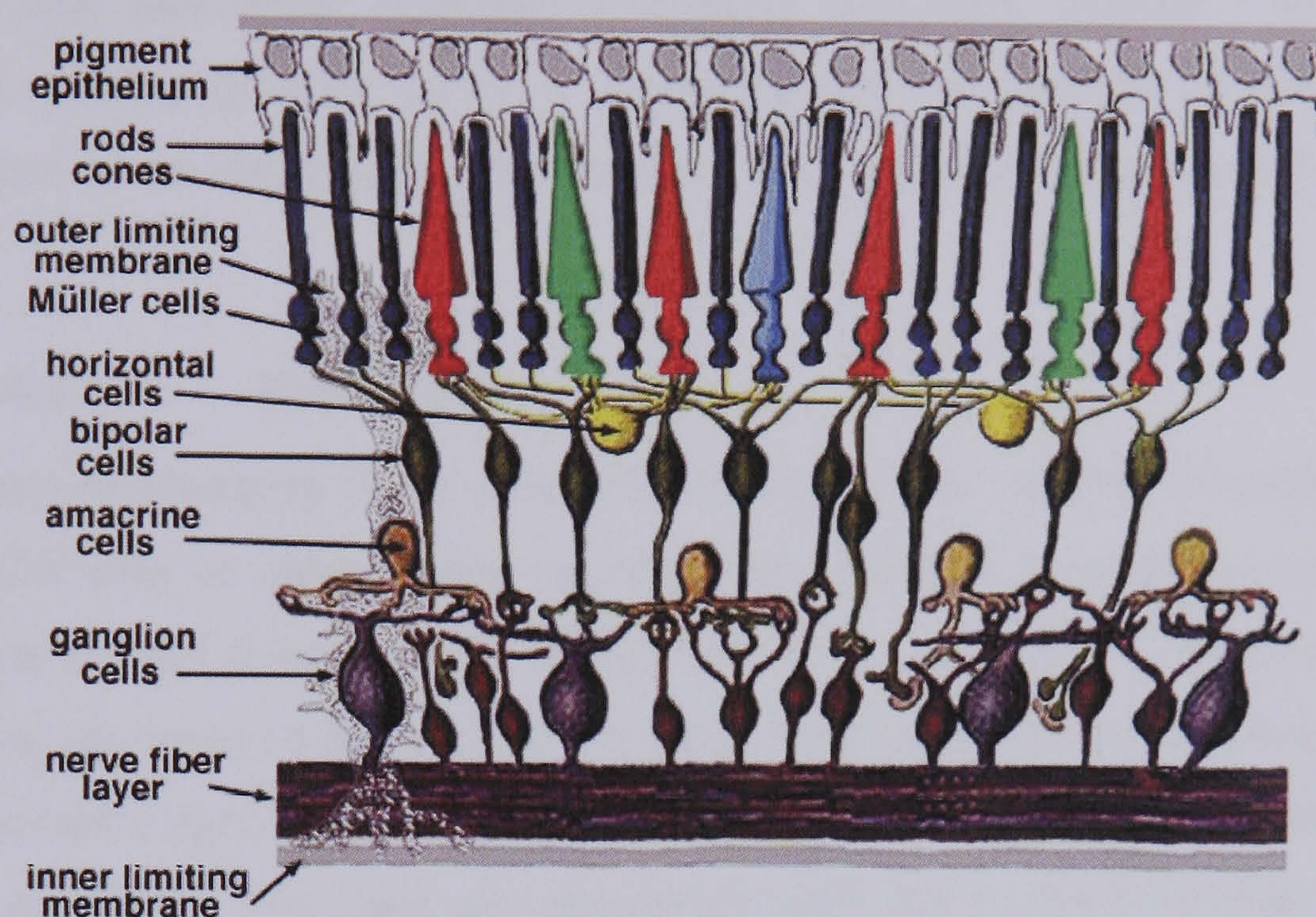


Figure 1-6: Schematic diagram of the human retina, (adapted from Foster, 2005)

### 1.4.3. Retina to the SCN

The rods and cones of the outer retina detect light, with the cells of the inner retina providing the initial stages of the visual processing, before ganglion cells (RGC) convey information to the brain via optic nerves. A small number of mammalian retinal ganglion cells act as photoreceptors for regulating certain non-image forming photo responses (Roenneberg and Foster, 1997; Hattar et al., 2002). These intrinsically photosensitive RGC express the novel photopigment melanopsin (Gooley et al., 2001; Hattar et al., 2002).

The photic information detected in the eye is conveyed from the RGC to the SCN primarily via a monosynaptic pathway, the retinohypothalamic tract (RHT) (Klein and Moore, 1979). The RHT which projects into the ventral



SCN is made up of a distinct, relatively small set of RGCs (Moore and Eichler, 1972). The primary neurotransmitter of the RHT is glutamate (review, Hannibal, 2002). The first stage of light detection involves the absorption of a photon by 11-cis-retinaldehyde, and the photoisomerization of this molecule to the all-trans state. This allows the opsin to trigger a phototransduction cascade that ultimately changes the cell's electrical activity. All opsin-vitamin-A photopigments have a characteristic absorption profile, which allows them to be identified on the basis of their spectral responses to light.

#### **1.4.3.1. Melanopsin**

Melanopsin expressing RGC relay information to the master circadian clock in the SCN and to other brain regions that mediate a range of non-visual responses to light (Hattar et al., 2002; Gooley et al., 2003). The non-visual effects are reported to be wavelength dependent such that short wavelength monochromatic light (e.g. 460 nm) is more effective than 555 nm light (long wavelength light). The data are consistent with the hypothesis that the non-visual effects are in part mediated by the recently discovered photopigment melanopsin which is expressed in light sensitive RGC (Brainard et al., 2001; Thapan et al., 2001).

Melanopsin is known to have a peak spectral sensitivity at approximately 480 nm that is distinct from the peak sensitivity of the rods and cones but it remains unclear to what extent the short-wavelength cones, with a peak sensitivity at 420 nm, contribute to these non-visual effects (Qiu et al., 2005). Melanopsin has some of the characteristics of invertebrate photopigments and it has been proposed that melanopsin is a bistable pigment. This implies that it has photosensory functions at shorter wavelengths and the ability to isomerise to regenerate bleached chromophore when subsequently exposed to longer wavelength light (Melyan et al., 2005).



#### **1.4.4. Intergeniculated leaflet and geniculohypothalamic tract**

Studies have indicated the presence of a large number of neurotransmitters in the SCN (Scott and Rusak, 1996; Hardeland et al., 2006). Two of the major incoming pathways that have been identified innervating the SCN; include the RHT and the geniculohypothalamic tract (GHT) (Sadun et al., 1984; Johnson et al., 1989).

The intergeniculated leaflet (IGL) is characterised by a population of neuropeptide Y (NPY) neurons coexisting with GABA containing neurones that project to the SCN core. The geniculohypothalamic tract (GHT) is a projection from the IGL to the SCN. The IGL also receives input from the SCN (Moore and Speh, 1994). The integrity of the GHT is not necessary for photic entrainment of circadian rhythms (Pickard et al., 1987) although lesions of the GHT cause change in circadian rhythmicity by slowing rate of entrainment after phase shift, reducing the magnitude of phase advances and reducing the free-running period under constant light conditions (Johnson et al., 1989).

##### **1.4.4.1. Glutamate**

The signalling pathway from the retina to the SCN comprises the activation of photoreceptors in the retina, glutamate release in the SCN from terminals of the RHT and subsequent resetting of the pacemaker. Light causes the release of glutamate, which initiates a signal transduction cascade in SCN neurones (Golombek et al., 2004).

Electrophysiology has also provided evidence that glutamate excites the SCN neurons (Bos and Mirmiran, 1993). It is thought that glutamate, released by the RHT, acts on N-methyl d-aspartate (NMDA) receptors on postsynaptic cells in the SCN (review, Hannibal, 2002).

The RHT also projects to other regions of the brain, such as the lateral hypothalamic area, the anterior hypothalamic area and the retrochiasmatic area (Johnson et al., 1988). The retinal ganglion cells projecting to the SCN also project to the intergeniculate leaflet (IGL) of the thalamus, pretectal area (PTA), subparaventricular zone (SPZ) and ventrolateral preoptic nucleus of the hypothalamus (Pickard, 1985).

#### **1.4.5. Y-aminobutyric acid**

GABA (Y-aminobutyric acid) is the major transmitter in the SCN and GABA is found in at least half of all pre-synaptic terminals in the SCN (Hardeland et al., 2006). With an abundance of GABA and GABA receptors in the SCN, this suggests a prominent role for this transmitter (Moore and Eichler, 1972). Administration of GABA agonists causes phase shifts of behaviour during the day and alters photic regulation of circadian systems during the night (Ralph and Menaker, 1989) thus highlighting its importance as an endogenous neurotransmitter.

#### **1.4.6. Vasointestinal peptide**

The anatomical organization of vasointestinal peptide (VIP) and its receptors provided early indications that this peptide may be important for circadian function. Studies in the rat revealed that the SCN had large amounts of VIP and VIP receptor-containing neurons (Roberts et al., 1980; Besson et al., 1986; Vertongen et al., 1998). These VIP-neurons are primarily located in the ventrolateral aspect of the nucleus (Abrahamson and Moore, 2001). Neurons in this region receive retinal input from the RHT and express both VIP and GABA (Kalsbeek et al., 2006).



### **1.5. Pineal gland**

The pineal gland produces the hormone melatonin. The pineal gland is a small neuroendocrine gland located between the mesencephalon and the diencephalon of the brain. It consists mainly of pinealocytes and its normal weight in humans is approximately 50-200 mg (Arendt, 1995).

The influence of the pineal gland on the circadian system seems to be more important in lower vertebrates than in mammals (Arendt, 1998b). The very earliest indication that the pineal gland contained a biologically active substance came from work showing that bovine pineal extracts applied to frog skin could produce a skin lightening response (McCord and Allen, 1917). In non-mammalian vertebrates, the melatonin rhythm is necessary for clock function; in several species pinealectomy results in attenuation of free running daily rhythms, and rhythmic melatonin infusions restore rhythmicity (Vanecek, 1998). Pinealectomy abolishes the rhythm of circulating melatonin in mammals, including humans (Arendt, 1995). Pinealectomy also abolishes the ability of the organism to respond to changing day length in photoperiodic species and suitable melatonin administration is able to replace lost time cues (Arendt, 1998b).

In humans the main functions of the pineal gland are transmission of information concerning day length or photoperiod via melatonin for the organisation of seasonal responses, transmission of information concerning phase and strength of the daily light-dark cycle for appropriate synchronisation of the circadian system. The pineal gland also has effects on physiological and behavioural rhythms such as sleep, temperature and reproduction (Arendt, 1995).

The pineal gland is connected to the SCN indirectly by the sympathetic nervous system.



### 1.5.1. Melatonin synthesis

Melatonin is synthesised from dietary tryptophan, which is first converted to 5-hydroxytryptophan by tryptophan-5-hydroxylase, and then into 5-hydroxytryptamine (5-HT, serotonin) by 5-HTP-decarboxylase. 5-HT is converted to N-acetylserotonin (NAS) by serotonin-N-acetyltransferase (AANAT), this is the rate limiting step of melatonin synthesis. NAS is converted to melatonin by hydroxyindole-O-methyltransferase (Axelrod and Weissbach, 1960; Klein, 1985). The pathway of melatonin synthesis is illustrated in Figure 1-7.

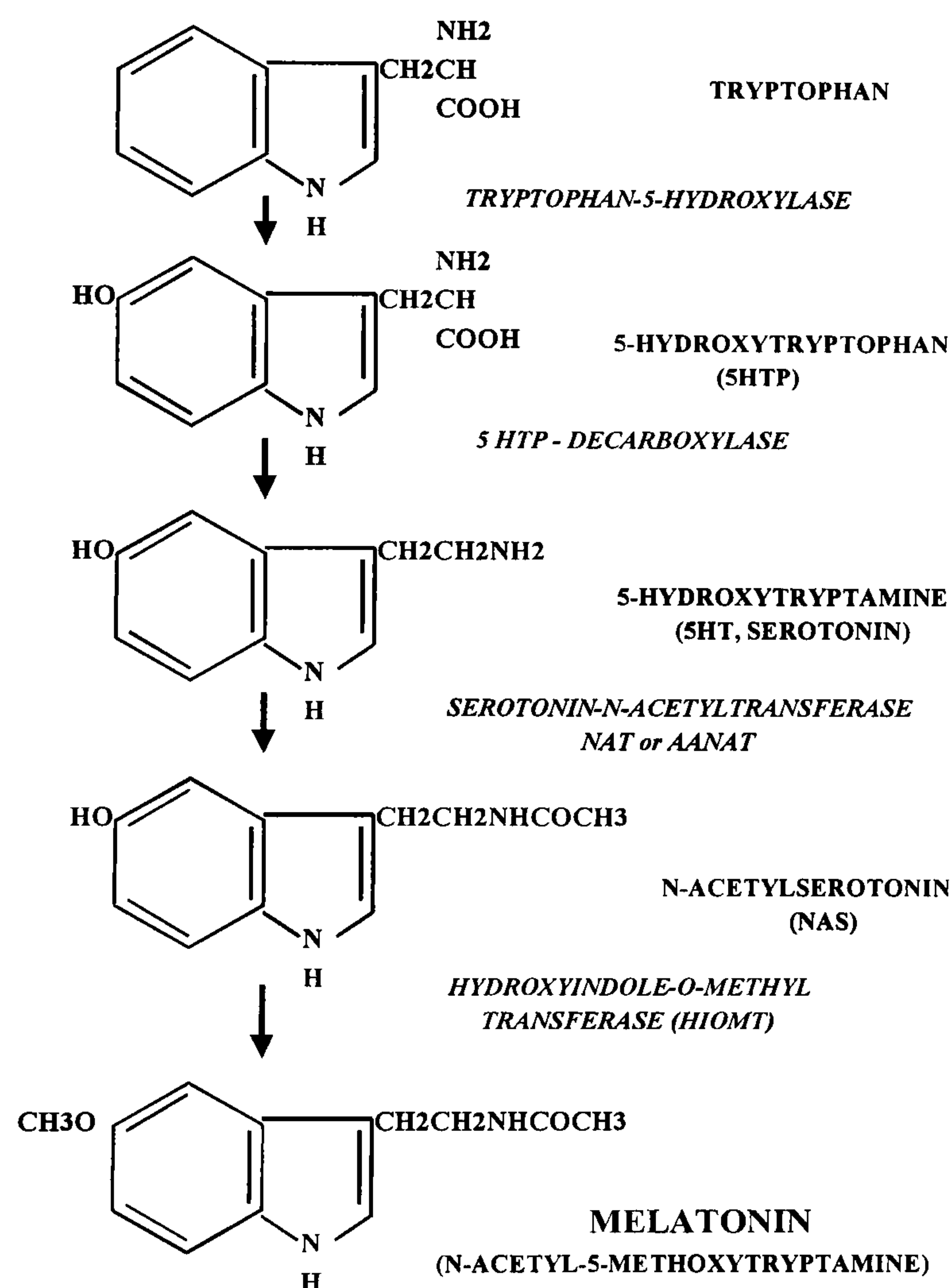


Figure 1-7: Synthesis pathway of melatonin (www.endocrine-source.com)

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Melatonin is often referred to as the 'hormone of the night' as its synthesis is inhibited by light and it is normally produced during the dark phase. The secretion of melatonin from the pineal is probably the most direct peripheral link to the central circadian clock (review, Arendt, 2005). Melatonin produced

in the pineal gland acts as an endocrine hormone since it is released into the blood and more recently reported to diffuse into the cerebrospinal fluid (CSF) (Tricoire et al., 2002).

### **1.5.2. Melatonin rhythm**

In most species, including humans (in the right circumstances), melatonin secretion is related to the length of the night, the longer the night, the longer the duration of secretion (Arendt, 1998b). The pattern of melatonin secretion changes with day length so that the duration of melatonin secretion can be used as a seasonal time cue by photoperiodic species such as sheep and hamsters (Arendt, 1998b).

### **1.5.3. Melatonin receptors**

Melatonin is a lipophilic molecule which readily crosses the blood-brain barrier, and lacks any group which would be significantly ionized at physiological pH. It is a rather flexible molecule with a number of atoms around which rotation is possible, with the indole ring forming a plane of symmetry. For such a small and simple molecule, melatonin shows remarkably high affinity binding to its receptors (review, Sudgen et al., 2004). Melatonin receptors have been identified and characterised in a number of tissues by in-vitro autoradiography and conventional binding assays using [<sup>125</sup>I] iodomelatonin (I-MEL) as a ligand (Vanecek, 1998).

The structural motif of a melatonin receptor consists of 7 membrane spanning domains connected by a series of extracellular and intracellular loops. The ability of melatonin to bind to, activate, and modulate its own receptors depends on the interaction of melatonin with specific amino acids and/or domains. There are two principal melatonin receptors (MT<sub>1</sub>, MT<sub>2</sub>) which have been discovered. MT<sub>1</sub> and MT<sub>2</sub> are both G-protein coupled receptors. MT<sub>1</sub> is expressed in the SCN of the hypothalamus and cardiac



vessels and MT<sub>2</sub> receptors are mainly found in the cerebellum, SCN, retina, kidney, ovary, cardiac vessels (Witt-Enderby et al., 2003). Radioligand binding studies in the rat SCN have found an inverse relationship between receptor density and serum melatonin levels (Gauer et al., 1993; Tenn and Niles, 1993).

In normal adults the night time peak concentration (acrophase) of plasma melatonin occurs between 02.00h and 04.00h. The nadir of core body temperature occurs within 1h of the peak of melatonin together with the peak of fatigue and the trough in ability to perform certain tasks, and this association may be part causal (Arendt, 2005). Whether you are a morning or evening type ('lark or owl') also affects when the peak of night time melatonin is going to occur. Morning types have been shown to have a significantly earlier peak in melatonin and temperature rhythm with an earlier bed and wake-time when compared to evening types (Baehr et al., 1999; Duffy et al., 2001; Mongrain et al., 2004). This suggests that time of day preference may be influenced by the circadian clock driving these physiological daily rhythms. There is also high inter-individual variability in levels of nocturnal endogenous melatonin (Arendt, 1988) and in the pharmacokinetics of exogenous melatonin (Aldous et al., 1985).

Melatonin in addition to blood, saliva, and urine has been detected in the CSF of mammals and in peripheral tissues (Arendt et al., 1977; Martin et al., 1992; Reiter and Tan, 2002). Melatonin is metabolised primarily in the liver but also in the kidney, undergoing hydroxylation via the P450 enzyme CYP1A (Facciola et al., 2001; Skene et al., 2001) and then conjugation producing 6-sulphatoxymelatonin (aMT6s) and glucuronide (minor metabolite), its urinary concentration accounts for 50-90% of administered melatonin (review, Arendt, 1995).



#### 1.5.4. Marker of the circadian clock

The melatonin rhythm, illustrated in Figure 1-8, is arguably the best marker of the circadian phase (review, Klerman, 2005). In laboratory and field studies the rhythm of urinary 6-sulphatoxymelatonin (aMT6s) the primary urinary metabolite of melatonin correlates well with plasma melatonin (Arendt et al., 1985; Bojkowski et al., 1987; Ross et al., 1995). The acrophase of aMT6s occurs approximately 2h after the peak of melatonin production. Melatonin is a good marker of circadian phase because it has a few 'masking' factors. Light suppresses the synthesis of melatonin, hence clinical trials and in-house laboratory studies are conducted in dim light to exclude this masking effect. Posture is also known to have a small effect on melatonin concentrations hence subjects are asked to stay in a semi-recombinant position during a laboratory constant routine (Deacon and Arendt, 1994).

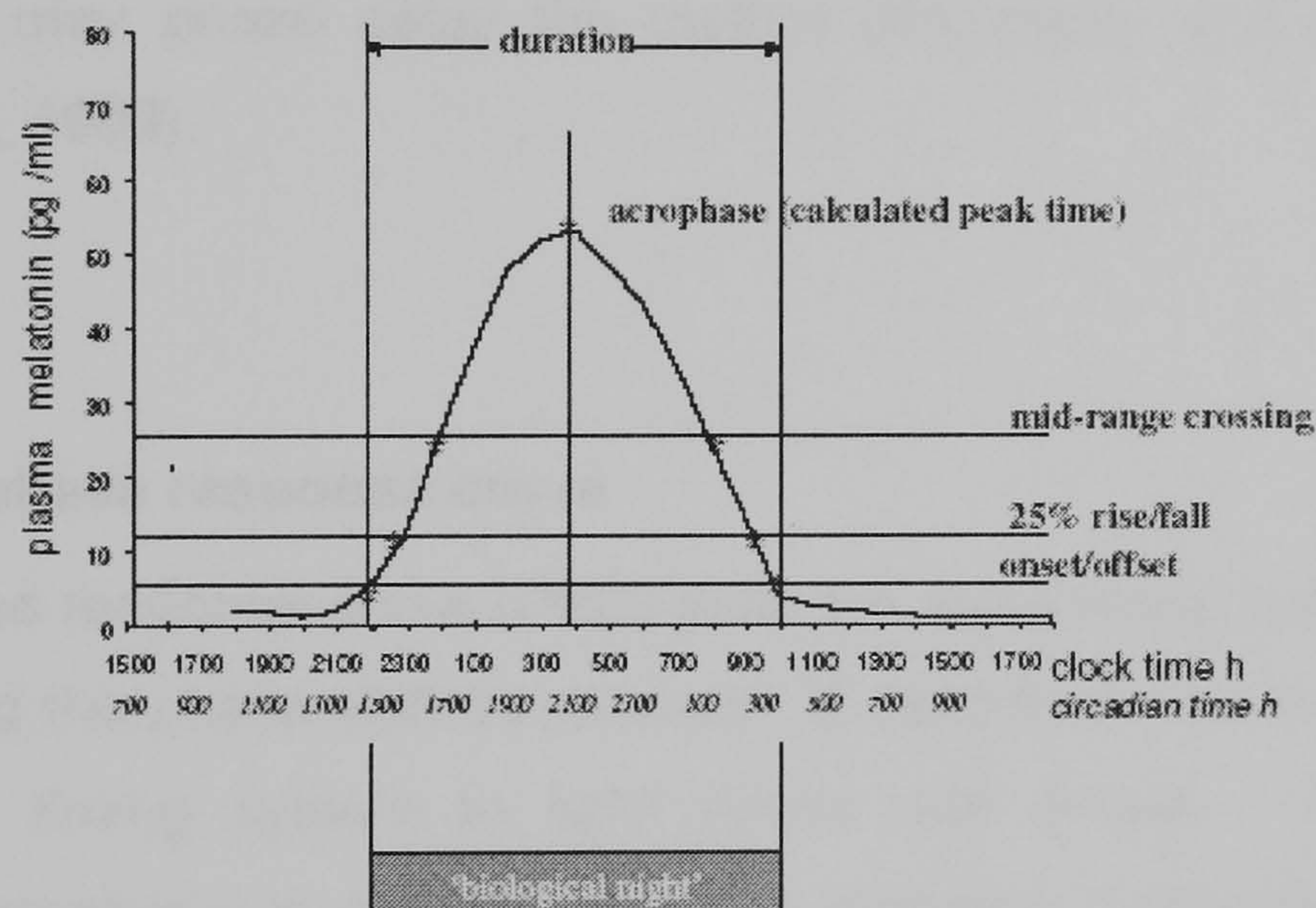


Figure 1-8: Schematic plasma melatonin profile, including parameters of the rhythm (adapted from Arendt and Skene, 2005).

The time of the onset of melatonin synthesis in dim light (dim light melatonin onset (DLMO), measured in saliva or plasma) and maximum (acrophase) are frequently used as markers for the phase of the circadian clock (Burgess et al., 2002) and see Figure 1-8.



#### **1.5.4.1. Light induced melatonin suppression**

Light has two main effects on melatonin, it is able to phase shift the circadian clock and hence the melatonin rhythm and secondly light is able to acutely suppress nocturnal melatonin. The discovery that high intensity bright (2500 lux) light can cause melatonin suppression (Lewy et al., 1980) triggered studies investigating the phase shifting effects of artificial high-intensity light (review, Eastman et al., 1995). More recently the effects of lower intensities have been investigated and it is clear that domestic intensity illumination (ca 100 lux) has substantial effects on suppression and phase shifting of melatonin (Zeitzer et al., 2000). It is well established in animals and humans that light pulses can shift the phase of circadian rhythms, and that the direction (advance or delay) and magnitude (hours) of the shift depends on when the light is applied within the circadian cycle. It is known that suitably timed morning light phase advances the whole melatonin rhythm, while evening light may phase delay the rhythm (Broadway and Arendt, 1986; Czeisler et al., 1989).

#### **1.6. Light phase response curve**

The light phase response curve (PRC) plots the relationship between a timed light pulse and the phase shift it produces. It describes how the response of the circadian timing system to light varies with phase. The entrained circadian pacemaker is in equilibrium with a light-dark (LD) cycle. Each daily pulse occurs at the phase when a light pulse induces a phase shift that is equal to the difference between the free running period ( $\tau$ ) and the period of the zeitgeber (Johnson, 1999). There are two different types of PRCs that can be generated, type 1 (or weak resetting) and type 0 (strong resetting). Type 1 is a low amplitude PRC that displays relatively small phase shifts (usually less than 6h) and has a continuous transition between delays and advances. A type 0 is a high amplitude PRC with phase shifts up to 12h in size (Johnson, 1999). The amplitude of the PRC directly depends on strength of the stimulus.

The PRC to light (Figure 1-9) indicates that phase-shifting responses are reduced during the subjective day. Phase shifts are plotted on the abscissa. Advances in phase shift are plotted as positive and phase delays are plotted as negative.

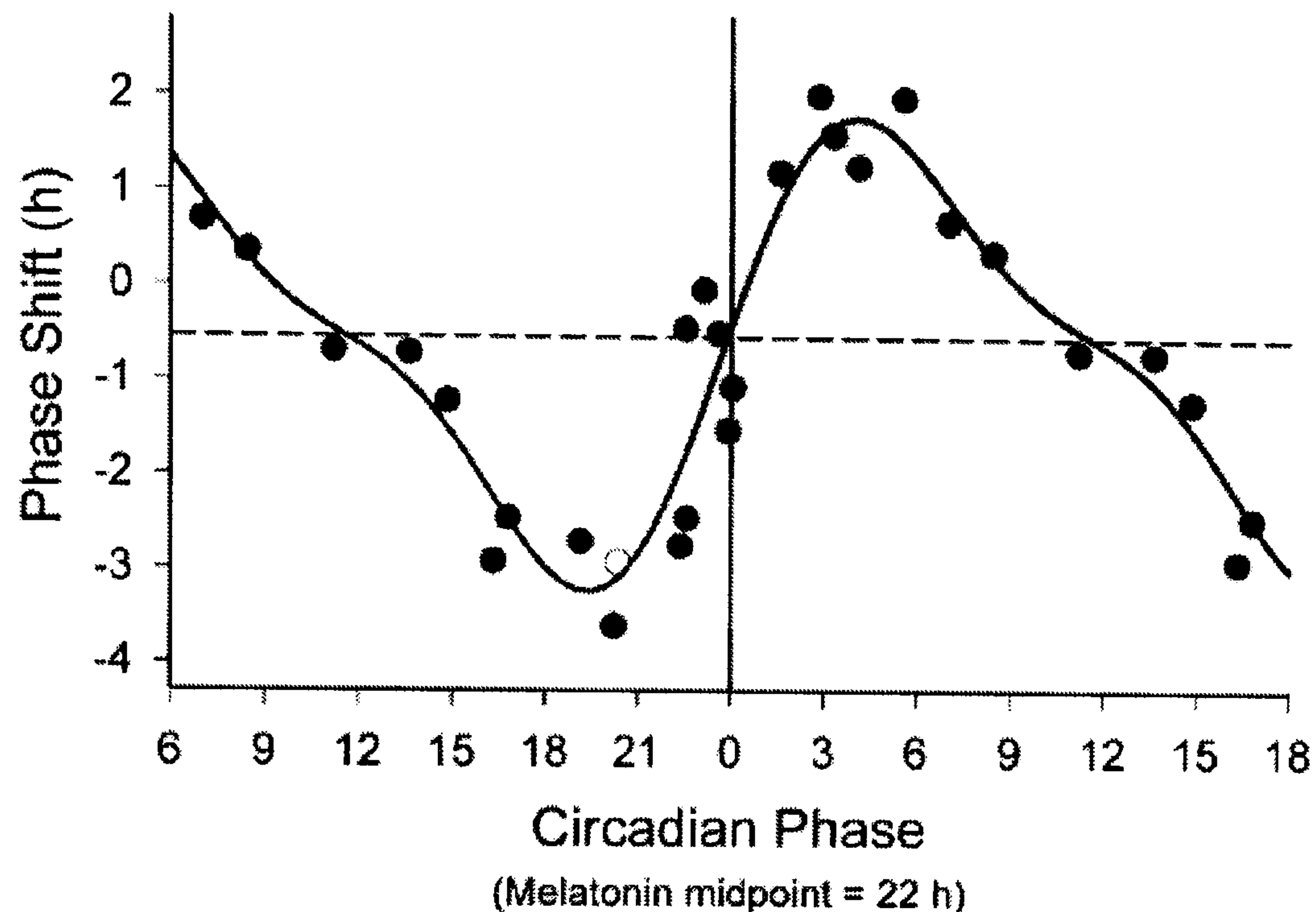


Figure 1-9: Illustration of a light PRC (Khalsa et al., 2003).

Typically, a light PRC is generated from laboratory animals free-running in constant conditions. The free running period is divided into 24 circadian hours to mark the x-axis; circadian time (CT) 0 corresponds to the beginning of the subjective day (lights on in a 12h light dark cycle), and CT12 corresponds to the beginning of the subjective night. The largest phase shifts are obtained when light is given during the subjective night, with phase delays induced by light at the beginning of the night and phase advances by light at the end of the night. Light during the subjective day has little phase-shifting effect. Khalsa et al. (2003), showed no evidence of a significant dead zone in their study. However the existence, or not, of a dead zone in the light PRC is controversial in human studies. Phase advances diminish gradually during the course of the early subjective day, ultimately yielding to gradually increasing phase delays in the subjective night. Increasing the intensity or duration of the light pulse can increase the magnitude of the phase shift (Eastman and Martin, 1999).



### **1.6.1. Light PRC studies**

There have been 5 previous reports of a light PRC in humans. The first was conducted by Honma and Honma (1988) which revealed a PRC without a significant phase delay region. These results were inconsistent with later reports from other laboratories that have routinely demonstrated phase delays to single light pulses. Czeisler et al. (1989) showed phase delays as large as 12h when the stimulus was centred near the CBT minimum (3 day light pulse, not a single light pulse, which may explain the large phase shifts, plus this protocol involved displacing the sleep period). Minors et al. (1991) showed a poorly defined delay region of the PRC, using single light pulses of 5000 and 9000 lux. Jewett et al. (1994) presented analysis of strong, weak, and critical bright-light resetting trials in humans, and they were the first to report not only phase but also amplitude data. Van Cauter et al. (1994) only reported shifts for a region spanning less than the circadian cycle. Khalsa et al. (2003) studied entrained subjects under constant routine conditions, and administered a 6.7h bright light (~10,000 lux) at different circadian phases.

PRCs are hard to compare, as each study has often used a different number of light pulses at varying light intensities, each at different circadian times in order to produce the phase shift required. PRCs are beneficial as 'they can be used to predict the timing of light treatment to enable adaptation to environmental changes, such as those seen in shift work and transmeridian travel' (Rajaratnam and Arendt, 2001) and studies have used this knowledge to develop bright light treatment to speed up adaptation to shifts in night workers and time zone travellers (section 1.6.1).

### **1.7. Shift work**

Shift work encompasses work outside the conventional daytime and thereby covers fixed evening and night work, roster work, and rotating shift work. Åkerstedt defined shift work as "an arrangement of work hours which employs two or more teams (shifts) of workers in order to extend the hours of



operation beyond that of conventional office hours” (Åkerstedt, 1990). In the UK shift work is the way of life for approximately 12% of the working population as illustrated in Table 1-1.

Type of Shift work	Population	Population (%)
All shift workers	3,551,000	12.0
Rotating shifts	2,722,000	9.2
Permanent night shifts	343,000	1.2
Sometimes nights, sometimes days	449,000	1.5
Weekend shifts	37,000	0.1

Table 1-1: Percentage of UK working population working shifts (UK Annual Labour Force Surveys, Office for National Statistics, 2005).

### 1.7.1. Shift work and light

Light has been suggested as a counter measure against night work impairment of sleep and alertness (Czeisler et al., 1990). This is because light is the most powerful zeitgeber. Light has the ability to both phase advance and delay the circadian clock (section 1.4.5.1) and this knowledge may benefit shift workers through the use of appropriately timed light therapy, together with the appropriate avoidance of conflicting light, which could impede adaptation when adaptation is desirable. It is possible that bright light may be sufficiently powerful to overcome all other synchronisers, and shift workers’ sleep habits may be largely irrelevant in determining the effectiveness of bright light intervention (Horowitz et al., 2001).

### 1.7.2. Simulated shift work

Simulated shift work allows a controlled laboratory protocol to be followed, physiological markers can be measured, meal times, exercise and sleep patterns can all be controlled, as all of these may mask the effects that are being investigated e.g. the effect of the light treatment. The appropriate use



of high intensity light can increase the rate of circadian adaptation. Several night shift simulation studies have successfully used light to phase shift rhythms to realign to the new shifted sleep period (review, Burgess et al., 2002). Table 1-2 outlines simulated night shift studies which have studied the effects of light on circadian timing.



INTERVENTION	OUTPUT MEASURES	RESULTS	STUDY BY:
<ul style="list-style-type: none"> <li>▪ 54 subjects participated in a shift work simulation of 4 day and 3 night shifts.</li> <li>▪ Subjects received 2,500 lux or 150 lux during night shifts and were scheduled to sleep (at home in darkened bedrooms) from 08.00 - 16.00h (fixed sleep).</li> </ul>	DLMO measured before and after the night shifts	<ul style="list-style-type: none"> <li>▪ Both fixed sleep and bright light conditions significantly phase delayed DLMO.</li> <li>▪ Treatments combined additively, with light leading to larger phase shifts.</li> </ul>	(Horowitz et al., 2001)
<ul style="list-style-type: none"> <li>▪ Study investigated whether melatonin can facilitate phase shifts in a simulated night-work protocol.</li> <li>▪ Thirty two subjects slept in the afternoons/evenings before night work (h advance of the sleep schedule).</li> </ul>	CBT, DLMO	<ul style="list-style-type: none"> <li>▪ Melatonin produced larger phase advances than placebo in DLMO and temperature rhythms.</li> <li>▪ Average phase advances (<math>\pm</math> SD) of the DLMO were <math>1.7 \pm 1.2</math>h (placebo), <math>3.0 \pm 1.1</math>h (0.5 mg), and <math>3.9 \pm 0.5</math> h (3.0 mg).</li> </ul>	(Sharkey and Charmane, 2002)
<ul style="list-style-type: none"> <li>▪ 67 subjects in 5 consecutive simulated night shifts (23.00-07.00h) and then slept at home (08.30-15.30h) in darkened bedrooms.</li> <li>▪ Participants wore sunglasses whilst outside during the day. Participants took placebo or melatonin (1.8 mg sustained release) before daytime sleep.</li> <li>▪ During the night shifts, participants were exposed to a moving (delaying) pattern of intermittent bright light (approximately 5000 lux).</li> </ul>	DLMO assessed before and after the night shifts (baseline and final), and 7h was added to estimate temperature minimum ( $T_{min}$ ).	<ul style="list-style-type: none"> <li>▪ With bright light during the night shift, almost all of the participants achieved complete re-entrainment. The phase delay shift was so large that darker sunglasses and melatonin could not increase its magnitude.</li> <li>▪ Recommend the combination of intermittent bright light during the night shift, sunglasses (as dark as possible) during the commute home.</li> </ul>	(Crowley et al., 2003)
<ul style="list-style-type: none"> <li>▪ 24 male subjects had their sleep schedules shifted 12h to accommodate eight or more consecutive simulated night shifts. They were exposed to artificial 5,000 lux, 3 to 6h durations) each night, slept at home in very dark bedrooms during the day, and wore dark welders goggles whenever they went outside during daylight</li> </ul>	CBT, sleep diaries and mood questionnaires	<ul style="list-style-type: none"> <li>▪ The CBT rhythm phase shifted, usually approximately 2h/day, in 21 of 24 subjects.</li> <li>▪ In the subjects whose temperature rhythms shifted, the direction of shift (advance or delay) depended on the timing of light exposure relative to their baseline temperature phase, consistent with a PRC.</li> </ul>	(Eastman, 1992)
<ul style="list-style-type: none"> <li>▪ 12 nursing night shift staff.</li> <li>▪ Study consisted of 3 treatment procedures; room light, bright light (10,000 lux) and bright light and sunglasses.</li> </ul>	Alertness measured by visual analogue scale. Sleep diaries and actigraphy.	<ul style="list-style-type: none"> <li>▪ Most significant improvement in sleep was seen in the group with light treatment and sunglasses.</li> </ul>	(Yoon et al., 2002)



<ul style="list-style-type: none"> <li>▪ 6 subjects received 4 hour light pulse, 6,000 lux between 24.00-04.00 h light pulse on the first night shift and then dim light for the remainder of the study.</li> <li>▪ 7 subjects in control group were under dim light conditions for the entire duration of the study.</li> </ul>	CBT	<ul style="list-style-type: none"> <li>▪ Treatment group delayed by 5-6hrs.</li> <li>▪ Control group delayed 2-3hrs</li> </ul>	(Dawson and Campbell, 1991)
<ul style="list-style-type: none"> <li>▪ 12 subjects (4 males and 8 females) studied on three occasions at the same clock time (13.30h), but at different body clock times, after consuming test meals, first in their normal environment, secondly after a forced 9 h phase advance (body clock time approximately 22.30h) and then again 2 days later in the normal environment.</li> </ul>	Plasma glucose, non-esterified fatty acids (NEFAs), TAGs, insulin, and urinary aMT6s.	<ul style="list-style-type: none"> <li>▪ Basal plasma NEFAs were lower immediately after the phase shift (<math>P &lt; 0.05</math>). Incremental (difference from basal) TAG responses were significantly higher (<math>P &lt; 0.05</math>) immediately after the phase shift compared with baseline. Two-day post-phase shift responses showed partial reversion to baseline values. This study suggested that it takes at least 2 days to adapt to eating meals on a simulated night shift,</li> </ul>	(Ribeiro et al., 1998)
<ul style="list-style-type: none"> <li>▪ This study was designed to investigate postprandial responses to a mixed meal in simulated shift work conditions. 9 normal healthy subjects were studied on two occasions at the same clock time (13.30h) after consuming test meals, first in their normal environment and secondly after a 9 h phase advance (body clock time 22.30h).</li> </ul>	Plasma glucose, insulin, triacylglycerol (TAG)	<ul style="list-style-type: none"> <li>▪ Significantly higher postprandial glucose responses were observed in the last 4h of the study after the phase shift than before. Peak plasma TAG levels occurred 5 h postprandially before the phase shift. Postprandial rises in plasma TAG were significantly delayed after the phase shift.</li> </ul>	(Hampton et al., 1996)
<ul style="list-style-type: none"> <li>▪ Volunteers were initially subjected to a gradual 9-h delay phase shift over 5 days (D1-D5) using a combination of bright light and darkness/sleep. Melatonin treatment was timed to phase advance and bright light to phase delay.</li> </ul>		<ul style="list-style-type: none"> <li>▪ Bright light delayed the aMT6s rhythm in all subjects. With the subsequent 9h advance shift two subjects delayed and five phase advanced with both MT and melatonin and bright light. Melatonin consistently improved subjective sleep, alertness and performance even in the presence of inappropriate bright light and before phase readaptation had occurred.</li> </ul>	(Deacon and Arendt, 1996)
<ul style="list-style-type: none"> <li>▪ Simulated night work field study included 8 consecutive night shifts followed by daytime sleep/dark periods (delayed 9 h from baseline).</li> <li>▪ 33 subjects in a 2 × 2 design that compared 1)</li> </ul>	CBT	<ul style="list-style-type: none"> <li>▪ Intermittent bright light groups had significantly larger phase delays than dim light groups, and 94% of subjects who received bright light had phase shifts large enough for the temperature minimum to</li> </ul>	(Baehr et al., 1999)



intermittent bright light (6 pulses each, 40-min long each, at 5,000 lx) versus dim light and 2) intermittent exercise (6 bouts, 15-min long, at 50-60% of maximum heart rate) versus no exercise.		reach daytime sleep. ▪ Exercise did not affect phase shifts; neither facilitating nor inhibiting phase shifts produced by bright light.	
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Table 1-2: Summary of simulated shift work studies that have studied the effects of light on circadian timing.

### 1.7.3. Field studies of shift work and bright light

Intense artificial light can shift the phase of human circadian rhythms (section 1.4.5) and has been successfully used to induce phase shifts and to improve sleep, performance and alertness (Czeisler et al., 1990; Dawson and Campbell, 1991; Deacon and Arendt, 1996). Bright light as a counter measure for circadian desynchrony has been used in the field, though the number of studies are limited.

One of the first field studies to use light to help subjects adapt back to the day shift after working nights was conducted by Midwinter and Arendt (1991) in Antarctica. The study involved 11 healthy men, mean age 21-33 years with and without morning and evening bright light treatment (2500 lux) between 08.00-09.00h and 16.00-17.00h. The acrophase of the melatonin rhythm was significantly delayed from 5.22h to 14.54h in summer and 8.73h to 13.23h in winter during a week of night shift work. The study demonstrated the importance of light treatment to shift the circadian clock to aid readaptation.

Field studies on NASA astronauts carried out by Stewart et al. (1995) used light as a counter measure to help adaptation to shift work. The study involved NASA astronauts (18 subjects, mean age  $37 \pm 11$  years) and ground crew using long duration high intensity light exposures (light 3000-12,000 lux) to produce an adaptive phase delay shift of 3-9h. Light treatment was administered a week prior to launch along with a slow or rapid shift in



sleep to pre-adapt the workers to the shift schedules required during the mission. Light treatment began 3 to 4 nights before the first night shift, during the first half of the subjective night to induce large phase delays. Light treatment had beneficial effects on night shift personnel. Subjects in the light treatment group rated their speed of work, concentration, and alertness while on duty significantly higher than the control group who did not receive light treatment.

Deacon and Arendt (1996) subjected 8 healthy volunteers to pulses of moderately bright light (1,200 lux) day 1: 18.00-03.00h, day 2: 21.00-06.00h and day 3: 24.00-09.00h. As a result of the light pulses a progressive shift in rhythms was evident, with a maximal shift in aMT6s of just over 9 hours.

A study conducted by Lowden et al. (2004) showed a clear effect of bright light during breaks on night shift alertness, sleep and melatonin levels. Eighteen workers at a truck production plant were exposed to either bright light (2500 lux) during breaks or normal light for 4 consecutive weeks. In the study 20-minute breaks were initiated by 67% of the workers between 03.00-04.00h. The results showed that bright light administration significantly suppressed melatonin levels during night work and most strongly at 02.00h. The results suggested that intermittent bright light combined with normal work light exposure strengthens the photic drive as opposed to continuous normal work light conditions. The study showed positive results of bright light during night work on alertness and sleep. This gives further support for the use of light for night work, with total sleep time being positively affected by light exposure (Lowden et al., 2004).

These studies described above demonstrate the abilities of appropriately timed light to help shift work adaptation in the field using the knowledge that has been acquired in laboratory studies (section 1.6.2).



#### **1.7.4. Shift schedules operated offshore**

A number of different shift schedules are operated offshore which have a variety of impacts on adaptation to night shift. One of the most common shift schedules operated offshore is the 12h shift from 18.00-06.00h for 2 weeks. Barnes et al. (1998a) have shown that the aMT6s rhythm of oil rig workers on this 2-week night shift (18.00-06.00h) adapts to the shift by phase delay. Another shift schedule is the 12h shift from 12.00-00.00h for 1 week followed by a 12h shift from 00.00-12.00h for 1 week, called a swing shift. When this shift was studied in the winter (November) months, no adaptation of the aMT6s rhythm was seen in the night shift (Barnes et al., 1998b). On the other hand when this shift was studied in spring (March) there was a significant phase advance of the rhythm during the night shift (Barnes et al., 1998b). Gibbs et al. (2002) studied a swing shift schedule of 7 nights 18.00-06.00h followed by 7 days 06.00-18.00h. A significant difference was found between the mean aMT6s acrophase at the start and the end of the night shift week. Most subjects adapted to the night shift but there was a wide inter-individual variation when readapting back to day shift.

#### **1.7.5. Studies using light and melatonin on offshore shift workers**

Bjorvatn et al. (1998) studied adjustment to 14 days of consecutive night work on a North Sea oil platform and the readjustment to day life at home, using the Karolinska sleep/wake diary during April and May. The subjective ratings from the study indicated that workers adapted to night work within a few days and had more problems re-adapting to day life offshore as had previously been found in Antarctic field studies, (Midwinter and Arendt, 1991; Ross et al., 1995). The platform workers adapted to night work within a few days as indicated by the rapid reduction of night work sleepiness, and by the gradual delay of bedtime. Readaption to day life was slower and more difficult adding to the evidence of complete adaptation to night work. It was concluded that the lack of conflicting exposure to daylight in the morning may have facilitated the rapid adjustment to night work.



A later study was conducted by Bjorvatn et al. (1999) on oil rig workers in the North Sea, with subjects working for 2 weeks on a 12h day; 07.00-19.00h, followed by 2 weeks off. The study used bright light treatment where the light exposure was scheduled on an individual basis on the assumption that the CBT nadir was located 2h before the subject's habitual time of awakening. The light treatment (10,000 lux white light) consisted of 30 minutes per day and was applied during the first 4 nights of the night shift and the first 4 days following the return home. Between subjects, the start of bright light exposure ranged from 03.30h to 05.30h during the first night of the night shift, and from 14.00h to 15.30h during the first day at home. Subjects were told to avoid outdoor light exposure after the scheduled bright light treatment. The study concluded that a few days of scheduled exposure to bright light could be used to facilitate adaptation back to day life following their return home.

In a more recent study Bjorvatn et al. (2006) studied the adaptation and readaptation processes from one week of nights followed by one week of days on an offshore oilrig, in a placebo controlled crossover field study. Seventeen male subjects were studied, using both subjective and objective measures, Karolinska sleepiness scale (KSS), simple serial reaction time test, sleep diary and actigraphy. Subjective and objective measures improved gradually during night work. The return to day work after one week of nights led to increases in subjective sleepiness.

Bjorvatn et al. (2007) also evaluated the effects of bright light and melatonin on adaptation to night work offshore. Seventeen subjects working a schedule of 2 weeks on a 12h shift schedule, with the first week on night shift (19.00-07.00h) and the second week on day shift (07.00-19.00h) (swing shift schedule) participated in a randomized controlled crossover design. Subjects received a placebo, melatonin (3 mg, 1h before bedtime), or bright light (30-minute exposure, individually scheduled) during the first 4 days on the night shift and during the first 4 days on the day shift. Subjective and objective measures of sleepiness (KSS and a simple serial reaction-time test) and sleep (diary and actigraphy) were obtained. Subjective measures



indicated that melatonin modestly reduced sleepiness at work during the day shift and increased sleep by 15–20 minutes per day. Melatonin and bright light modestly improved sleep and sleepiness in this field study.

These studies demonstrate the use of light intervention to encourage phase adaptation in shift workers.

#### **1.7.6. Adaptation to the night shift**

Circadian adaptation in terms of adapting to a night shift or a new time zone can be defined as a significant shift of the melatonin rhythm into the daytime (Nowak et al., 1987). Exceptions to the general rule that shift workers do not adapt to night work have been shown to occur where night workers work and sleep at unusual isolated locations such as offshore oilrigs or Antarctic stations (Broadway and Arendt, 1988; Midwinter and Arendt, 1991; Ross et al., 1995; Barnes et al., 1998a; Gibbs et al., 2002). In these unusual work environments, workers are less subject to the external light dark cycle and competing social demands. Moreover there also seems to be a large inter-individual variability in shift workers who actually adapt to shift work in a 'normal' environment (Burgess et al., 2002).

Offshore North Sea oil installations are located far enough North (61°N) to study the effects of the presence (in summer) and absence (in winter) of natural bright light during the night shift. Light exposure during the winter period on night shift has been reported to range between 50 and 400 lux and consisted of only artificial light (Barnes et al., 1998b). Exposure during the summer period on night shift ranged between 50 and 2000 lux (Barnes et al., 1998b). Studies in Antarctica (Broadway and Arendt, 1988; Midwinter and Arendt, 1991) have shown that different seasons influence the ability of shift workers to adapt to night shift.



### **1.7.6.1. Individual differences and adaptation**

Individual differences in the ability to adapt to a night shift, and hence adapt the internal body clock to sleep at a new time schedule are influenced by many factors. Evening types (whose T<sub>min</sub> occurs at a later clock time) generally work better at night work as they have later bedtimes already, hence they do not have to shift the timing of their sleep period as much as those who are morning types (whose T<sub>min</sub> occurs at an earlier clock time) (Duffy et al., 2001) and who have to shift their sleep period further in comparison to evening types. Secondly, evening types are known to be capable of larger phase delays than morning types, which allows evening types to adapt to the night shift faster than morning types (Mitchell et al., 1997). Most recently, Gibbs et al. (2007) have shown that initial phase, early or late, at the beginning of night shift can predict both rate of adaptation to nights (18.00-06.00h) but also rate and direction of re-adaptation to days (06.00-18.00h).

### **1.7.6.2. Age and adaptation**

Age also influences the rate at which one can adapt to the night shift. Older people tend to have an earlier sleep-wake cycle compared to young people, with respect to clock time (Duffy and Czeisler, 2002). Therefore older night shift workers would have to shift their sleep-wake cycle further to adapt to the night shift compared to younger night shift workers (Dijk et al., 1999). Older people have been shown to wake up at an earlier circadian phase of the body temperature and plasma melatonin rhythm (Dijk et al., 1999; Duffy and Czeisler, 2002). Wake maintenance and sleep initiation are not markedly affected by age except that sleep latencies are longer in older people when sleep initiation is attempted in the early morning (Dijk et al., 1999). Sleep in older people is particularly disrupted when scheduled on the rising limb of the temperature rhythm, indicating that the sleep of older people is more susceptible to arousal signals generated by the circadian pacemaker (Dijk et al., 1999).



## **1.8. Shift work and its effect on health**

The general consensus is that shift work is associated with greater health problems than 'normal' day work (Harrington, 2001). Night shift workers are required to work and eat at the 'wrong' phase of their circadian cycle. This results in complaints of sleepiness, reduced performance and disturbed sleep due to lack of adjustment of the circadian timing system (Åkerstedt and Gillberg, 1990; Kecklund and Åkerstedt, 1993; Bjorvatn et al., 1999). During consecutive night shifts, the internal circadian rhythms gradually shift to adapt to the new schedule (Crowley et al., 2003). Attempts to sleep at inappropriate phases of the circadian cycle, for example during the declining phase of melatonin and rising phase of core body temperature, usually results in shorter sleep episodes and more awakenings (Kecklund and Åkerstedt, 1995).

Night and rotating shift workers are most likely to report disrupted eating habits and poorer diets than those who work day or afternoon shifts, as well as a higher prevalence of gastric and peptic ulcers, gastritis and constipation (Gordon et al., 1986). However there has been no report of a link between shift work, eating and disease (Knutsson, 1989). Lennernas et al. (1995) reported that energy intake and the quality of food intake (% of energy from macronutrient and density of micronutrients) were not affected by shift work. Recently an association between increased risk of cancer and shift work has been reported due to the hypothesis of exposure to light at night and suppression of melatonin (Schernhammer et al., 2006).

### **1.8.1. Shift work and cardiovascular disease**

Shift work has been long associated with cardiovascular disease (CVD).

#### **1.8.1.1. Epidemiology**

Heart and circulatory disease is the UK's biggest killer. In 2002, CVD caused 39% of deaths in the UK, and killed just fewer than 238,000 people.



Coronary heart disease (CHD), the main form of CVD, caused over 117,000 deaths a year in the UK; approximately one in five deaths in men and one in six deaths in women. Death rates for CHD have been falling rapidly in the UK since the late 1970s. Despite this, death rates from CHD in the UK are still amongst the highest in Western Europe ([www.heartstats.org](http://www.heartstats.org) British Heart Foundation statistics website).

#### **1.8.1.2. Circadian aspects of CVD**

Circadian control of normal physiology will inevitably lead to circadian variation in disease. The increased risk of heart disease in those who work shifts is thought to be due to the disruption of internal circadian mechanisms (review, Hastings et al., 2003) as illustrated in Figure 1-10.



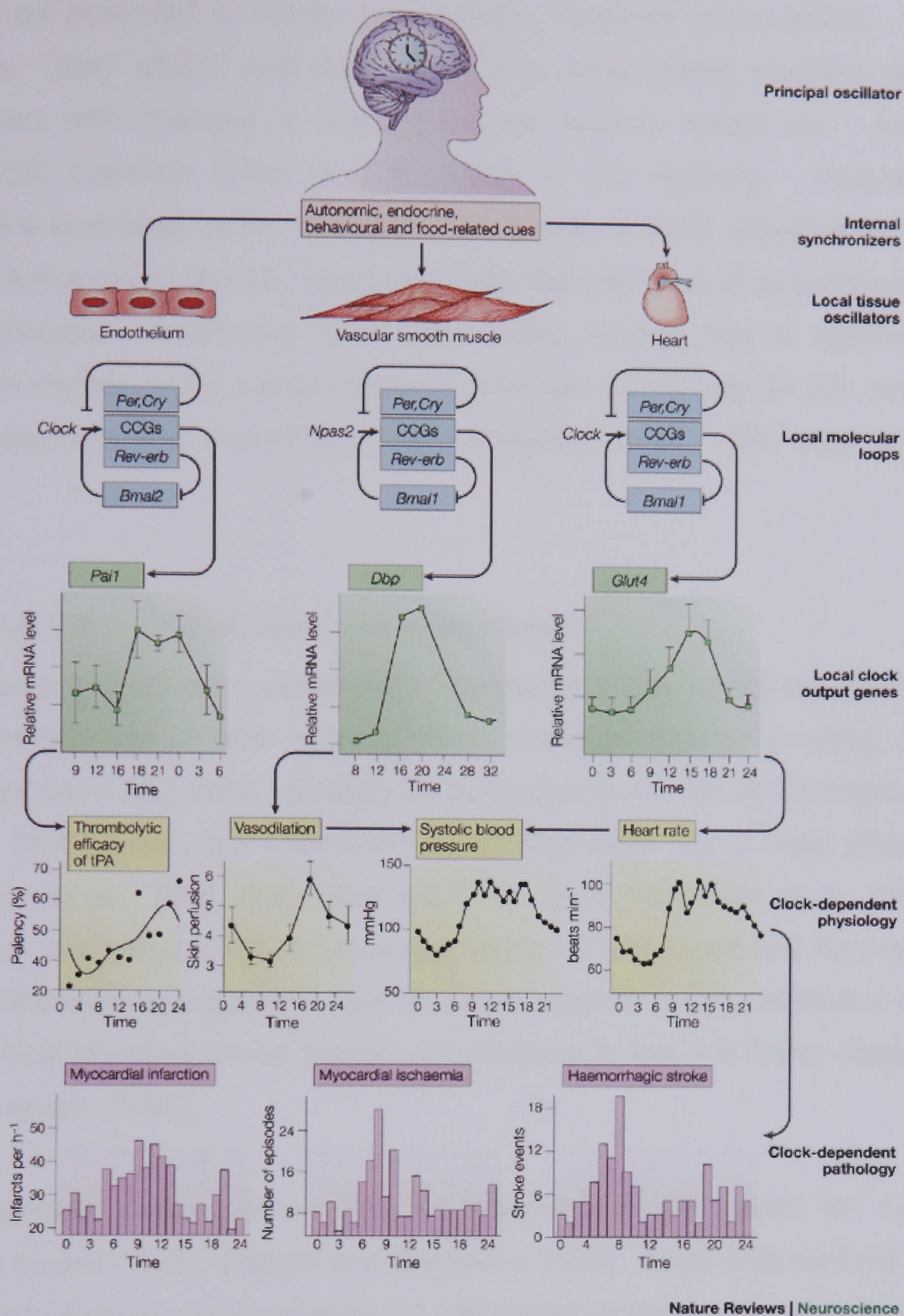


Figure 1-10: Circadian mechanisms of CHD (review, Hastings et al., 2003).

Circadian oscillators in the vascular endothelium, smooth muscle and myocardium, are based on clock gene feedback loops, they are synchronized by signals from the SCN. As a result infarctions, ischemia and strokes can be seen as consequences of actions of the circadian system in the vascular



tissues, as illustrated in Figure 1-10 (review, Hastings et al., 2003). For example, heart attack and strokes show a pronounced morning peak associated with changes in blood pressure, cardiac output etc. Acute myocardial infarction (AMI) is 40% higher in the morning. Peripheral oscillators contribute to the circadian prevalence of CVD (Hastings et al., 2003). A number of studies have shown that the incidence of all subtypes of stroke (lacunar, thrombotic, and hemorrhagic stroke) has a significant circadian rhythm, with a higher frequency between 06.00h and 24.00h, and a lower frequency from midnight to 06.00h (Gallerani et al., 1996; Lago et al., 1998).

#### **1.8.1.3. Shift work and heart disease**

The causes of CVD are multifactorial. The psychosocial factors of sedentary work, monotonous or other stressful work conditions, passive smoking, and shift work have also been identified as occupational risk factors (Kristensen, 1989). Shift workers are known to have an increased risk of heart disease (Hampton et al., 1996; Steenland and Fine, 1996; Tenkanen et al., 1997; Nicholson and D'Auria, 1999) of approximately 40% (Bøggild and Knutsson, 1999). Sociotemporal patterns, lack of social support, stress, behaviour and biochemical changes are all thought to contribute to this risk factor (Bøggild and Knutsson, 1999).

Triacylglycerol concentrations are an independent risk factor for heart disease (Austin, 1997; Bøggild and Knutsson, 1999). Night shift workers are reported to have an approximately 1.5 fold higher incidence of heart disease risk (Bøggild and Knutsson, 1999) and also demonstrate higher TAG levels compared with matched day workers (Lund et al., 2001; Morgan et al., 2003). Simulated shift work studies have also demonstrated this same principle (Ribeiro et al., 1998; Sopowski et al., 2001). If shift workers repeatedly consume meals at night, during the time of maximal insulin insensitivity, the cumulative postprandial effects may predispose individuals to display other abnormalities of the metabolic syndrome (Morgan et al., 2003). One study



found that cholesterol correlated with the distribution of meals and cholesterol levels were higher when a larger proportion of the diet was ingested at night (Lennernas et al., 1994).

Several studies have confirmed the well-known clinical experience that the onset of both myocardial infarction and angina pectoris is more common in the early morning. This chronobiological rhythm has been explained by a mismatch between oxygen supply and oxygen requirement at time of awakening (Bøggild and Knutsson, 1999). A study conducted by Karlsson et al. (2001) concluded that obesity, high triacylglycerol (TAG) levels and low concentrations of high density lipoprotein (HDL) cholesterol seem to cluster more often in shift workers. There is strong evidence showing that the metabolic syndrome is a combination of obesity, dyslipidemia, with high TAG and low HDL cholesterol and glucose intolerance, which are all risk factors for heart disease (Haffner, 1999). Knuttson and Bøggild. (2000) reported that these factors appear to cluster together more often in shift workers than day workers. It has been claimed that when people make a decision to apply for shift work, they have probably estimated their ability to withstand irregular work hours and therefore differ in terms of health from those choosing not to apply (Kivimaki et al., 2006).

### **1.8.2. Insulin resistance**

Insulin resistance is the condition in which normal amounts of insulin are inadequate to produce a normal insulin response from fat, muscle and liver cells (Bonadonna et al., 1993). Insulin resistance refers to a reduced ability to utilise insulin to control glycaemia. In insulin-sensitive individuals, insulin promotes glucose uptake in target tissues and inhibits glucose production by the liver. Insulin resistance in muscle reduces glucose uptake whereas insulin resistance in liver reduces glucose storage, with both effects serving to elevate blood glucose. It is known that insulin sensitivity is lower during the night than in the day (Van Cauter et al., 1992). High plasma levels of



insulin and glucose due to insulin resistance often lead to the metabolic syndrome and type II diabetes (Balkau, 2005).

### **1.8.3. Metabolic syndrome**

Clinical conditions including hypertension, truncal obesity, dyslipidaemia and insulin resistance (Nakamura et al., 1997; Ha and Park, 2005) are collectively known as the 'metabolic syndrome'. The metabolic syndrome is poorly understood because it is multifactorial including environmental, genetic and epigenetic aspects (Magliano et al., 2006). It has been shown under experimental conditions, that following an abrupt change in the timing of sleep and work, postprandial glucose and lipid tolerance and insulin secretion are significantly altered, to an extent that may contribute to atherogenesis and hence cardiovascular disease (Foret et al., 1998; Ribeiro et al., 1998; Morgan et al., 2003) (as well as metabolic syndrome). These findings suggest that it takes at least 2 days to adapt to eating meals on a simulated night shift.

#### **1.8.3.1. Shift work, the metabolic syndrome and insulin resistance**

Shift work is associated with the metabolic syndrome (Assman et al., 1999). These problems are believed to be due largely to desynchrony of circadian rhythms (Lund et al., 2001) although health problems, which arise from shift work are multi-factorial. Collectively all these factors may contribute to the health risks associated with shift work.

### **1.8.4. Shift work and sleep**

The sleep-wake cycle is one of the most important circadian rhythms. Sleep is usually initiated when body temperature is falling (Dijk and von Schantz, 2005; Waterhouse et al., 2005). Shift work that includes night work is associated with shortened sleep and increased sleepiness (Åkerstedt, 2003).



One reason for night shift sleepiness is that the individual is required to work at the nadir of the well-established circadian pattern of wakefulness. Alertness, performance and metabolism peak in the late afternoon, and reach a nadir in the early morning. The period of maximum alertness will also strongly interfere with sleep, whereas the nadir of CBT will equally promote sleep (Åkerstedt and Gillberg, 1981; Czeisler et al., 1990).

#### **1.8.5. Sleep problems in shift workers**

Health problems associated with shift workers may be mediated directly by sleep problems as well as desynchrony. Shift workers are known to sleep less than day workers and to be more fatigued (Åkerstedt, 1990; Ohayon et al., 2002). However, there is limited knowledge on a possible relationship between lack of sleep and CVD, although sleep apnoea syndrome is a risk factor for CVD (Bøggild and Knutsson, 1999).

The majority (60-70%) of shift workers complain about their sleep; most of them report difficulties falling and staying asleep, poor quality of sleep, short duration of sleep and difficulties staying awake (Knauth et al., 1980; Marquie and Foret, 1999). Sleep after night work is usually shorter and more fragmented than sleep after day work (Wyatt and Marriott, 1953; Smith et al., 1982; Ahasan et al., 2001). Sleep after the night shift is usually initiated 1h after the termination of the shift (Knauth et al., 1981; Tepas, 1982), and studies using sleep diaries of workers have shown that the sleep period during the day is 1-4h shorter than that of sleep at night (Torsvall et al., 1989; Tepas and Carvalhais, 1990). Sleep studies have also reported that the total sleep duration is related to the body temperature rhythm at bedtime (Czeisler et al., 1980; Gillberg and Åkerstedt, 1982). Sleep difficulties may be partly social but there is a strong circadian influence on sleep latency that makes early initiation of sleep difficult following a night shift (review, Ahasan et al., 2001).



Sleep in shift workers can be altered by both endogenous (i.e. circadian, metabolic, hormonal) and exogenous (traffic, children playing, household noise) factors. Social stress factors (disruption of family organisation, parenting role) also contribute to sleep fragility in shift workers (Barton et al., 1995; Ahasan et al., 2001). In the day, there is a reduction of the 'deep sleep', which is essential for recovering from physical fatigue (Åkerstedt, 1984)

#### **1.8.6. Stress and shift work**

Another consequence of shift work is problems in family life, as the work hours interfere with social activities and family responsibilities.

Shift work reduces both the time available for family and recreation, and potentially leads to social isolation and subsequent stress. Married night workers with family commitments typically do not retain a day sleep regime during their off duty break, as they want to interact with day-orientated family, rather than being awake when everyone else is asleep (Monk, 2000). Stress is probably one of the key elements in the relationship between shift work and health problems (Bøggild and Knutsson, 1999).

Work efficiency during the night is also not the same as during the day. Wakefulness and activity is associated with daylight, whilst rest and sleep is associated with the night. The circadian fall in psycho-physical performance at night, in association with sleep deficit and strong feelings of fatigue, decreases the work efficiency of the night worker and increases the possibility of errors and accidents (Kelly and Schneider, 1982; Folkard et al., 2006).

#### **1.8.7. Shift work and cancer**

Recently there has been a hypothesis that has linked shift work and cancer, due to light exposure suppressing melatonin levels at night (Blask et al.,



2005; Schernhammer et al., 2006). This mechanism has been supported by animal studies (Van den Heiligenberg et al., 1999; Blask et al., 2005) and longitudinal and retrospective studies (Davis et al., 2001; Schernhammer et al., 2001).

#### **1.8.7.1. Animal studies**

Several experimental animal studies have provided evidence for an association between melatonin levels and the risk of breast cancer. For example, rodent studies found that pinealectomy increased tumour growth (Tamarkin et al., 1981) and that constant light exposure had growth-promoting effects on chemically induced tumours (Van den Heiligenberg et al., 1999). More recently a study by Blask et al. (2005) showed that rats bearing rat hepatomas or human breast cancer xenografts that were exposed to increasing intensities of white light during each 12h dark period resulted in a dose-dependent suppression of nocturnal melatonin blood levels and a stimulation of tumour growth and linoleic acid uptake/metabolism by the mitogenic molecule 13-hydroxyoctadecadienoic acid.

#### **1.8.7.2. Circadian mechanisms and cancer**

The biological mechanism underlying the association of light at night and breast cancer is assumed to be the suppression of normal nocturnal melatonin production in the pineal gland. The result is decreased levels of melatonin in the blood. Melatonin synthesis is regulated via the retina by the light/dark cycle (section 1.4.1) and influences the biological regulation of circadian rhythms, sleep and probably tumour growth (Blask et al., 2005). Filipski et al. (2006) demonstrated that rhythms of clock genes *per2* and *reverb- $\alpha$*  were suppressed in the liver and in the tumour of jet lagged mice. This functional disturbance of the molecular clock resulted in down regulation of *p53* and over expression of c-Myc, two effects which may favour cancer growth. These results indicated that the circadian system could play an important role in malignant growth control.



Women who are totally blind with no light perception are known to have a decreased risk of breast cancer, of approximately 50% (Hahn, 1991; Feychting et al., 1998) thus giving some support for this claim. However those who are blind may lead a different type of lifestyle which may contribute to the reduced risk.

### 1.8.7.3. Epidemiological studies

Persons who engage in night work, as a result of desynchrony, may have altered melatonin levels and reproductive hormone profiles that could increase the risk of hormone related diseases such as breast cancer (Davis et al., 2001; Hansen, 2001; Moser et al., 2006; Schernhammer et al., 2006). However this is largely speculative at present. There are numerous factors which affect the relative risk of developing breast cancer; these are shown in Figure 1-11.

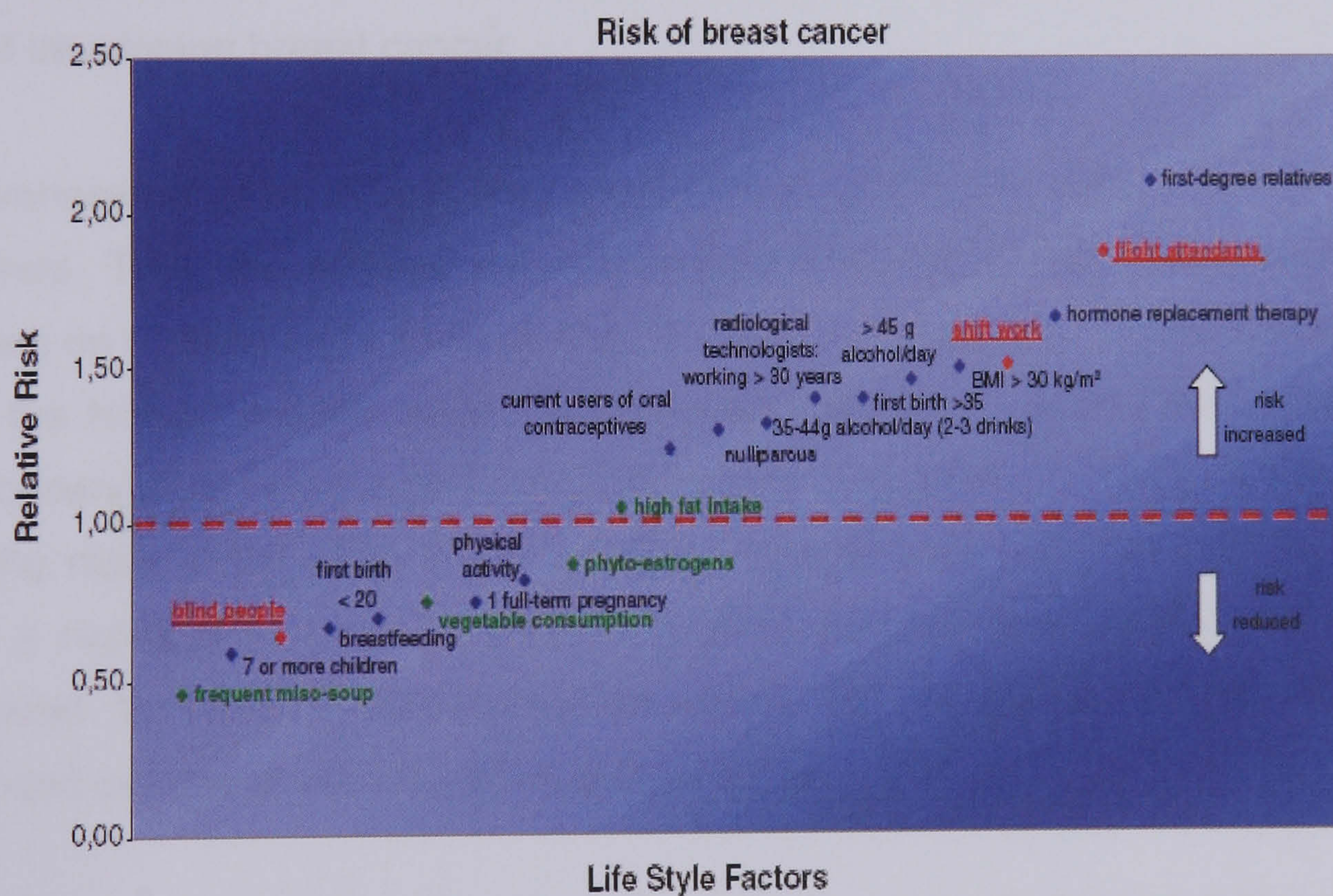


Figure 1-11: Factors affecting the breast cancer risk (Moser et al., 2006).

Hansen (2001) looked at 7035 Danish women aged between 30-54 years, who worked predominantly at night. In this Danish study, they found a



positive trend of increasing duration of work at night with a 1.5 fold increase in risk for primary breast cancer, in those who had worked for at least half a year in any trade with predominantly night work.

Davis et al. (2001) investigated the relationship between light exposures at night to see if it was associated with an increased risk of breast cancer. They assessed sleep habits and the bedroom environment of those who had worked the “graveyard shift”, which was defined as work beginning after 19.00 h and leaving work before 09.00h. The findings showed that breast cancer risk was increased in subjects who frequently did not sleep during the period of night where melatonin levels are at their highest. The graveyard shift was associated with increased risk of breast cancer (OR=1.6; 95% CI=1.0-2.5) with a trend of increased risk with increasing duration of doing shifts and with more hours per week of the graveyard shift. The study concluded, without the use of light measures, that the results provide evidence that exposure to light at night may be associated with an increased risk of developing breast cancer.

Schernhammer et al. (2001) conducted one of the largest cohort studies in this field. They investigated the relationship between breast cancers and working on rotating night shifts during 10 years of follow up in 78,562 women from the Nurses’ Health study. A moderate increase in breast cancer risk was observed among women who had worked 1-14 years or 15-29 years on rotating night shifts. The study concluded that women who had worked at least 3 nights per month, in addition to days and evenings in that month, appeared to have a moderately increased risk of breast cancer after extended periods of working rotating night shifts.

More recently Schwartzbaum et al. (2007) has reported that night shift work is not linked to an increased risk of breast cancer or prostate cancer. Schwartzbaum et al. (2007) came to this conclusion after analysing 20 years worth of data that compared people who reported jobs that required night shift work. Altogether 2,102,126 male and 1,148,661 female workers were



identified who worked in both 1960 and 1970. Their jobs were classified according to the percentage of shift workers, and they were followed from 1971 through 1989 or until they were diagnosed with cancer or died. There is much controversy and debate in this subject area.

#### **1.8.8. Summary of shift work and its effect on health**

There is substantial evidence suggesting that night shift work is detrimental to health. The increased risk of disease may be related to desynchronisation of internal bodily rhythms from the 24h light-dark cycle and/or from internal desynchronisation. This thesis investigates the utilisation of light treatment to reduce the time of circadian desynchronisation.



## **1.9. Objectives of the study**

With the support of the industry regulator (Health and Safety Executive) and the UK petrochemical industry offshore operators, this project was established with the following objectives:

### **1.9.1. Shift schedule effect on sleep and adaptation**

Adaptation to the 18.00-06.00h 2 week night shift offshore has been documented in previous studies (Barnes et al., 1998a). The 19.00-07.00h 2 week night shift has previously been unreported in terms of adaptation.

The aims and hypothesis of the study were to:

- Investigate the effects of shift schedule timing on sleep, circadian timing and light exposure. Two shift schedules; 2 weeks 18.00-06.00h and 2-3 weeks 19.00-07.00h were studied in the summer (chapter 3).

The null hypothesis stated there will be no effect of shift schedule timing on sleep and circadian adaptation.

### **1.9.2. Light box study**

Previous studies (Broadway et al., 1987; Eastmann and Martin, 1999; Revell et al., 2005) have shown that light in the morning can phase advance the circadian system. The Litebook<sup>®</sup> is a small portable light treatment device: whether or not the light box could cause a phase shift was investigated.

The aims and hypothesis of the study were to:

- To investigate the ability of a light box to phase advance the melatonin rhythm in healthy young male volunteers in controlled laboratory conditions (chapter 4). The aim of the study was to investigate the effects of a single bright light pulse to change the time of the internal clock (shift circadian phase).



- The null hypothesis stated there would be no effect of light treatment on the phase of melatonin (aMT6s acrophase, DLMO and DLMOff).

### **1.9.3. Light treatment in offshore shift workers**

Adaptation to the night shift is beneficial in terms of sleep and cognitive performance, however as a result of this adaptation problems arise at the end of the night shift concerning readapting back to day shift/home life. The use of light treatment, appropriately timed, at home may alleviate/reduce the physiological and behavioural problems caused by circadian rhythm disturbance.

The aims and hypothesis of the study were:

- To assess the use of timed light treatment to hasten circadian adaptation of offshore adapted night shift workers when they return home to day life and improve subjective and objective sleep (chapter 5).

The null hypothesis stated there would be no effect of light treatment on the phase of melatonin and subjective and objective sleep.

#### *Secondary objective: Light exposure and aMT6s excretion*

Subjects who have fully adapted to the night shift will return home onshore with their aMT6s rhythm peaking during the day. Light exposure during the day will probably suppress the subject's aMT6s rhythm.

The aims and hypothesis of the study were:

- To assess the effects of light exposure on the total production of melatonin (as aMT6s) upon returning home onshore (chapter 6).

The null hypothesis stated there would be no effect of light exposure on the suppression of aMT6s.



## 2. MATERIALS AND METHODS

### 2.1. Light and activity analysis by actigraphy

Light and activity data were collected by the use of an Actiwatch-L (Cambridge Neurotechnology Ltd), shown in Figure 2-1. This is a wrist type monitor, which is worn on the non-dominant wrist, outside the clothing. Measurements of light and activity were taken in one-minute epochs. The data is transferred telemetrically and analysed using Cambridge Neurotechnology software packages (Sleep Analysis 5, version 5.42). Light exposure (measured in lux) was calculated in half hourly bins, sleep periods were identified by use of sleep diaries (Section 2.6). The following parameters were studied: sleep efficiency, sleep latency, fragmentation index, sleep onset, offset and sleep duration.



Figure 2-1: Diagram of an Actiwatch-L

### 2.2. Assessment of diurnal preference





The Horne Östberg (HÖ) morningness-eveningness sleep questionnaire (*Appendix A*) is designed to assess the diurnal preference of individuals (Horne and Östberg, 1976). The questionnaire is able to identify differences in sleep and wake preference (chronotype) on the basis of score. Subjects answer a series of questions, the overall score is tallied up and diurnal preference is indicated as follows:



HÖ score	Diurnal Preference
16-30	Extreme evening type
31-41	Moderately evening type
42-58	Neither type
59-69	Moderately morning type
70-80	Extreme morning type

### 2.3. Mood assessment

Subjective alertness, anxiety, mood and energy were self-rated using a visual analogue scale by marking a point on a 10 cm line of paper. The following questions were answered within an hour of awakening:

Very sad		Very happy
Very anxious		Very calm
Very lethargic		Very energetic
Very drowsy		Very alert

### 2.4. Sleep diaries

Sleep diaries (*Appendix B*) were completed throughout the duration of the studies. The diaries obtained the following information:

1. Date (date woke up on)
2. Time of retiring to bed (e.g. 23:00h)
3. Time of trying to sleep (e.g. 23:15h)
4. How long it took to fall asleep (minutes)



5. Number of night awakenings
6. The total duration of night awakenings (minutes)
7. Time of awaking
8. Time of getting up
9. Sleep quality rated on a scale of 1-9 (1 best ever sleep, 9 worst ever sleep).
10. Naps, time and duration

### **2.5. General principles of radioimmunoassay**

Radioimmunoassay (RIA) is an *in-vitro* method of measurement, whereby a fixed amount of radioactively labelled antigen is allowed to react with a fixed but limited number of specific antibodies in such relative concentrations that only 50% approximately of the added label is bound to the antibody fraction. Once equilibrium is reached during the incubation period, the portion of the label, which is bound to antibody, is separated, e.g. using charcoal that binds to free antigen. A distribution between bound and free antigen is shown for different concentrations of unlabelled antigen. If a quantity of unlabelled antigen, present in either a standard solution or sample, is added to the mixture of labelled antigen and antibody before incubation commences, the unlabelled antigen will compete with the labelled antigen for the binding sites on the limited number of antibodies present. This results in fewer labelled antigen molecules being bound by the antibody which in turn results in there being fewer counts in the antibody bound fraction. The more unlabelled antigen molecules present, the fewer the labelled molecules will be bound to the antibody. The bound and unbound antigens are separated and the radioactivity in either bound or unbound fractions is counted. The samples are compared to a standard curve, which is constructed using a set of aMT6s or melatonin standards of a known concentration. The radioactivity is counted by a gamma counter or beta scintillation counter in counts per minute (cpm).



## 2.6. Radioimmunoassay of melatonin in human saliva

Salivary melatonin was determined by RIA with a protocol modified from English et al (1993).

### 2.6.1. Sample collection

A non-citrated cotton Salivette (Starstedt Ltd, Leicester, UK) was used to collect saliva. The samples were immediately frozen upon collection and stored at  $-20^{\circ}\text{C}$  until analysis.

### 2.6.2. Reagents

**Tricine buffer (0.1M):** The assay buffer was freshly prepared each week. This buffer consisted of tricine (0.1 mol/l), 0.9% NaCl and 0.2% gelatin, made up in double glass-distilled water (DGDW) to give a stock solution of pH 5.5, this was adjusted to pH 8.0 by 1 M NaOH and stirred on a heater to  $50^{\circ}\text{C}$  for 30 mins to dissolve the gelatin.

**Antiserum:** The antimelatonin antiserum used in the assay was supplied freeze dried. Rabbit anti-melatonin antibody (product no: AB/R/031) was obtained from Stockgrand Ltd, University of Surrey, UK. These aliquots (50  $\mu\text{l}$  aliquot to 10 ml tricine buffer) were reconstituted to achieve a final working solution of 1:30,000.

**Separation system for bound and unbound antigen:** The solid phase, double antibody coated cellulose suspension was purchased from IDS, Bolden, Tyne and Wear, UK (product no. AA-SAC-1). A solid phase second antibody-coated cellulose suspension (SAC-CEL) was used. Brij/Saline solution (0.15 M NaCl, 2% Brij 35) was used as a detergent to wash the suspension.

**Radiolabelled melatonin:**  $^{125}\text{I}$ -2-iodomelatonin was purchased from Amersham International (Amersham, Buckinghamshire, UK).  $^{125}\text{I}$ -2-



iodomelatonin was diluted in tricine buffer to give approximately 10,000 cpm in 100  $\mu$ l.

**Melatonin standard:** this was supplied by Sigma Ltd (Bedford, UK). The melatonin stock solution was made up to 1 mg/ml by dissolving 10 mg melatonin in 0.5 ml absolute ethanol and adjusting the volume to 10 ml with DGDW.

The working standard was freshly prepared from the stock solution, as follows:

100  $\mu$ l of stock solution (1 mg/ml) to 100 ml DGDW to give 1  $\mu$ g/ml

500  $\mu$ l of 1  $\mu$ g/ml solution to 50 ml DGDW to give 10 ng/ml

50  $\mu$ l of 10 ng/ml to 2.5 ml tricine buffer to give 0.2 ng/ml (200 pg/ml top standard).

Melatonin concentration (pg/tube)	Assay Buffer ( $\mu$ l)	Melatonin standard 200 pg/ml ( $\mu$ l)
0	500	0
1	495	5
2	490	10
4	480	20
8	460	40
14	430	70
20	400	100
40	300	200
100	0	500

Table 2-1: Preparation of melatonin assay standards.

### 2.6.3. RIA procedure

Duplicate tubes were prepared for all samples, standards; total count tubes, non-specific binding and quality control (QC) samples, zero binding tubes (0



pg/ml melatonin) were prepared in quadruplicate. Saliva samples were thawed and centrifuged at 3000 rpm for 10 mins. 500 µl aliquots of saliva samples and QCs were incubated with 100 µl of antiserum, the samples were vortexed and left to incubate for 30 mins at room temperature. 100 µl of <sup>125</sup>I-2-iodomelatonin was added to each sample and incubated overnight at 4°C. The free and antibody bound fractions were separated using solid-phase antibody precipitating system (100 µl of SAC-CEL). The assay was intermittently mixed for 1h at room temperature. Then 100 µl Brij/Saline solution was added to each of the tubes and centrifuged at 3000 rpm for 4°C (Beckman J6 centrifuge, Beckman-Coulter, High Wycombe, UK). The supernatant was discarded and concentrations of samples were measured in a gamma counter from the standard curve using a "RIACalc" program, RIACalc Wallac International, Finland.

The RIACalc program automatically determines the percentage of the total counts bound or free, and plots this as a function of known concentrations of melatonin standards. A curve is fitted through the standard points based on the smoothing spline calculation method. This fits a continuous curve to each pair of standard points using the conventional methods of least squares, but in addition, it calculates the best-fitting curve with the minimum number of turning points and is as smooth as possible. The concentrations of the unknown samples are determined from this curve by extrapolation.

Quality Controls (QCs) were included in each assay to assess intra-assay and inter-assay variation. Quality control samples contain known concentrations of the antigen. A range of QCs were used to read low, medium and high across the standard curve. Comparison of the QC values at the start and the end of each assay was performed to ensure that there was no assay drift; variation in measured values due to reagents not being added at the same interval to all tubes and subsequent variation of incubation times. Comparison of the QCs after the standard curve were used to ensure there was minimal inter and intra-assay variation, and that the



assay results are reliable, with a tolerance of variation of <15% was accepted.

#### **2.6.4. Calculation of dim light melatonin onset**

The dim light melatonin onset (DLMO) was calculated using the twice individual baseline value method.

For example, if baseline values for 3 time points were 20:00h- 5 pg/ml, 20:30h -5.3 pg/ml and 21:00h – 6 pg/ml for an individual, the mean of these 3 time points (5.4 pg/ml) is the mean baseline value. Twice the baseline is thus 10.8 pg/ml. The time associated with this value is between time point 21:00h (6 pg/ml) and 21:30h (13.2 pg/ml). The change in melatonin concentrations during this period is calculated as (13.2-6) and the change in concentration per min is ((13.2-6)/30). The number of minutes after the start of this time period (21:00h) that the twice baseline value occurs was calculated as follows:

No. of mins = (twice baseline value-lowest melatonin concentration in time period) / (change in melatonin concentration)/mins.

In this example  $(10.8-6) / [(13.2-6)/30] = 20$  mins

The number of mins is then added to the start time period 21:00h + 20 mins = 21:20 h (h:mins) = (21.33 decimal h). Therefore, the DLMO calculated using the twice baseline method in this example occurs at 21:20h (21.33 decimal h).

#### **2.7. RIA of 6-sulphatoxymelatonin (aMT6s) in human urine**

The urinary melatonin metabolite aMT6s was measured by a specific radioimmunoassay (Aldhous and Arendt, 1988), adapted from Arendt et al. (1985).



### 2.7.1. Sample collection

Circadian status was determined by measurement of the aMT6s rhythm in urine. All urine was collected every time the subject urinated using the urine collection protocol (*Appendix C*). The time and volume of urine was recorded, a 5 ml sample was aliquoted into a sample collection tube and frozen until further analysis.

### 2.7.2. Reagents:

**Tricine buffer (0.1M):** The assay buffer was freshly prepared each week. The buffer consisted of tricine (0.1 mol/l), 0.9% NaCl and 0.1% gelatin made up in double glass-distilled water (DGDW) to give a stock solution, this was adjusted to pH 5.5 by 1M NaOH and heated to 50°C for 30 mins to dissolve the gelatin.

**Antiserum:** Specific polyclonal sheep anti-aMT6s antiserum was supplied freeze dried by Stockgrand Ltd, University of Surrey and was reconstituted with 1ml DGDW and 9 ml of tricine buffer to provide an intermediate dilution of 1:100. 50 µl aliquots were stored at -20°C. 50 µl aliquots were diluted up to 20 ml with tricine buffer and provided enough antiserum for 100 assay tubes at an initial dilution 1:20,000.

**Radiolabel:** <sup>125</sup>I-aMT6s was prepared and provided by Stockgrand Ltd, University of Surrey, UK. It was diluted in buffer to give approximately 10,000 cpm in 100 µl.

**Charcoal stripped urine:** This was provided by Stockgrand Ltd in freeze-dried vials (100 µl) and was reconstituted with 25 ml of tricine buffer to give a dilution of 1:250.

**aMT6s standards:** 500 pg aMT6s was supplied by Stockgrand Ltd at 100 pg/ml diluted 1:250 in charcoal stripped urine and this was stored at 4°C. Standards were further diluted for the standard curve.



**Separation system:** dextran coated charcoal containing 2% activated charcoal (product code C5260) was suspended in tricine with 0.02% dextran (product code D1390, Sigma Ltd, UK) and stirred overnight at 4°C.

### 2.7.3. RIA Procedure

Urine samples were diluted 1:250 with tricine buffer by use of an automatic diluter (Hamilton Microlab, USA). Urine samples, QC's and standards in 500 µl aliquots were incubated with 200 µl of anti-aMT6s antiserum, which binds to the antigen present in the sample and/or to radioactive label. The reaction was incubated at room temperature for 30 mins. 100 µl of <sup>125</sup>I-aMT6s was then added, mixed and the assay incubated overnight at 4°C. The free fraction of aMT6s was separated by addition of charcoal, which before adding was continually stirred for 5 mins to ensure that it was suspended properly in the buffer. The tubes were incubated at 4°C for 15 mins and then centrifuged for 3,500 rpm for 15 mins at 4°C. The supernatant was discarded and the radioactivity of the pellet counted by a gamma counter, by way of counts per minute (cpm). The measured radioactivity is associated with the free antigen proportional to the concentration of the test antigen, when measured against standards. The standard aMT6s concentrations are shown in Table 2-2.



aMT6s standard 200 pg/ml (µl)	aMT6s free urine (1:250) (µl)	aMT6s concentration (ng/ml)
0	500	0
5	495	0.5
10	490	1
20	480	2
40	460	4
70	430	7
100	400	10
200	300	20
500	0	50

Table 2-2: Preparation of aMT6s assay standards.

#### 2.7.4. Cosinor Analysis

Cosinor analysis is a curve fitting procedure that assumes the rhythm is sinusoidal in shape and fits the best fitting cosine curve to the data. Software to do this analysis was obtained from Dr D Minors, Manchester University, UK. This method of analysis makes two estimates of 'goodness of fit', which are then used to determine the validity of the cosinor-derived parameters of the acrophase (peak time) and amplitude. The first is the percentage variability in the data accounted for by the cosine curve and is given as a percentage rhythm (%R). A 100% rhythm would mean all the points fall on the curve so the larger the percentage rhythm the better the fit. The analysis also checks the likelihood of the data points fitting a straight line as opposed to a cosine curve, or how probable it is that the data could fit the cosine curve by chance. A 'significant fit' to the cosine curve is deemed to be when there is <5% probability that the data would fit a straight line i.e.  $p < 0.05$  (Nelson et al., 1979). Acrophase values were only accepted if the cosinor fit was significant at the 95% level or if the fit was significant at >80% level and the variance (percentage rhythm) accounted for by the cosine curve was greater than 50%.



## **2.8. Statistical analyses**

Methods for statistical analysis will be detailed in each chapter separately.



### 3. THE EFFECTS OF CIRCADIAN ADAPTATION ON OFFSHORE SLEEP

#### 3.1. Introduction

Shiftwork leads to desynchronisation of internal biological rhythms and as a result there are problems of physiology (e.g. digestive problems) and behaviour (e.g. sleep) (section 1.8). Sleep is one of the major complaints that are associated with working night shifts (section 1.8). The majority (60-70%) of night shift workers complain about their sleep; most of them report difficulties falling and staying asleep, poor quality of sleep, short sleep duration and difficulties staying awake (Knauth et al., 1980; Marquie and Foret, 1999). Attempts to sleep during the day at inappropriate phases of the circadian cycle, for example during the declining phase of melatonin and rising phase of core body temperature, usually results in shorter sleep episodes, more awakenings, night time sleepiness and reduced performance (Åkerstedt and Gillberg, 1990; Kecklund and Åkerstedt, 1993; Bjorvatn et al., 1999).

Complete circadian adaptation to the night shift is rare (section 1.7.6). It is observed in situations where night shift workers work and sleep in isolated locations such as Antarctica (Broadway and Arendt, 1988; Midwinter and Arendt, 1991; Ross et al., 1995) and on offshore oil installations (Barnes et al., 1998a; Gibbs et al., 2002). In these situations social influences, which can impede night shift adaptation, are removed. Inappropriate morning bright light exposure, which counters adaptation (Eastman et al., 1994; Koller et al., 1994) can be minimised when workers do not have to travel home after night work. Nevertheless the presence of early morning natural light (in summer) may hinder and its absence (in winter) may aid adaptation to the night shift.

Daily rhythms in sleep and waking performance are generated by the interplay of multiple external and internal rhythms. These rhythms include the light-dark and social cycles, to circadian oscillators and homeostatic factors which are driven primarily by sleep-wake behaviour (section 1.2.1). Internal



oscillators contribute to variation in many aspects of sleep and wakefulness (e.g., sleep timing and duration and performance parameters, including attention and memory) (Dijk and von Schantz, 2005). With respect to the relationship between circadian adaptation and sleep, there is ample evidence for a close temporal relationship between the melatonin secretory phase, thermoregulation and circadian sleep propensity (Cajochen et al., 2003). Therefore if these factors are not carefully aligned then the duration and quality of sleep will be affected.

Due to the circadian sleep propensity rhythm, sleep after working night shift is reported to be more fragmented and of shorter duration due to the unadapted internal biological clock (Wyatt and Marriott, 1953; Smith et al., 1982; Torsvall et al., 1989; Åkerstedt, 2003). Compared to those working night shifts onshore, the reasons for improved night shift sleep quality offshore might be that there are no family or lifestyle disturbances. Home and family duties may also explain why day time sleep is reduced in onshore night shift workers; offshore there is less to do in free time (watch TV or go to the gym) and therefore there is more time that can be dedicated to sleeping.

### **3.2. Aims and hypothesis**

This study evaluated sleep (subjective and actigraphic), light exposure (actigraphy) and circadian phase (using the urinary metabolite of melatonin, 6-sulphatoxymelatonin, aMT6s) on 2 shift schedules differing by only one hour in work start and finish time, 18.00-06.00h and 19.00-07.00h, but with potential of different natural light exposure.

The null hypothesis stated there will be no effect of shift schedules on sleep and circadian adaptation.



### **3.3. Study design and methods**

Methodologies specific to this study are outlined here, for full details see chapter 2, Materials and Methods. Individual methods are cross referenced.

#### **3.3.1. Subject recruitment**

Ethical permission was obtained from the University of Surrey Ethics Committee (Ethics number: ace/2002/95SBLs). Five offshore installations were used in the North Sea, at geographical latitudes 58, 59 and 60°N. Subjects were free of medications indicated by the protocol exclusion criteria, and gave written informed consent.

##### **3.3.1.1. Summer 19.00-07.00h subjects**

Ten subjects were studied in the summer months (May-August, 2005) for the last 7 days of a 2 or 3 week night shift, 19.00-07.00h, on 3 offshore installations at 60°N. They were aged  $46 \pm 10$  years (mean  $\pm$  SD), with a body mass index (BMI) of  $27.9 \pm 2.3 \text{ kg/m}^2$ , and a HÖ score of  $57 \pm 8$ .

##### **3.3.1.2. Summer 18.00-06.00h subjects**

Seven subjects were studied in the summer months (May-August, 2003) for the last 7 days of a 2 week night shift, 18.00-06.00h, on 2 offshore installations at 60°N. They were aged  $41 \pm 12$  years (mean  $\pm$  SD), with a BMI of  $26.6 \pm 3.6 \text{ kg/m}^2$ , and a HÖ score of  $56 \pm 9$ .

#### **3.3.2. Study design**

Subjects kept daily sleep diaries and wore Actiwatch-L's to monitor light and activity/sleep (epoch length 1 mins) throughout the last week offshore of a 2 or 3 week night shift. Sequential urine samples (approximately every 4 hours and over the sleep period) (*Appendix C*) were collected throughout the last 3 days offshore (72h). Circadian phase was determined by measuring



urinary aMT6s (section 2.2.3) and calculating the peak time of excretion by cosinor analysis (section 2.2.4).

### **3.3.3. Statistics**

Statistical analysis: correlations were done by Pearson's *r*. Other statistics were sleep analysis by RM-ANOVA (Statistica), and aMT6s data by unpaired Student's *t*-test (Prism) or otherwise as stated in the text.

## **3.4. Results**

Sleep and circadian adaptation between two offshore shift schedules (18.00-06.00h and 19.00-07.00h) for the last week offshore of a two or three week night shift was compared. No significant difference was observed between the two shift schedules in terms of age, BMI and HÖ score.

### **3.4.1. Summer, 19.00-07.00h subjects**

#### **3.4.1.1. Circadian adaptation**

The mean aMT6s acrophase for the adapted subjects working the 19.00-07.00h shift schedule during the summer was  $14.6 \pm 0.55$ h, mean  $\pm$  SEM (N=7). Two of the ten subjects did not adapt to the night shift as their peak aMT6s acrophase was outside of the sleep period. These subjects CT-01 and CT-02 had offshore acrophases of  $4.3 \pm 0.22$ h and  $5.3 \pm 0.29$ h respectively. One other subject was excluded due to insufficient data (one data point only) and the possibility of being free-running or non-adapted with an acrophase of 18.4h. He was excluded from further analysis.



### 3.4.1.2. Actigraphic and subjective sleep

Objective sleep parameters derived from actigraphy and subjective sleep parameters derived from sleep diaries are shown below in Table 3-1, for those working 19.00-07.00h.

		Adapted (N=7)		Non-adapted (N=2)	
Sleep Parameters		Mean	SEM	Mean	SEM
<b>Objective sleep</b>	Sleep Duration (Dec. h)	5.71	0.27	4.24	0.39
	Sleep Efficiency (%)	77.9	2.3	80.8	5.1
	Fragmentation Index	36.3	5.7	25.8	6.2
	Sleep Onset (Dec. h)	9.51	0.31	10.76	0.11
	Sleep Offset (Dec. h)	16.27	0.37	16.11	0.04
	Sleep Latency (Dec. h)	0.40	0.08	0.16	0.04
<b>Subjective sleep</b>	Sleep Duration (Dec. h)	6.58	0.57	5.15	0.23
	Sleep Onset (Dec. h)	9.28	0.35	10.57	0.11
	Sleep Offset (Dec. h)	16.41	0.36	16.11	0.06
	Sleep Latency (Dec. h)	0.13	0.02	0.14	0.05

Table 3-1: Objective sleep and subjective sleep parameters for the last 6 days of a 2 or 3 week nightshift for adapted (N=7) and non-adapted (N=2) subjects working a 19.00-07.00h shift schedule.

### 3.4.1.3. Circadian adaptation and sleep duration

Sleep duration determined by actigraphy for the last 6 days of a 2 or 3 week night shift was shorter in non-adaptors ( $4.69 \pm 0.20\text{h}$ ,  $3.78 \pm 0.41\text{h}$ ), than adaptors ( $5.71 \pm 0.20\text{h}$ , N=7) with no other differences in the objective and subjective sleep parameters studied. These differences between adapted and non-adapted subjects are illustrated in Figure 3-1.



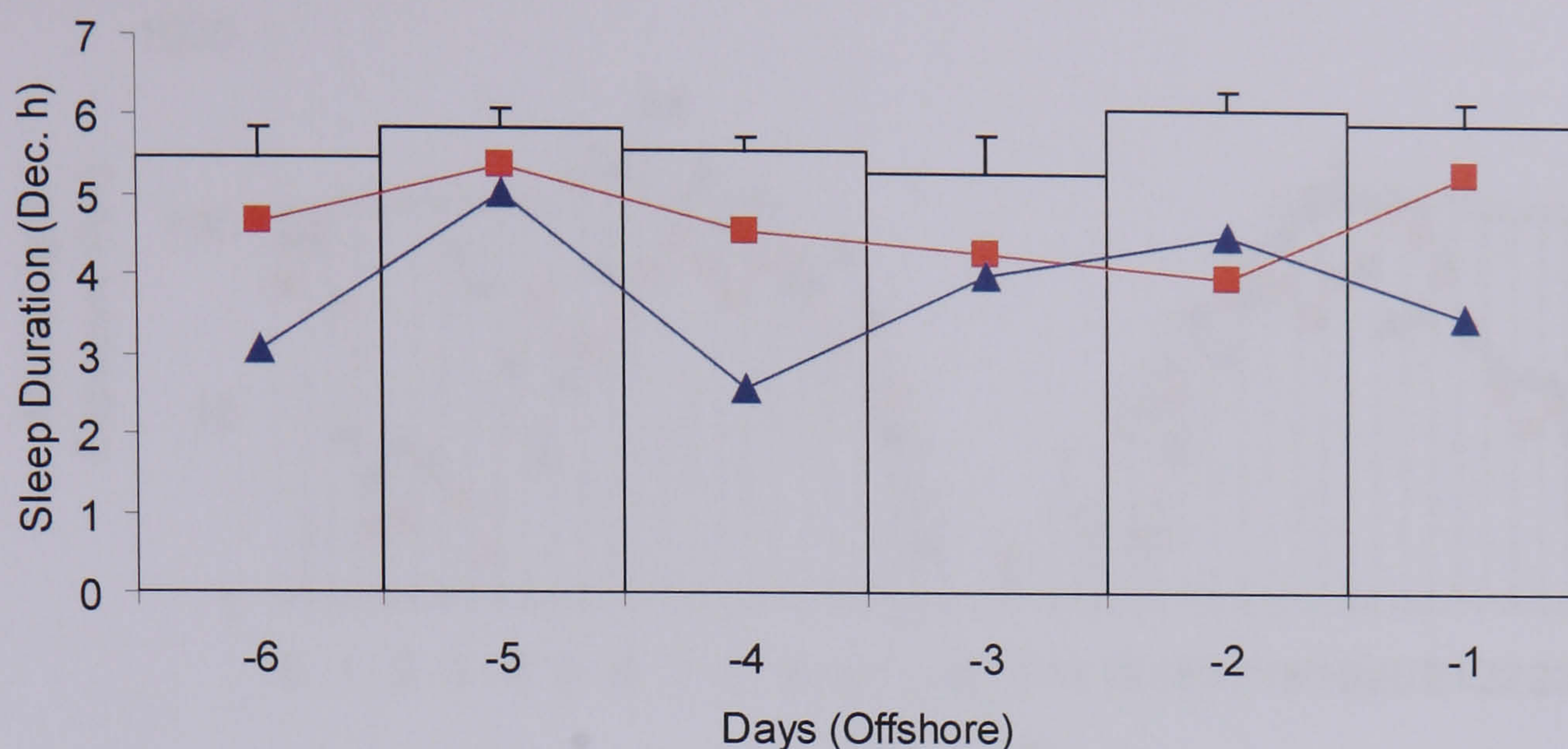


Figure 3-1: Sleep duration between non-adapted subjects (blue squares (CT-01) and red triangles (CT-02)) and adapted subjects (white blocks, mean  $\pm$  SEM) (N=7).

#### 3.4.1.4. Circadian adaptation and light exposure

Light exposure profiles (analysed in half-hourly bins) derived from actigraphy data showed that there were differences between adapted (N=7) and non-adapted (N=2) subjects working the 19.00-07.00h shift schedule. Adapted subjects had higher mean light levels at night (01.30-02.30h,  $121 \pm 27$  lux) and lower light exposure in the morning between 09.30-11.00h ( $15 \pm 8$  lux) compared to non-adaptors ( $5 \pm 1$  and  $145 \pm 49$  lux respectively). These differences between adapted and non-adapted light exposure profiles are illustrated in Figure 3-2.



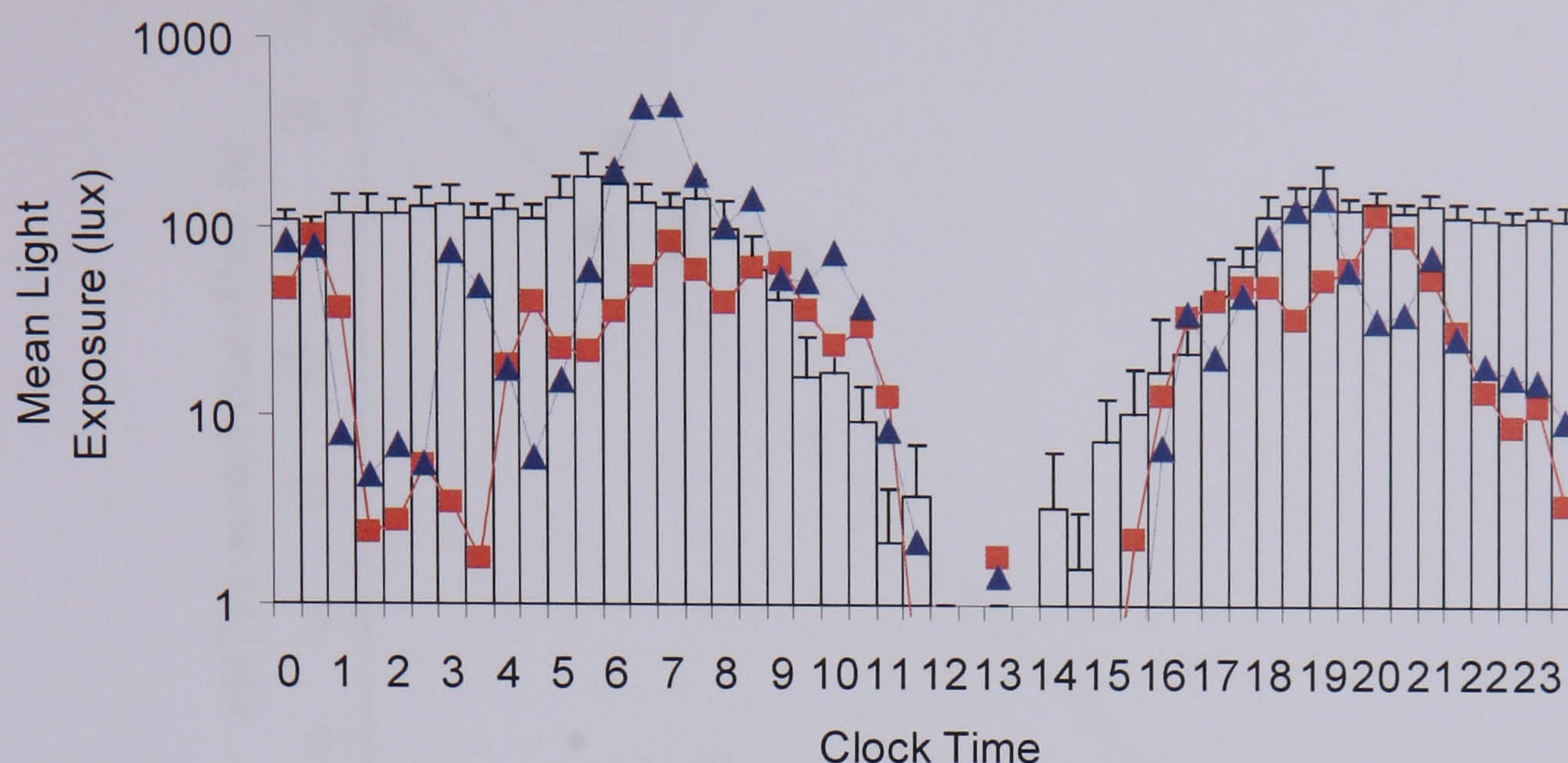


Figure 3-2: Light exposure between non-adapted subjects (blue triangles (CT-01) and red squares (CT-02)) and adapted subjects (white blocks, mean  $\pm$  SEM, N=7). Light exposure on the y-axis is on a log scale.

#### 3.4.1.5. Circadian adaptation and age

The two subjects (CT-01 and CT-02) who did not adapt to the nightshift were aged 54 and 65 years. Those who did adapt to the night shift had a mean age of  $43.3 \pm 7.4$  years (mean  $\pm$  SD).

#### 3.4.1.6. Diurnal preference (HÖ) and adaptation

For those working 19.00-07.00h in the summer months a significant correlation ( $r = -0.60$ ,  $p = 0.01$ ) was seen between mean aMT6s acrophase for the last 2 days offshore and HÖ score, which evaluates morningness and eveningness. Early aMT6s acrophases were associated with morningness. This data is illustrated graphically in Figure 3-3.



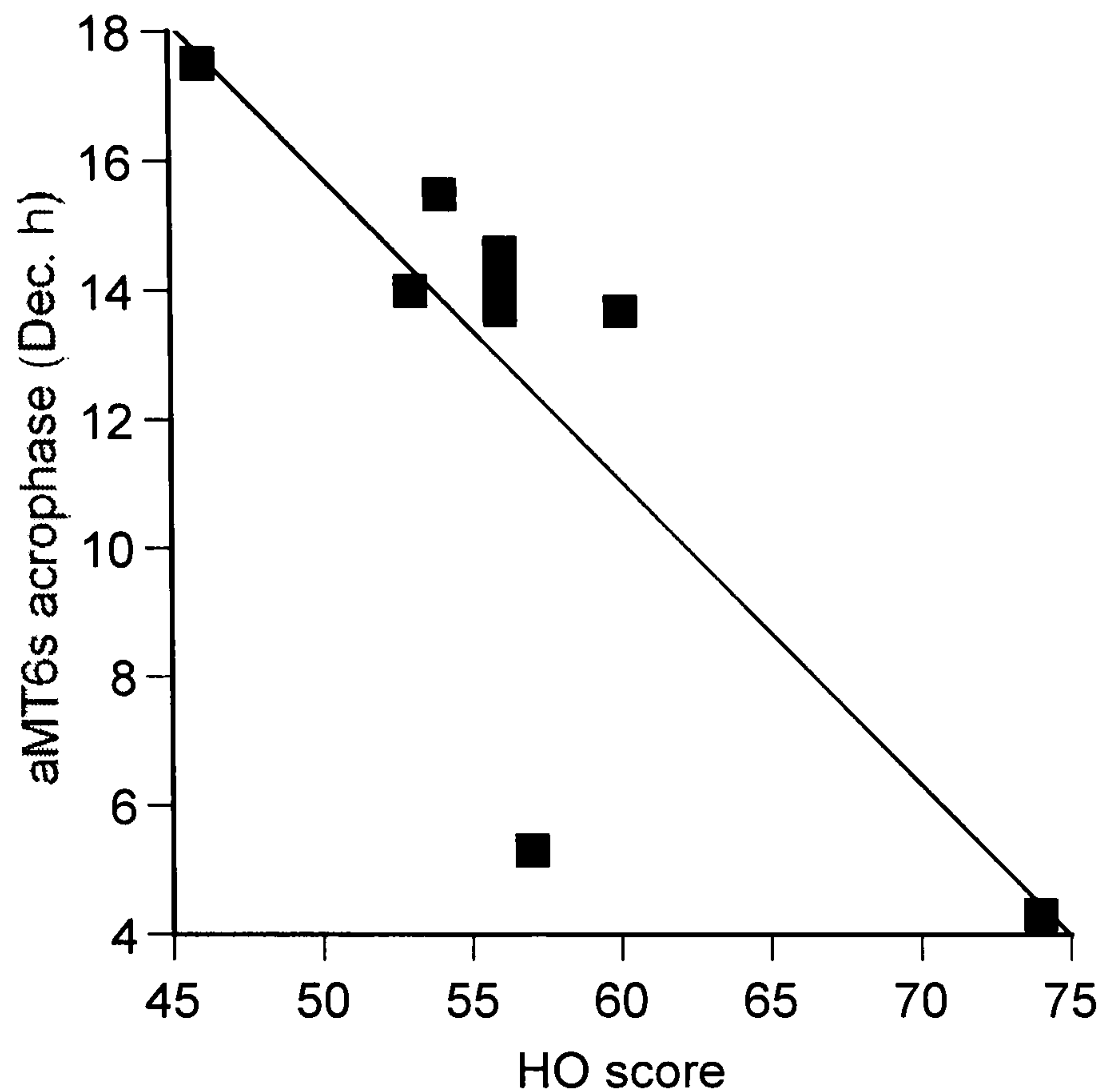


Figure 3-3: Relationship of individual Hö scores with aMT6s acrophases for subjects working 19.00-07.00h in summer (N=9,  $r=-0.60$ ,  $p=0.01$ ). One subject was excluded due to no Hö score.

### 3.4.2. Summer, 18.00-06.00h subjects

#### 3.4.2.1. Circadian adaptation

All of the 7 subjects working 18.00-06.00h during the summer were adapted, with a mean aMT6s acrophase of  $11.7 \pm 0.77$ h (N=7).

#### 3.4.2.2. Actigraphic and subjective sleep

Objective sleep parameters derived from actigraphy and subjective sleep parameters derived from sleep diaries are shown below in Table 3-2.



		<b>Sleep Parameters</b>	<b>Mean</b>	<b>SEM</b>
<b>Objective Sleep</b>		Sleep Duration (Dec. h)	6.60	0.30
		Sleep Efficiency (%)	82.2	2.2
		Fragmentation Index	36.0	2.7
		Sleep Onset (Dec. h)	9.23	0.37
		Sleep Offset (Dec. h)	16.48	0.24
		Sleep Latency (Dec. h)	0.35	0.13
<b>Subjective Sleep</b>		Sleep Duration (Dec. h)	7.09	0.16
		Sleep Onset (Dec. h)	9.13	0.38
		Sleep Offset (Dec. h)	16.80	0.19
		Sleep Latency (Dec. h)	0.31	0.12

Table 3-2: Objective and subjective sleep parameters (N=7) for the last 6 days of a 2 or 3 week night shift for subjects working 18.00-06.00h in the summer.

### **3.4.3. Comparison between 18.00-06.00h and 19.00-07.00h shift schedule**

#### **3.4.3.1. Subject demographics**

Diurnal preference did not differ significantly between the two groups with mean HÖ scores of  $57 \pm 2$  and  $56 \pm 4$  for the 18.00-06.00h and 19.00-07.00h subjects respectively. There were no significant differences in age or BMI between the two groups or for those working 2 or 3 weeks of a 19.00-07.00h night shift.

#### **3.4.3.2. Diurnal preference and adaptation**

No significant correlation ( $r=-0.48$ ,  $p=0.08$ ) was observed when both shift schedule groups (18.00-06.00h and 19.00-07.00h) were analysed together between HÖ score and aMT6s acrophase.

#### **3.4.3.3. Circadian adaptation**

Circadian adaptation to the night shift was observed in all the subjects working the 18.00-06.00h (N=7) and the majority (7/10) of those working the



19.00-07.00h shift schedules. Significant differences were observed between the mean aMT6s acrophase for subjects working 18.00-06.00h, which was  $11.7 \pm 0.77$ h (N=7), and adapted subjects working 19.00-07.00h, which was  $14.6 \pm 0.55$ h (N=7) ( $p=0.01$ ), shown in Figure 3-4.

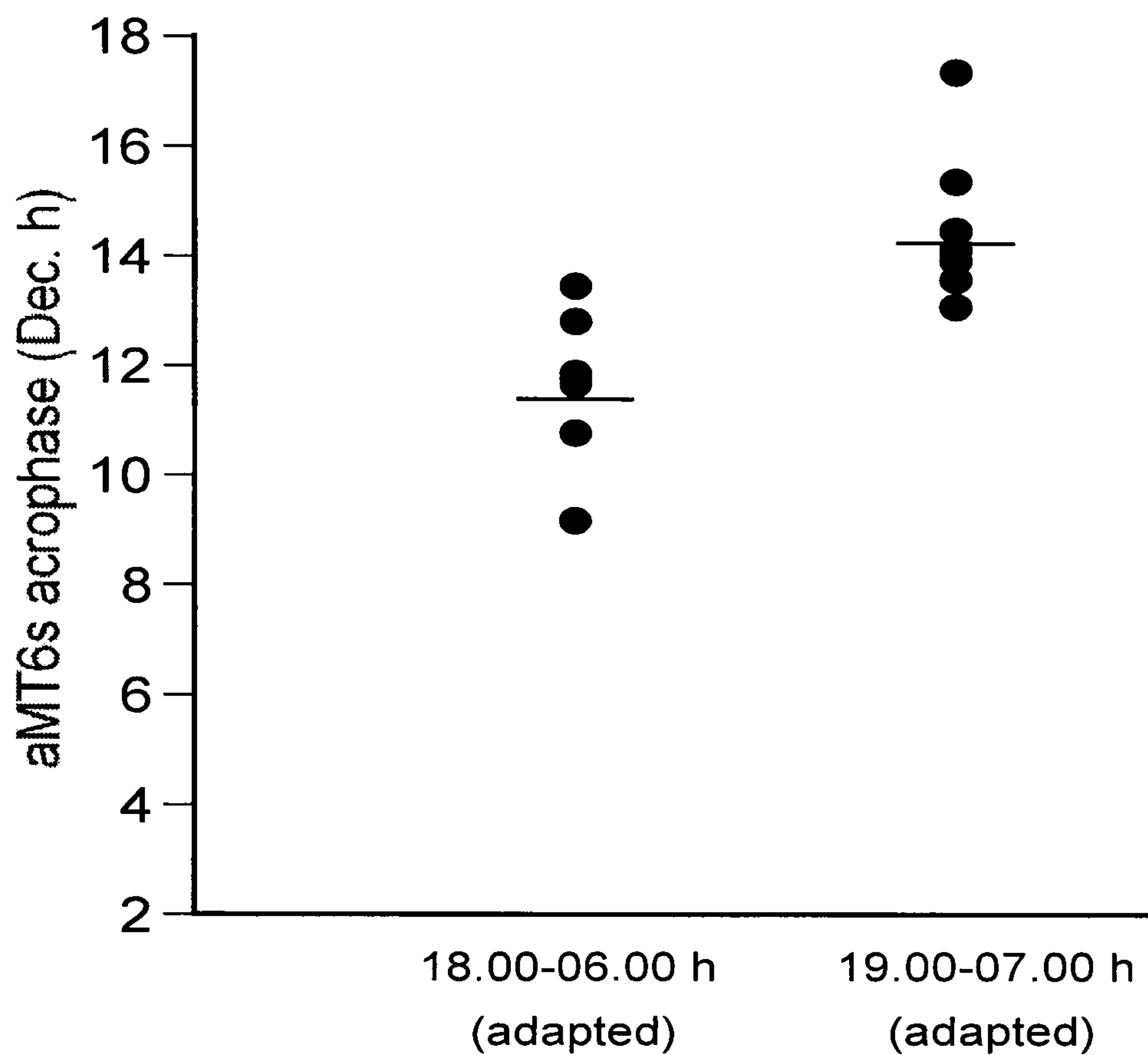


Figure 3-4: Mean aMT6s acrophase for the last 3 days offshore of a 2 or 3-week night shift. (●, individual data; –, mean aMT6s acrophase) for adapted subjects working 18.00-06.00h (N=7) and adapted subjects working 19.00-07.00h (N=7).

#### 3.4.3.4. Actigraphic sleep parameters

Actigraphy analysis of the last 6 days of night shift offshore showed significant differences in sleep duration between the two shift schedules. The sleep duration for adapted subjects working 19.00-07.00h ( $5.71 \pm 0.27$ h mean  $\pm$  SEM, decimal hours) was significantly shorter than for those working 18.00-06.00h, where actual sleep time was  $6.60 \pm 0.30$ h. There was a significant difference in sleep duration between the two groups ( $F_{(1,12)} = 6.20$ ,  $p < 0.05$ , two way RM-ANOVA) illustrated in Figure 3-5.



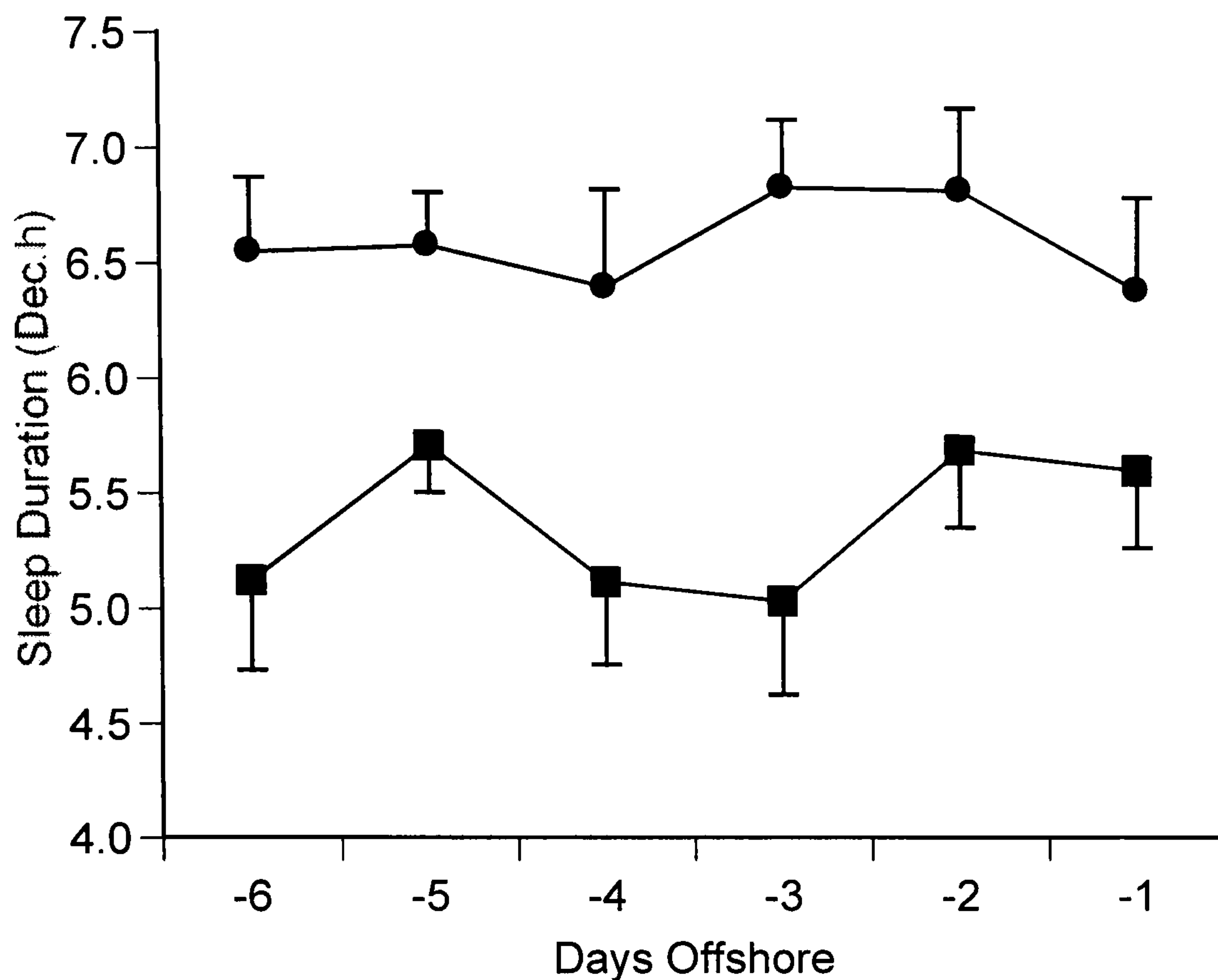


Figure 3-5: Sleep duration (mean  $\pm$  SEM) working 18.00-06.00h (circles) and 19.00-07.00h (squares) shift schedules on the last 6 days of the night shift.

All other derived actigraphic sleep parameters were not significantly different between the two groups, but those working 18.00-06.00h, along with higher sleep duration tended to have higher sleep efficiency ( $82.2 \pm 2.2\%$ ) compared to  $78.6 \pm 2.4\%$  for those working 19.00-07.00h. There were no differences in fragmentation index ( $36.0 \pm 2.7$  and  $36.3 \pm 5.7$ ), sleep latency ( $0.35 \pm 0.13$ h and  $0.40 \pm 0.08$ h), sleep onset ( $9.23 \pm 0.37$ h and  $9.51 \pm 0.31$ h) and offset ( $16.48 \pm 0.24$ h and  $16.27 \pm 0.37$ h), for the 18.00-06.00h and 19.00-07.00h shifts respectively. Although, whilst not significant, the workers on the 19.00-07.00h shift schedule went to bed later and thus may have been exposed to more morning light (section 3.4.3.5).

#### 3.4.3.5. Subjective sleep

Subjective sleep from sleep diaries showed slight trends for improved sleep in the 18.00-06.00h shift schedule compared to 19.00-07.00h. Sleep duration was  $7.09 \pm 0.16$ h and  $6.58 \pm 0.57$ h, sleep onset,  $9.13 \pm 0.38$ h and  $9.28 \pm 0.35$ h, sleep offset,  $16.80 \pm 0.19$ h and  $16.41 \pm 0.36$ h, and sleep



latency,  $0.31 \pm 0.12\text{h}$  and  $0.13 \pm 0.02\text{h}$  for those working 18.00-06.00h and 19.00-07.00h, respectively.

### 3.4.3.6. Light exposure

There was a trend observed with subjects working 19.00-07.00h compared to 18.00-06.00h. This group were exposed to more morning light between 07.30-10.30h, where mean light exposure ( $\pm$  SEM) was  $56 \pm 22$  lux, in comparison to subjects working 18:00-06:00 h (N=6, one subject was excluded due to the actiwatch not being worn during the night) where mean light exposure was  $17 \pm 15$  lux. ( $F_{(1,14)}=3.80$ ,  $p=0.07$ , two way-ANOVA, time and group as factors) illustrated in Figure 3-6.

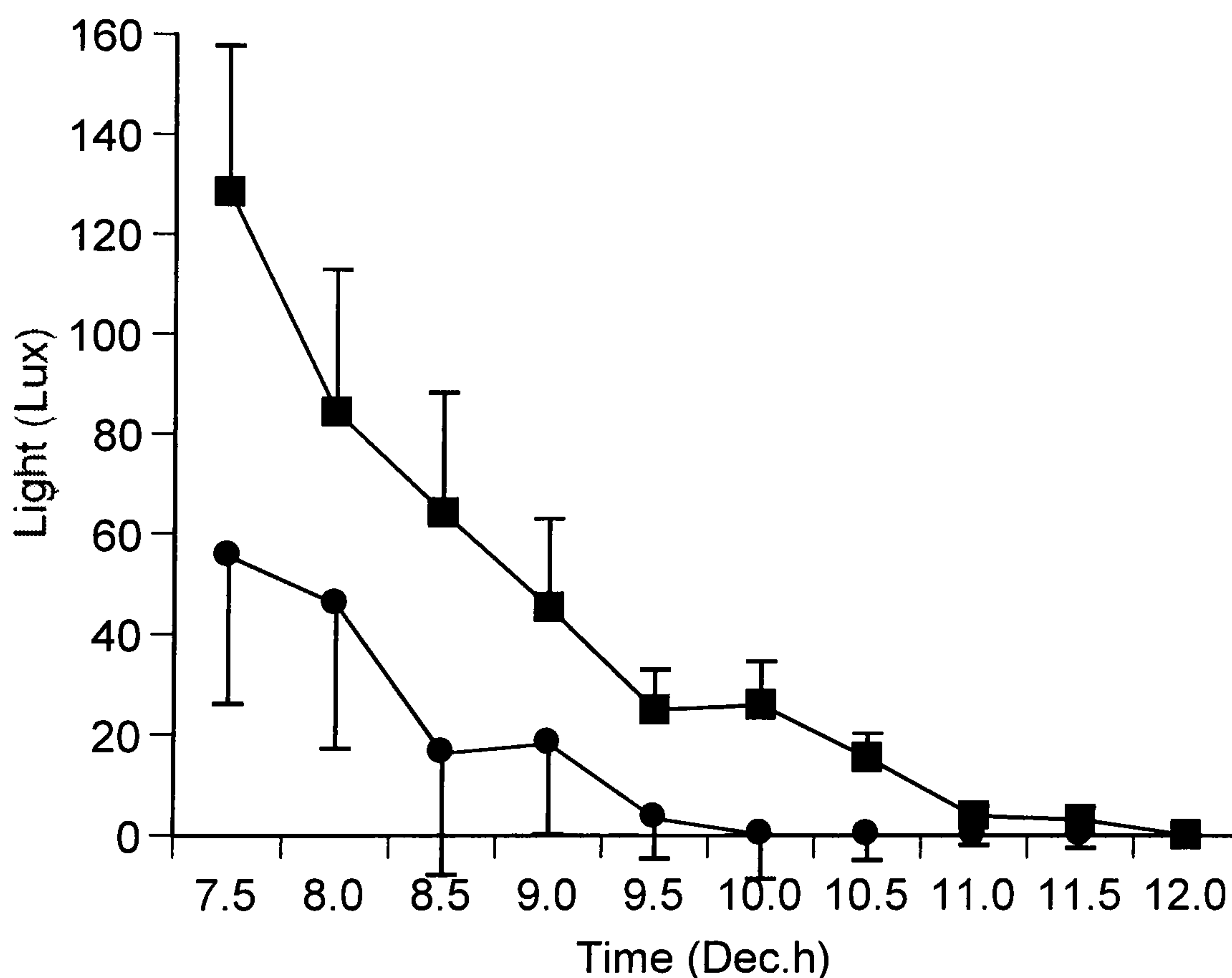


Figure 3-6: Light exposure (mean  $\pm$  SEM) between 07.30 and 10.30h for the 6 days of a night shift for those working 19.00-07.00h (squares) and 18.00-06.00h (circles) in summer.



#### **3.4.3.7. Phase angle**

The phase angle between sleep onset and aMT6s acrophase differed between the two shift schedules. Adapted subjects working 18:00-06:00 h had a smaller phase angle difference (aMT6s acrophase – sleep onset) of  $2.47 \pm 0.7$  h compared to the 19:00-07:00 h shift schedule whose phase angle difference was  $5.09 \pm 0.46$  h ( $p < 0.01$ , unpaired Student's t-test).

#### **3.4.4. Circadian adaptation and sleep**

The effects of circadian adaptation on sleep offshore in summer were analysed. The data of both shift schedules (18.00-06.00h and 19.00-07.00h) has been combined.

##### **3.4.4.1. Objective sleep onset and sleep duration**

Actigraphic sleep onset time significantly negatively correlated with sleep duration ( $p = 0.01$ ), illustrated in Figure 3-7. Those subjects (working both 19.00-07.00h and 18.00-06.00h in the summer) who start the timing of their sleep earlier have a longer sleep duration than those who start their sleep at a later hour of the day.



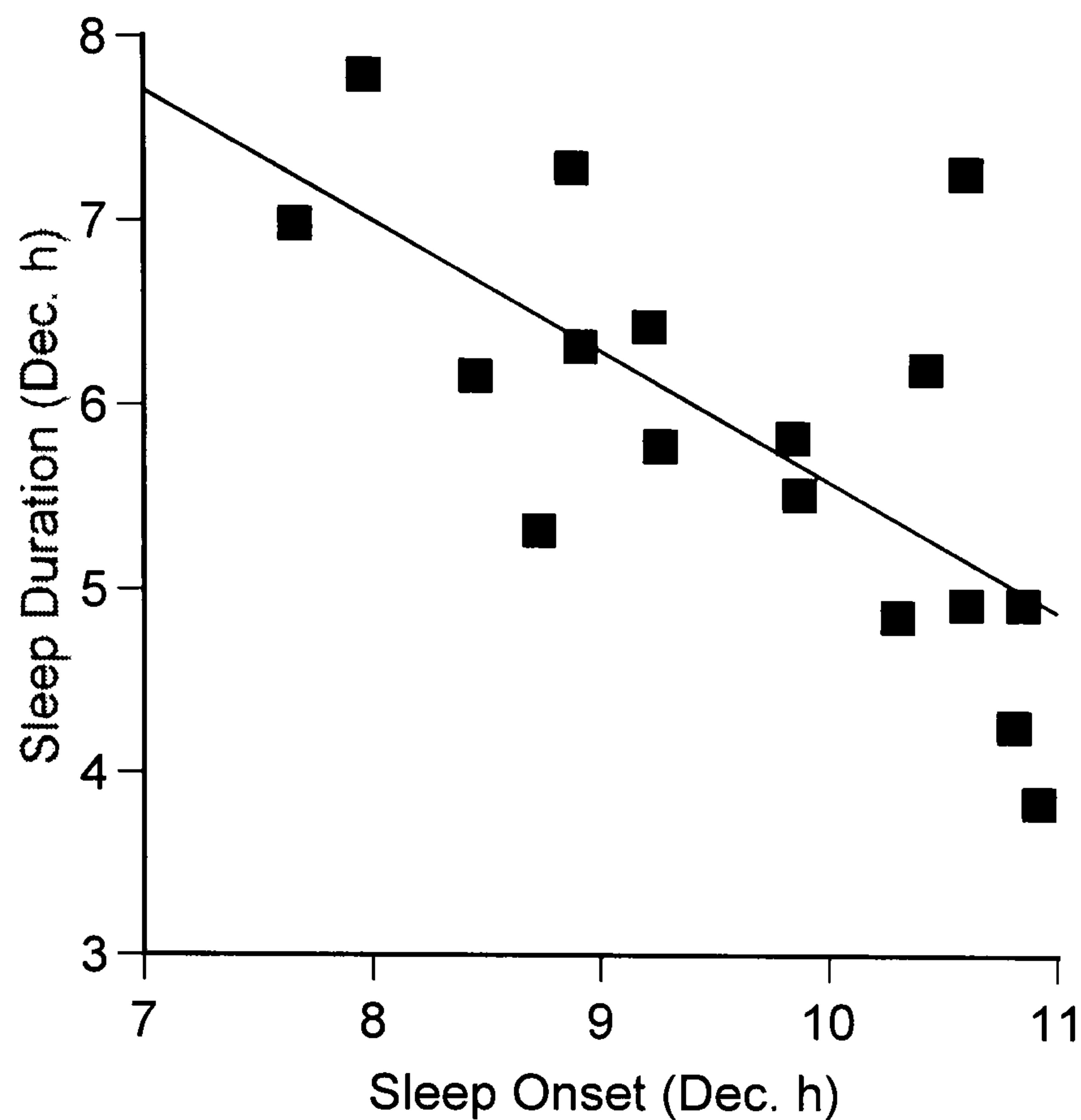


Figure 3-7: Correlation of actigraphic sleep onset time and sleep duration (N=17,  $r=-0.48$ ,  $p<0.05$ ). Subjects were studied in the summer months 19.00-07.00h, N=10 and 18.00-06.00h N=7.

#### 3.4.4.2. Objective sleep onset and sleep efficiency

Sleep duration and sleep efficiency are related sleep parameters. Long sleep duration should correlate with high sleep efficiency. There was a significant negative correlation between sleep duration and sleep efficiency, ( $r=-0.53$ ,  $p=0.03$ ). Subjects who had an earlier sleep onset had a higher sleep efficiency (Figure 3-8).



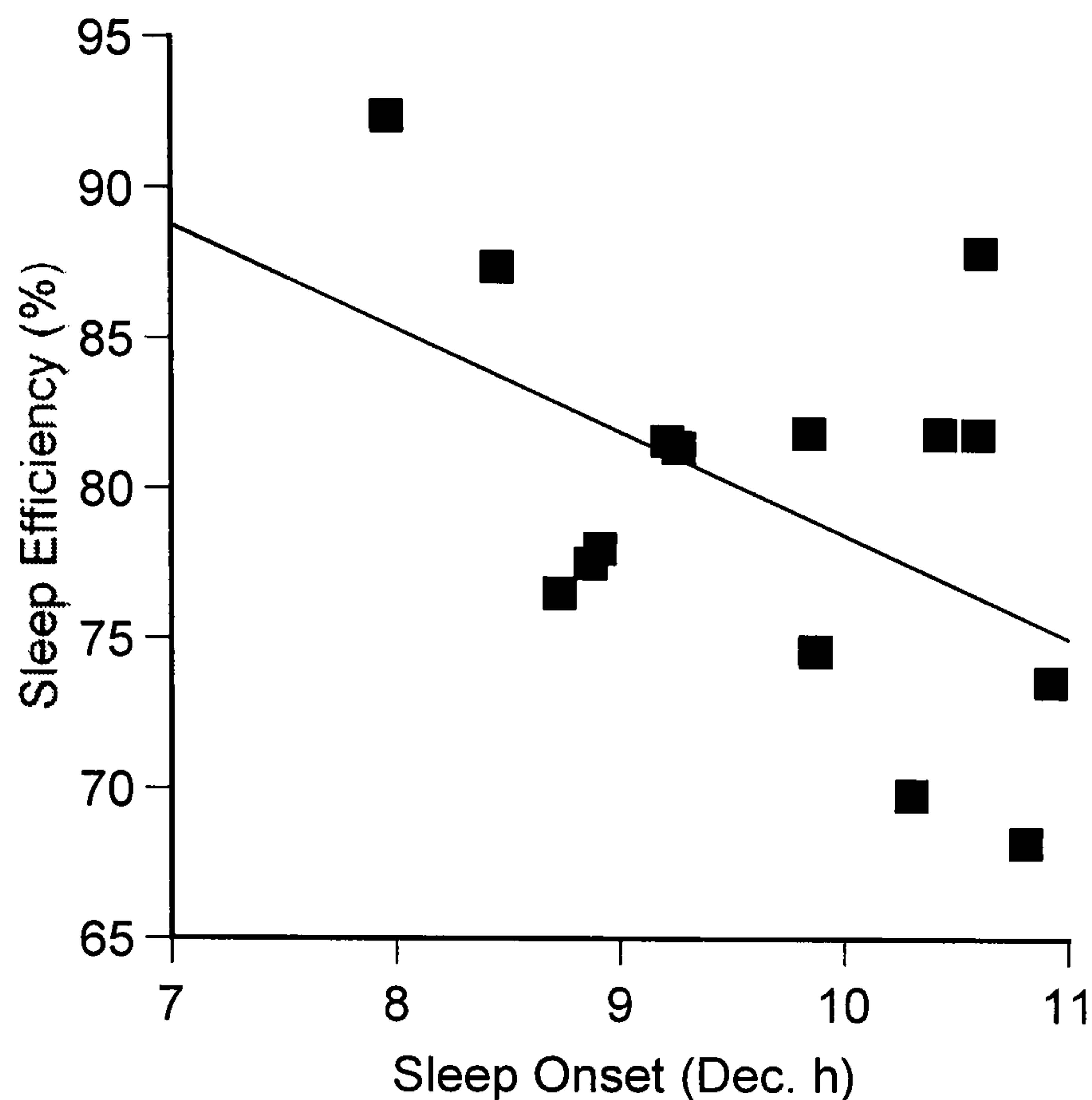


Figure 3-8: Correlation of sleep onset and sleep efficiency, (N=17,  $r=-0.53$ ,  $p=0.03$ ). Subjects were studied in the summer months, 19.00-07.00h, N=10 and 18.00-06.00h, N=7.

There were no differences in other actigraphic sleep parameters studied such as sleep latency and fragmentation index.

#### 3.4.4.3. Age and circadian phase

Age significantly correlated ( $p<0.001$ ) with circadian phase (as assessed by aMT6s acrophase). The older the subject the earlier was their aMT6s acrophase. This is graphically shown in Figure 3-9.



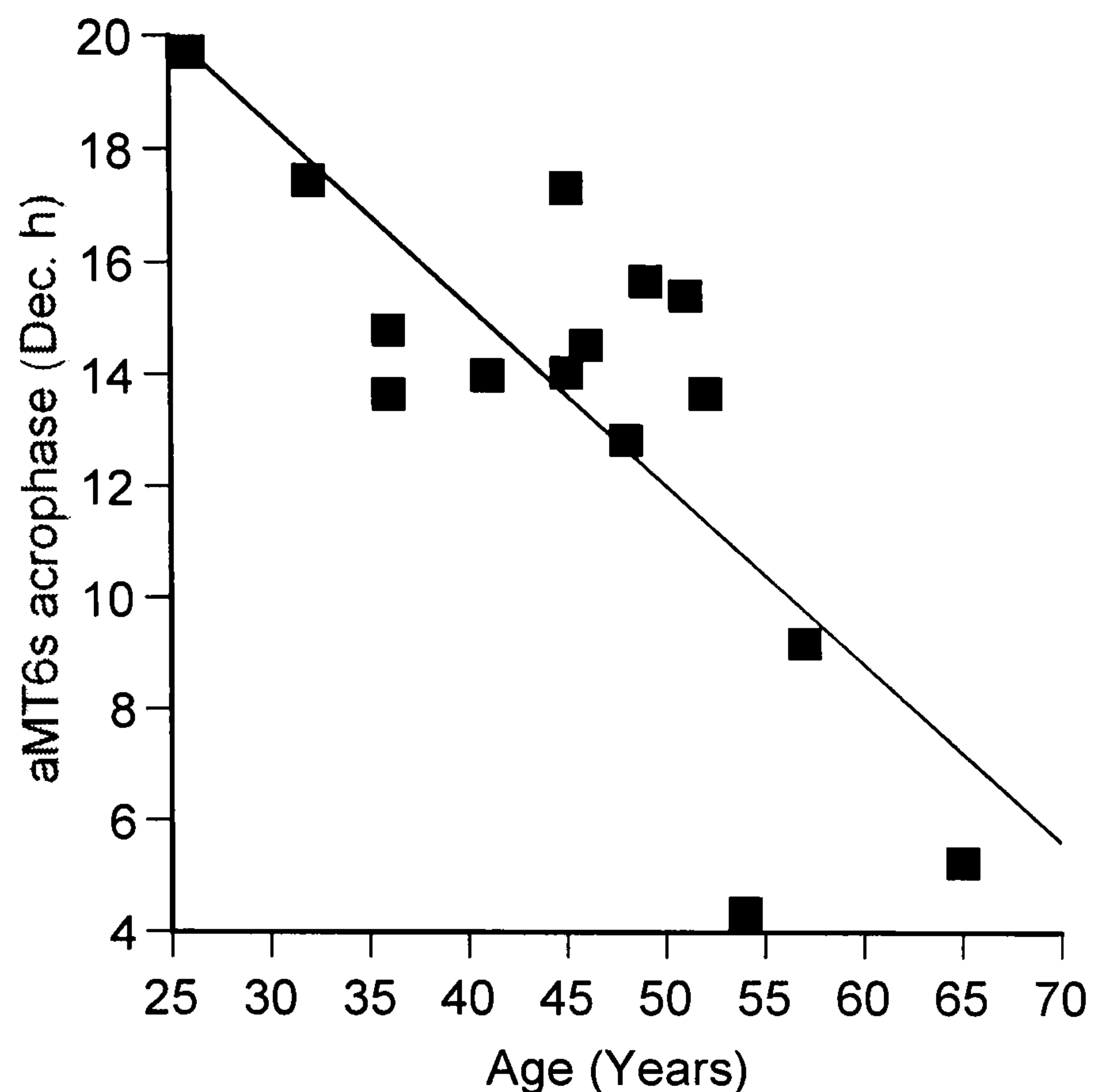


Figure 3-9: Correlation of age and circadian phase (N=15,  $r=-0.77$ ,  $p=0.001$ ). Two subjects provided no age data and were excluded.

Age also significantly correlates with phase angle (aMT6s acrophase – sleep onset), with older subjects having an earlier phase angle compared to those who are younger, non-adapted and adapted  $p=0.003$ .

### 3.5. Discussion

This chapter addresses the implications of shift schedule timing in terms of adaptation to the night shift, sleep and light exposure. The latter may directly influence adaptation and therefore influence the quality of sleep during the night shift. Previous studies have shown that numerous factors influence circadian adaptation to the night shift; these include timing of work schedule (Barnes et al, 1998a and b), season (Barnes et al, 1998b), light exposure (Koller et al., 1994; Dumont et al., 2001), age (Harma et al., 1994) and diurnal preference (Crowley et al., 2003; Mongrain et al., 2004). The results show significant differences between the 18.00-06.00h and 19.00-07.00h shift schedule in terms of timing of aMT6s acrophase and sleep.



### **3.5.1. Circadian adaptation (aMT6s acrophase)**

The timing of the aMT6s acrophase was significantly later in the 19.00-07.00h shift schedule compared to the 18.00-06.00h schedule. This may be due firstly to a later bedtime in the 19.00-07.00h shift and secondly that working on the 19.00-07.00h shift leads to an increased amount of morning light exposure (07.30-10.30h). In the case of adapted subjects this would phase delay the circadian clock and the aMT6s rhythm to a later timing (Eastman and Martin, 1999). For non-adapters, however, this morning light would fall on the phase advance portion of the light PRC (Khalsa et al., 2003) and would counter any circadian adaptation.

### **3.5.2. Circadian adaptation and sleep**

A partial explanation for the shorter sleep that was observed in the subjects on the 19.00-07.00h shift schedule, may be that two subjects did not adapt to the night shift (aMT6s acrophase 4.3h and 5.3h), whereas adapted subjects' mean acrophase was  $14.6 \pm 0.55$ h. However, sleep was also significantly shorter in those subjects who were adapted to the night shift working 19.00-07.00h compared to those working 18.00-06.00h. When looking at the light exposure of the two unadapted individuals working 19.00-07.00h compared to the adapted subjects, it is clear that these two subjects had lower light exposure around midnight and higher light exposure in the early morning. The mean light exposure between the two shift schedules indicated, as expected, that those working 19.00-07.00h were exposed to a greater amount of morning light between 07.30-10.30h due to the shift schedule finishing an hour later. Research investigating adaptation to night shifts has shown improved adaptation in those who avoid morning bright light (Eastman et al., 1994; Koller et al., 1994). Morning bright light would be expected to be most extreme in the summer months in comparison to winter.

In night shift workers sleep duration and sleep efficiency correlate well with sleep onset time; this may be predominantly due to the influence of the circadian system. Those who are adapted well to the night shift will have a



higher drive to sleep in the morning (subjective night, period of melatonin production), than those who are only partially or non-adapted. This was clearly shown by unadapted subjects CT-01 and CT-02 who went to bed later (10.60h and 10.92h compared to the 9.51h bedtime seen in adapted subjects working the 19.00-07.00h shift schedule). The circadian influence on sleep has been determined in several studies using forced desynchronisation (Czeisler et al., 1980; Dijk et al., 1999). The present field data reinforce these laboratory observations.

### **3.5.3. Circadian adaptation and age**

Harma et al. (1994) reported that age significantly affected adjustment to night work (rectal temperature minimum and sleepiness were used as phase markers). The circadian system is most sensitive to light in the short (blue) wavelength region (Brainard et al., 2001; Thapan et al., 2001) and the reduced transmission of short wavelength light with age (Herljevic et al., 2005) may result in reduced responsiveness of the circadian system to light. Ageing is characterised by changes in both sleep quality and circadian phase (Badia et al., 1991; Bliwise, 1993). The two non-adapters with very short sleep, working the 19.00-07.00h shift schedule were older than those who had adapted to the night shift and this factor may also have played a role.

Morningness increases with age (Åkerstedt and Torsvall, 1981; Jones et al., 1999; Taillard et al., 1999). The current findings of those working the 19.00-07.00h shift schedule show that if subjects are more morning-like, the timing of the aMT6s acrophase is earlier during the night shift. This does not seem to apply to the 18.00-06.00h shift schedule in this small number of subjects. If subjects adapt to the night shift by phase delaying, morning types have further to shift the timing of their aMT6s rhythm to adapt to the night shift. This may be one underlying reason why morning types find it harder to adapt to the night shift (Griefahn et al., 2002).



#### **3.5.4. Age and aMT6s phase angle**

Duffy et al. (1991) investigated the effects of ageing on melatonin and sleep. Their results showed that older subjects in comparison to younger subjects have an overall endogenous circadian rhythm of plasma melatonin that is at an earlier clock time in healthy older subjects than in younger subjects, but that the melatonin rhythm occurs later within the habitual sleep episode in older subjects. This does not seem to apply to the present shift work study.

Forced desynchrony protocols (Dijk and Czeisler, 1994) showed that when sleep is initiated approximately 6h before the endogenous circadian temperature minimum, sleep will be undisturbed for 8h.

The melatonin maximum is close to the core body temperature minimum (Cagnacci et al., 1992) and the aMT6s acrophase is approximately 2h later than the melatonin peak (Ross et al., 1995). Therefore theoretically subjects working the 19.00-07.00h shift schedule, in view of their phase angle difference between aMT6s acrophase and sleep onset (5.09h) should have had higher sleep duration, and in comparison to the 18.00-06.00h shift schedule where the phase angle difference was only 2.47h and sleep was initiated close to the (theoretical) melatonin peak. This was not the case in the present study, however in neither schedule was the phase angle difference optimal for sleep duration.

#### **3.5.5. Circadian adaptation and individual differences**

Individual differences in night shift adaptation have been consistently observed Gibbs et al. (2007) (section 1.8.6.1). Some individuals clearly have difficulties handling shift work; and employers should take this into account. The issue of fatigue and safety can be seen as of paramount importance.



### **3.5.6. Advantages and disadvantages of the different shift schedules**

If sleep is indeed worse when subjects are working 19.00-07.00h in comparison to 18.00-06.00h in the summer, a change in shift schedule by one hour could have huge benefits in terms of adaptation to the night shift. If adaptation to the night shift occurs then a better quality of sleep in terms of sleep duration, sleep efficiency and fragmentation index will follow. Maybe a compromise between the two shift schedules is to work 18.00-06.00h in the summer and 19.00-07.00h in the winter. This opinion is not shared by all, Tucker et al. (1998) reported in onshore shift workers that earlier (06.00h) onshore shift changeovers had deleterious effects on night sleeps, but were beneficial to day sleeps, whereas the effects were reversed when changeovers are later (07.00h).

The difference in actigraphic sleep duration between 18.00-06.00h and 19.00-07.00h appears to be due largely to a later bedtime but similar wake up time for 19.00-07.00h, compounded by two non-adaptors in the latter schedule. The difference in sleep duration overall was substantial (0.89h) between adapted subjects. If a simple change of schedule by one hour is sufficient to increase sleep time and thus decrease sleep deprivation, this may well have considerable benefits for health and safety.

### **3.6. Conclusions**

In conclusion, circadian phase was later and objective sleep appeared to be worse in those working 19.00-07.00h compared to those working the 18.00-06.00h shift schedule. The differences observed between the two schedules may relate to differences in morning light exposure countering circadian adaptation to the night shift and to the later bedtime in the 19.00-07.00h schedule. The 2 week 18.00-06.00h schedule allows for full adaptation to the night shift, as previously reported by Barnes et al. (1998a), whilst in the 19.00-07.00h shift schedule only 80% of subjects studied adapted to the night shift. This implies that the timing of the shift schedule may put a strain



on those who are pre-disposed to problems adapting to the night shift (e.g. older people, extreme morning types). Smith et al. (2005) proposed a path model of hypothesised effects on shift work, sleep and fatigue. They proposed a series of factors which will effect shift work outcomes, these include age and experience, morningness-eveningness and workload. Clearly the timing of schedules is also an important factor.

It could be predicted that the differences observed will be more prominent in summer compared to winter particularly at high latitudes with large changes in day length. In this scenario a simple change of schedule by one hour may have substantial benefits for sleep and health of offshore workers.



## 4. PHASE SHIFTING EFFECT OF A PORTABLE LIGHT BOX IN CONTROLLED LABORATORY CONDITIONS

### 4.1. Introduction

Light resets the circadian rhythm system and enables us to synchronise our daily biological rhythms to the light-dark cycle. Light has two main effects on melatonin, firstly it is able to phase shift the circadian clock and hence the melatonin rhythm (an output of the clock) and secondly light is able acutely to suppress nocturnal melatonin (section 1.5.4.1). The discovery that high intensity 'bright light' (~2500 lux) completely suppressed nocturnal melatonin secretion (Lewy et al., 1980) led to the idea that bright light could be used to used to phase shift the circadian system. It is well established in animals and humans that light pulses can shift the phase of circadian rhythms. The direction of the phase shift (advance or delay) and magnitude (hours) of the shift depends on when the light is applied within the circadian cycle (section 1.6). Previous studies (Broadway et al., 1987; Eastman and Martin, 1999; Khalsa et al., 2003; Eastman et al., 2005; Revell et al., 2005) have shown that suitably timed morning light phase advances the circadian system, while evening light may phase delay the rhythm (section 1.5.4.1).

With this knowledge it is possible to manipulate the timing of the circadian system to benefit situations where circadian rhythms are misaligned, for example when experiencing jet-lag or after shift work. In these situations people suffer from fatigue, poor performance and their sleep is disrupted due to their body clock being out of phase with the light dark cycle (Åkerstedt and Gillberg, 1990; Kecklund and Åkerstedt, 1993; Bjorvatn et al., 1999) (section 1.8).

### 4.2. Aims and hypotheses

This in-house laboratory study was designed to investigate the ability of light delivered by a portable light box to change the time of the internal clock (shift



circadian phase). The effects of a single bright light pulse (3000 lux, irradiance  $1000 \mu\text{W}/\text{cm}^2$ ) provided by a portable light box (Litebook<sup>®</sup>, Alberta, Canada) on phase advancing the melatonin circadian rhythm in healthy young male volunteers were ascertained.

The null hypothesis stated that there would be no effect of light administration on the phase of melatonin rhythm (aMT6s acrophase, DLMO and DLMOff), whilst the alternative hypothesis stated that there will be a significant effect of light administration on the phase of melatonin.

### **4.3. Methods**

#### **4.3.1. Subjects**

Ten healthy male volunteers, mean age  $26.3 \pm 6.3$  years (age  $\pm$  SD) with a BMI of  $24.0 \pm 2.4 \text{ kg}/\text{m}^2$  and a HÖ score of  $46 \pm 5$  participated in the study. Ethical permission was obtained prior to the study from the University of Surrey Advisory Committee on Ethics (ACE/2002/95/SBLS). Before starting the study subjects were asked to complete the HÖ (*Appendix A*). Extreme morning types (larks) ( $\text{HÖ} > 69$ ) and extreme evening types (owls) ( $\text{HÖ} < 31$ ) were excluded from the study. Other exclusion criteria included taking any sleeping medication, and drugs such as antidepressants, barbiturates, benzodiazepines and  $\beta$ -blockers, which are known to affect melatonin production. Subjects were also excluded if they had undertaken shift work or took a trans-meridian flight within 2 weeks of commencement of the study.

#### **4.3.2. Pre-study protocol**

Subjects were required to keep a regular sleep-wake cycle for 7 nights prior to the study (between 23.00–07.00h) and kept daily sleep diaries (*Appendix B*). Subjects also wore Actiwatch-Ls to measure light exposure and monitor activity (section 2.1) for the 7 days prior to and throughout the study. Twenty-four hours prior to the start and during the study, subjects were required to



avoid bright light and refrain from heavy exercise, alcohol and caffeine consumption.

### **4.3.3. Study**

The study was conducted in the Clinical Investigation Unit (CIU), School of Biomedical and Molecular Sciences at the University of Surrey, Guildford, Surrey, UK, by Penny Owens (PhD student) and Dr. Shelagh Hampton, and the data was analysed by myself. The unit is fully equipped with a kitchen, sleeping and toilet/shower facilities. Meals and non-caffeinated drinks were provided during the study. The subjects were supervised by the researchers at all times during the study.

The volunteers were provided with information sheets about the study and required to fill in a medical questionnaire (*Appendix D*). Subjects were then reimbursed for their time and inconvenience with a payment of £250 upon completion of both study legs. Subjects that did not complete the study received payment pro rata. All volunteer information was kept coded and held in the strictest confidence and in compliance with the Data Protection Act (1998).

The study consisted of two legs in a randomised crossover design, one study leg with light administration (07.00–08.00h) and the other study leg as a “no light” control. Environmental light exposure was kept constant through the whole study in the CIU, except during the light exposure periods and sleep times. Light levels (at eye level in the direction of gaze) were approximately 10 lux. Each study leg was a maximum of 5 days long during which time the subjects were confined to the CIU. In each leg, days 1 (D1) and 2 (D2) were baseline days with no extra light exposure. On day 3 (D3) subjects received light or no light, days 4 (D4) and days 5 (D5) following light administration, were post stimulus assessment days. The two study legs were separated by a period of at least two weeks. The protocol is illustrated in Figure 4-1.



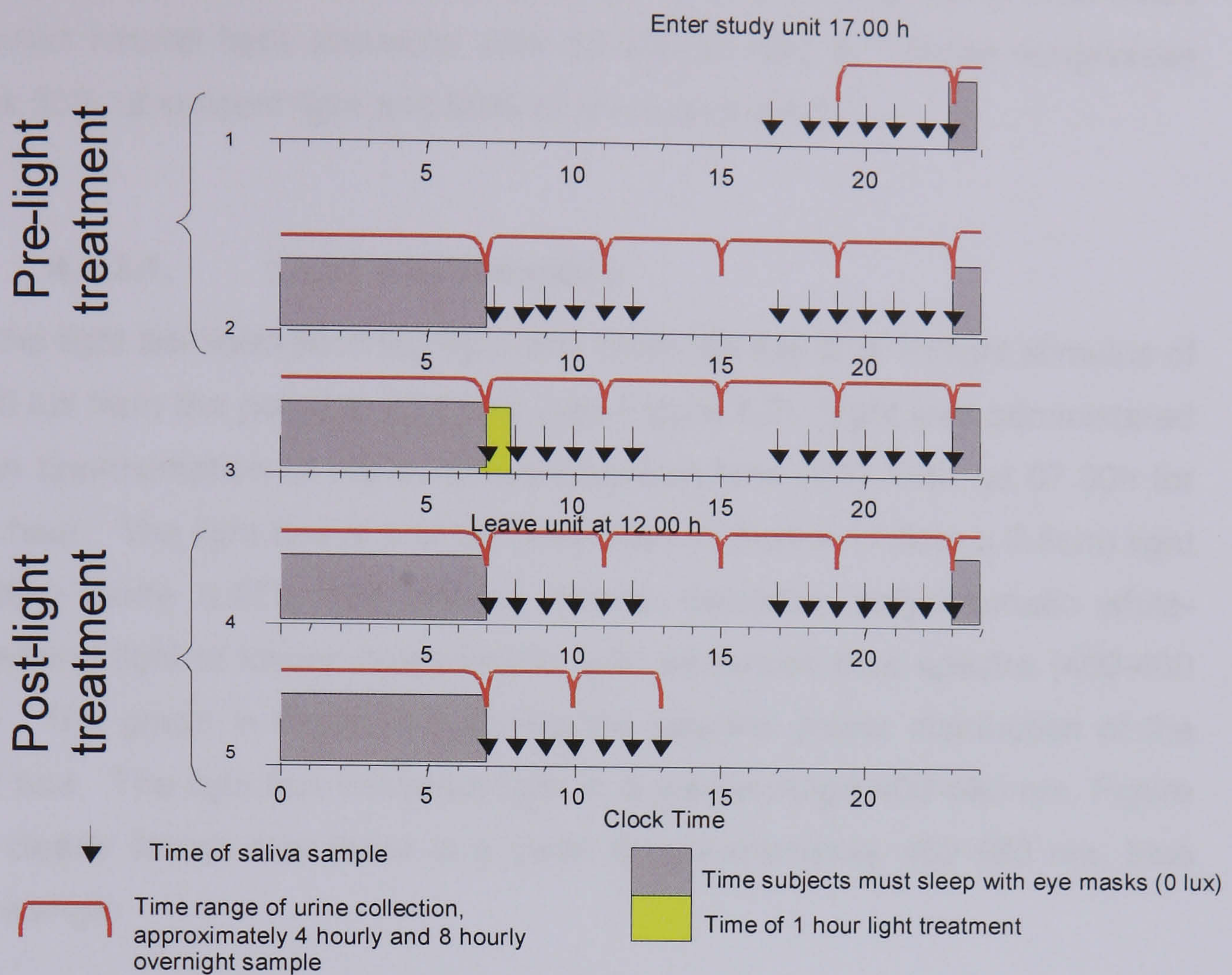


Figure 4-1: Experimental 5 day protocol of the light box study

Subjects arrived at the CIU at 17.00h on day 1 and remained in the CIU until 12.00h on day 4. Subjects were asked to remain in a sedentary or semi-recumbent position throughout the study, except during the sleep period (supine wearing eye masks, 0 lux 23.00-07.00h). Since the subjects were in a sedentary or semi-recumbent position, they were provided with equipment to be able to urinate *in situ*. However, subjects were allowed to get up and go to toilet to defaecate. Since during the waking periods subjects remained in a semi recumbent position on each day of the study, the subjects were instructed to move their legs and perform basic stretching exercises every 2 hours. During the study all meals and non-caffeinated drinks were supplied and water was provided *ad libitum* throughout. Breakfast was served at 07.00h on days 2, 3 and 4; lunch was served at 12.00h on days 2, 3 and 4 and dinner was provided at 19.00h on days 1, 2 and 3. On day 4 at 12.00h



when the subjects left the CIU, sunglasses were worn (Litebook<sup>®</sup>) at all times to avoid natural light exposure until 12.00h on day 5. These sunglasses block 95% of incident light and 99% of UV-A and UV-B.

#### 4.3.3.1. Light administration

On the light administration leg light was given on day 3: a 1h light stimulus of 3000 lux from the portable light box (see Figure 4-2). Light was administered at an approximation of the subject's circadian time (CT) 1-4h, at 07.00h for one hour. The light box is a small (less than 17.5cm x 17.5cm x 3.8cm) light emitting diode (LED) light therapy device delivering polychromatic white-appearing light of known composition, with enhanced blue spectra (460-480 nm). The graph in Figure 4-2 shows the spectral power distribution of the light box. The light box delivered light in a visible range 400-480 nm, Figure 4-2 clearly shows that there is a peak at approximately 460-480 nm, blue wavelength.

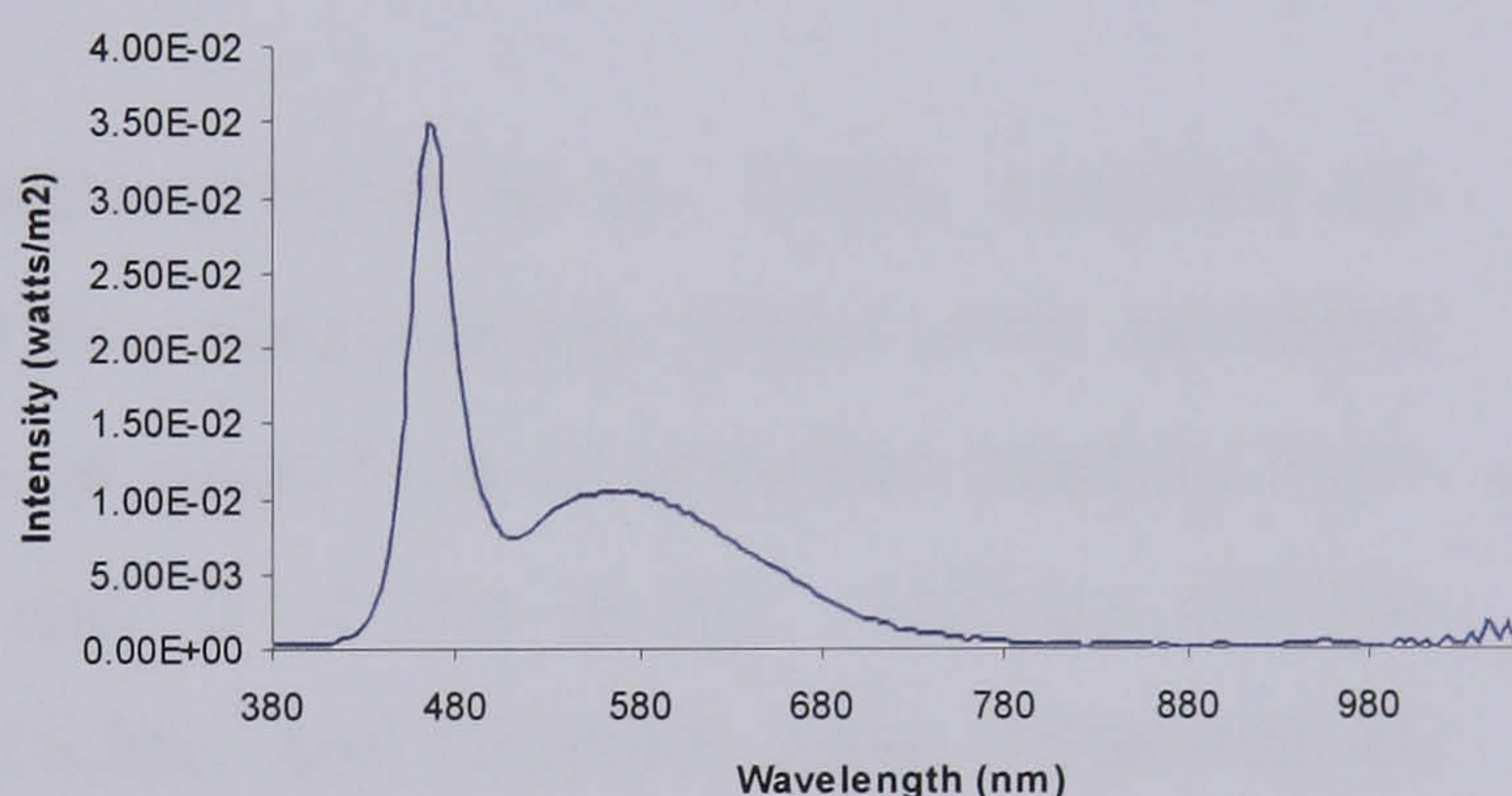


Figure 4-2: Portable light box (Litebook<sup>®</sup>) used to deliver the light (left) and the spectral power distribution of the light box (right).

Researchers continuously monitored the subjects to ensure the correct amount of light reached the subjects' retina (a distance of 70cm was maintained). The protocol allowed the subjects to read during the light exposure. In the "no light" condition exactly the same procedure was carried out with the light box as in the light administration condition, but the light was not switched on. The correct orientation (i.e. positioning of the light box with respect to the subjects eyes) was ensured by the researchers to guarantee that the correct amount of light was being delivered to the subject's retina.



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The intensity of the light at eye level (~3000 lux) was also measured using a power meter (Macam Photometric Ltd, Livingstone, Scotland). The subjects were asked to report any side-effects or discomfort to the investigators immediately.

#### **4.3.4. Determination of circadian phase and phase shift**

##### **4.3.4.1. Salivary melatonin and urinary aMT6s**

Salivary melatonin was measured by RIA with reagents supplied from Stockgrand Ltd, with a protocol modified by English et al., (1993). A non-citrated cotton Salivette (Starstedt Ltd, Leicester, UK) was used to collect saliva (section 2.2). The samples were immediately frozen upon collection and stored at  $-20^{\circ}\text{C}$  until analysis. Saliva samples were collected at 30 minute intervals between 07.00-12.00h and 17.00-23.00h on days 1-4 and between 07.00-12.00h on day 5, as illustrated in Figure 4-1. Sample collection ended at 12.00h on day 5.

Urinary aMT6s was measured by RIA) (Arendt et al., 1985, modified by Aldous and Arendt, 1988) (section 2.2). Sequentially timed urine samples were collected in separate containers at each time of urination starting from 07.00h on day 1, until 12.00h on day 5 of the study. Urinary aMT6s (acrophase) and salivary melatonin (DLMO and DLMOff) were measured to assess the pre- and post- light stimulus circadian phase positions of the melatonin rhythm under controlled sleep and wake conditions.

Subjects continued to collect sequential urine samples (4 hourly) (*Appendix C*) and saliva samples for 78h following light treatment. On day 4, the subjects left the CIU at 12.00h and carried out whatever activities they chose. Sunglasses were worn at all times, except during sleeping, until day 5. Activity was restricted with subjects being instructed not to exercise upon leaving the laboratory during saliva sample collection between 17.00-23.00h.



#### **4.3.5. Statistical analysis**

P values of  $<0.05$  were accepted as statistically significant. Paired Student's t-test (Prism, version 5) was carried out to determine significant differences in the effect of light treatment on circadian phase of aMT6s, DLMO and DLMOff. The data of the saliva was an average of three points to help smooth the salivary melatonin rhythm.



## 4.4. Results

### 4.4.1. Sleep diary data subjective sleep

#### 4.4.1.1. Sleep onset during light administration

Sleep onset time (Dec. h) determined by sleep diaries completed during the week prior to starting the light administration leg is shown in Table 4-1.

Subjects	N1	N2	N3	N4	N5	N6	N7	Mean N3-N7	SEM
1	22.95	0.98	22.88	23.22	22.70	23.32	22.80	22.98	0.12
2	02.75	04.53	25.10	0.35	0.27	23.85	1.42	24.60	0.29
3	1.52	23.55	23.10	23.33	23.43	23.37	23.45	23.34	0.06
4									
5	0.50	0.50	23.13	22.93	0.07	23.12	23.10	23.27	0.20
6	1.08	25.23	0.25	23.42	23.17	23.38	23.48	23.54	0.19
7	1.15	24.00	23.52	23.28	23.40	24.00	23.53	23.55	0.12
8									
9	23.93	23.52	23.23	23.43	22.93	23.72	23.65	23.39	0.14
10	1.00	1.63	23.50	22.98	23.78	23.22	23.13	23.32	0.14
							<b>Mean</b>	<b>23.50</b>	<b>0.16</b>

Table 4-1: Sleep diary sleep onset (Dec. h, N=8) for the 7 days preceding the laboratory study on the light administration leg (grey boxes = subject did not participate in this leg).



#### 4.4.1.2. Sleep offset during light administration

Sleep offset time (Dec. h) determined by sleep diaries completed during the week prior to starting the light administration leg, shown in Table 4-2.

Subjects	N1	N2	N3	N4	N5	N6	N7	Mean N3-N7	SEM
1	7.45	7.98	7.08	7.22	7.05	7.35	7.25	7.19	0.06
2	8.00	9.00	7.50	7.00	7.00	5.93	7.00	6.89	0.26
3	10.00	8.00	6.88	7.90	7.67	8.33	7.48	7.65	0.24
4									
5	9.17	10.50	7.20	7.25	6.98	6.83	7.85	7.22	0.17
6	9.50	9.50	7.50	8.45	7.50	7.50	7.00	7.59	0.24
7	7.25	8.23	8.02	6.98	6.97	8.00	7.00	7.39	0.25
8									
9	7.50	7.72	7.45	7.15	7.33	11.77	8.48	8.44	0.86
10	10.00	7.50	7.75	8.00	8.25	7.08	6.97	7.61	0.25
							<b>Mean</b>	<b>7.50</b>	<b>0.29</b>

Table 4-2: Sleep diary sleep offset (Dec. h, N=8) for the 7 days preceding the laboratory study on the light administration leg, (grey boxes = subject did not participate in this leg).



#### 4.4.1.3. Sleep onset during control condition

Sleep onset time (Dec. h) determined by sleep diaries completed during the week prior to starting the control condition, shown in Table 4-3.

Subjects	N1	N2	N3	N4	N5	N6	N7	Mean N3-N7	SEM
1	02.30	23.52	23.53	22.67	24.63	22.05	23.48	23.27	0.44
2	25.52	6.05	23.07	23.60	23.92	23.72	0.50	23.76	0.23
3	3.00	22.75	22.97	24.52	23.78	23.80	23.62	23.74	0.25
4	25.53	1.58	23.02	22.85	0.08	0.00	23.08	23.41	0.26
5									
6	0.50	1.48	23.82	24.25	24.50	0.47	022	24.25	0.12
7	02.50	02.61	0.80	24.25	24.37	23.80	Nd	24.31	0.18
8	23.13	0.32	22.78	22.50	23.53	23.28	23.33	23.09	0.19
9									
10									
							<b>Mean</b>	<b>23.67</b>	<b>0.24</b>

Table 4-3: Sleep diary sleep onset (Dec. h, N=7) for the 7 days preceding the laboratory study during the control condition on the no light treatment leg (nd = no data, grey boxes = subject did not participate in this leg).



#### 4.4.1.4. Sleep offset during control condition

Sleep offset time (Dec. h) determined by sleep diaries completed during the week prior to starting the control condition, shown in Table 4-4.

Subjects	N1	N2	N3	N4	N5	N6	N7	Mean N3-N7	SEM
1	10.98	8.42	7.75	7.75	7.67	7.33	7.33	7.57	0.10
2	6.97	8.35	7.00	7.47	7.50	7.75	7.50	7.44	0.12
3	10.25	8.33	7.83	8.25	8.28	7.50	7.33	7.84	0.19
4	8.17	7.17	6.92	7.08	7.33	7.02	7.15	7.10	0.07
5									
6	8.50	10.75	6.95	7.75	8.75	6.97	7.00	7.48	0.35
7	7.97	7.50	7.00	8.17	7.17	7.92	Nd	7.56	0.25
8	7.17	7.63	6.33	6.17	5.92	5.97	5.95	6.07	0.08
9									
10									
							<b>Mean</b>	<b>7.29</b>	<b>0.17</b>

Table 4-4: Sleep diary sleep offset (Dec. h, N=7) for the 7 days preceding the laboratory study on the no light treatment leg (grey boxes = subject did not participate in this leg, Nd = no data).

On the light administration leg for the 5 days prior to the study, subjects had a sleep onset time of  $23.50 \pm 0.16$ h (mean decimal time  $\pm$  SEM), with a sleep offset of  $7.50 \pm 0.29$ h. During the control condition subjects for the last 5 days prior to the study had a sleep onset time of  $23.67 \pm 0.24$ h and a sleep offset of  $7.29 \pm 0.17$ h. The first two days of the sleep diaries were excluded as these were weekend nights and were not deemed representative of the subject's sleep-wake cycle.

#### 4.4.2. Salivary DLMO

During the light treatment leg the mean DLMO on night 2 (N2) was  $21.80 \pm 0.37$ h (mean  $\pm$  SEM) compared to night 3 (N3) where a DLMO of  $21.52 \pm$



0.25h was observed. During the “control” no-light administration session a non-significant phase delay of the DLMO was observed between N2 ( $21.36 \pm 0.39$ h) compared to N3 where a DLMO of  $21.68 \pm 0.40$ h was observed. N2 was used as the baseline night to compare against N3 to ascertain the amount of phase shift. It was assumed that the second baseline night (N2) would be more consistent as subjects had been in dim light for over 24h, therefore previous light exposure would affect the DLMO less than if N1 was used.

Individual DLMO (Dec. h) for each subject is shown in Table 4-5 for the light (N=5) and no light treatment legs (N=5) for N1-N3.

Subject	LIGHT			NO LIGHT		
	N1	N2	N3	N1	N2	N3
1	Nd	Nd	Nd	Nd	21.11	20.24
2	22.33	23.00	22.45	Nd	Nd	Nd
3	21.56	21.19	21.11	21.05	21.01	21.42
4				22.23	22.34	22.33
5	22.18	21.23	21.08			
6	21.46	22.35	21.46	22.24	22.11	22.06
7	Nd	Nd	Nd	Nd	Nd	Nd
8				22.47	20.22	22.33
9	21.32	21.24	21.50			
10	Nd	Nd	Nd			
<b>Mean</b>	<b>21.77</b>	<b>21.80</b>	<b>21.52</b>	<b>22.00</b>	<b>21.36</b>	<b>21.68</b>
<b>SEM</b>	<b>0.20</b>	<b>0.37</b>	<b>0.25</b>	<b>0.32</b>	<b>0.39</b>	<b>0.40</b>

Table 4-5: Individual DLMO (Dec. h) for subjects during N1, N2 and N3 of the two study legs (light treatment and no light treatment). For subjects 7 and 10 DLMO could not be determined on the light treatment leg (Nd= no data, grey boxes = subject did not participate in this leg).

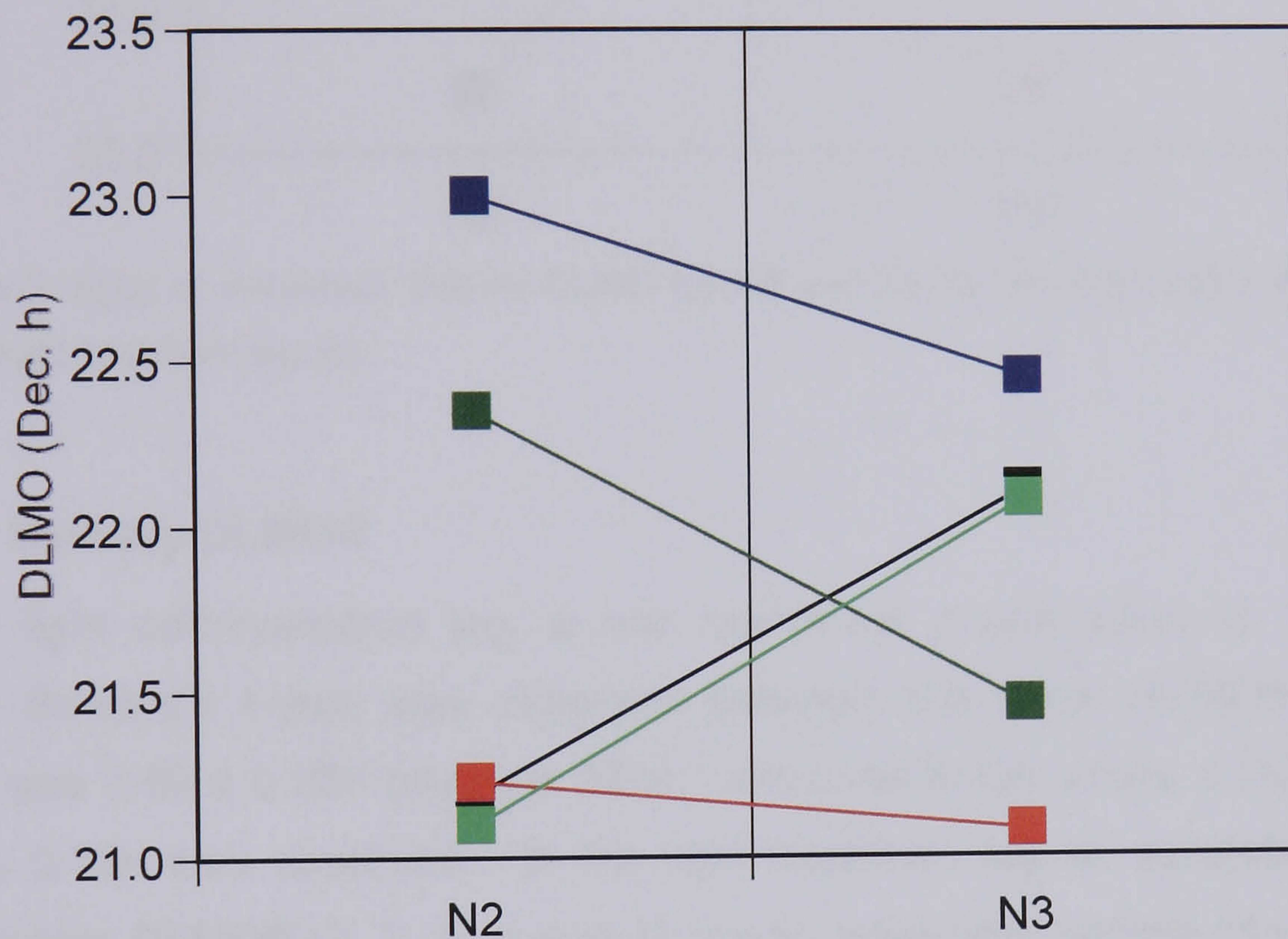
Individual data for the light administration and control no light administration leg is shown graphically in Figure 4-3 (a) and (b) respectively. Not all



subjects phase advanced their DLMO, in response to the light exposure, subjects 2, 3, 5 and 6 phase advanced whilst subject 9 showed a phase delay. The DLMO of subjects 1, 7 and 10 could not be ascertained after measuring the samples as a clear onset was not visible.

In the no light treatment leg the DLMO of subjects 3 and 8 phase delayed whilst subjects 1 phase advanced. Subjects 4 and 6 also phase advanced, but only by 1 and 5 minutes, respectively.

(a)





(b)

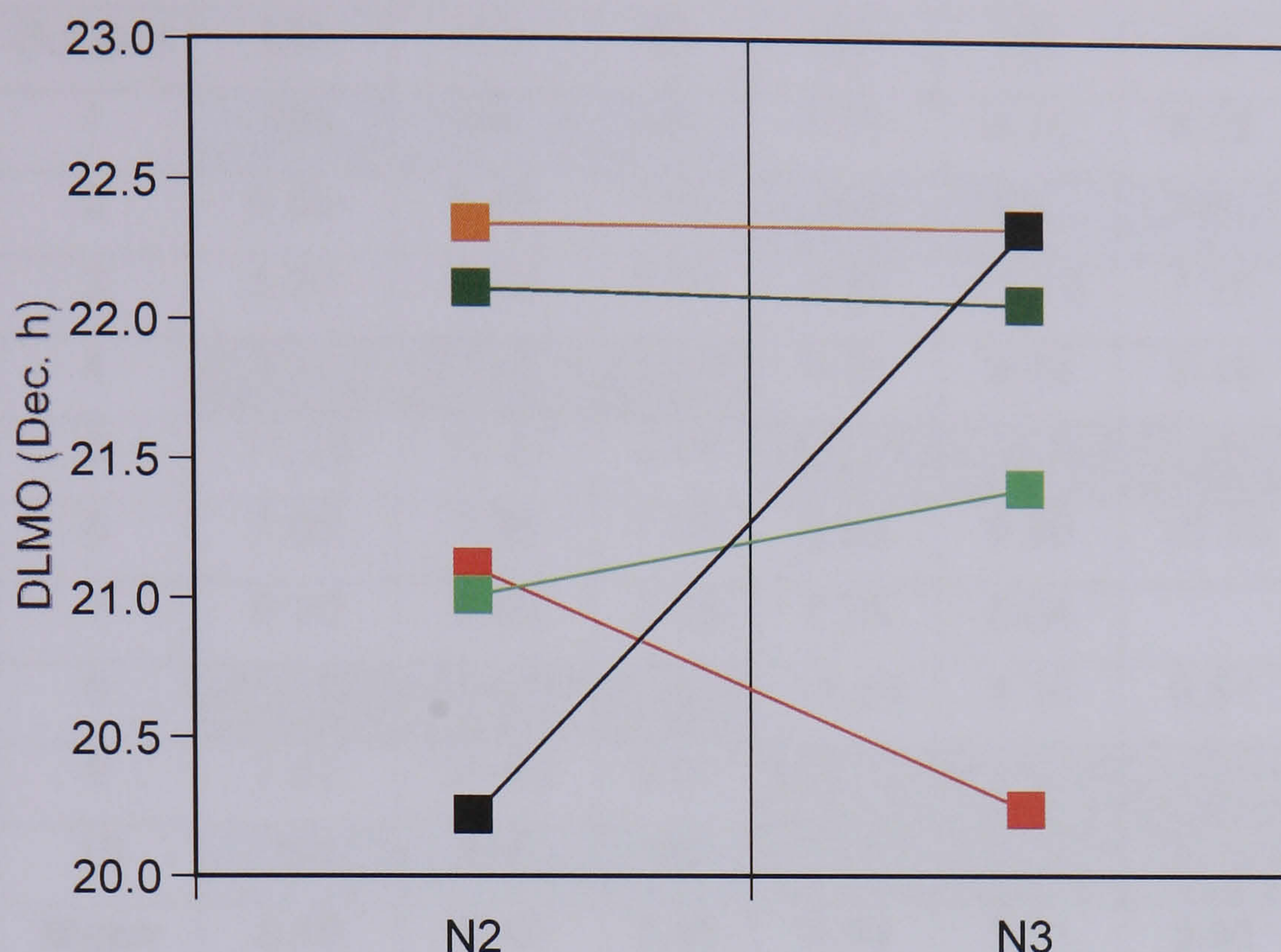


Figure 4-3: Plots of individual data for DLMO for N2 and N3, for the light treatment leg (a) and no light treatment leg (b).

#### 4.4.3. Salivary DLMOff

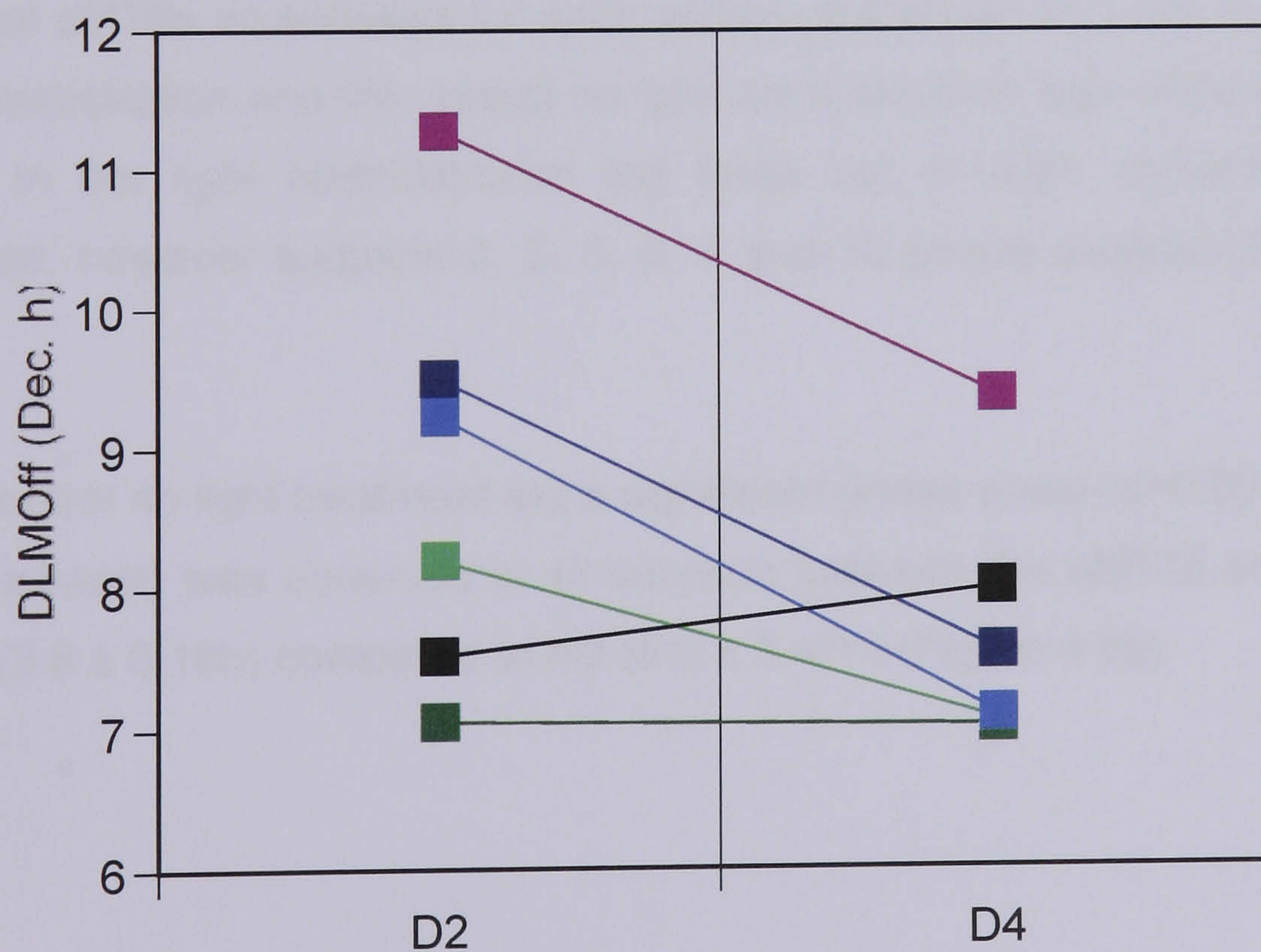
In the light administration leg, a non significant phase advance ( $p=0.06$ , paired Student's t-test) was observed between the mean DLMOff on D2 which was  $8.69 \pm 0.26h$  (mean  $\pm$  SEM) compared to D4 where a DLMOff of  $7.68 \pm 0.15h$  was observed. In the light treatment leg all subjects with a measurable DLMOff (2, 3, 5, 6 and 7) phase advanced, except one subject (9) who phase delayed their timing of DLMOff. In the control no light treatment leg no shift in phase of the DLMOff was observed between D2,  $8.93 \pm 0.27h$  compared to D4 where a DLMOff of  $8.91 \pm 0.25h$  was observed. The individual DLMOff (h) for each subject is shown in Table 4-6 for the Light (N=7) and no light treatment legs (N=7) for D2-4, and shown graphically in Figure 4-4 (a) and (b) respectively.



Subject	LIGHT			NO LIGHT		
	D2	D3	D4	D2	D3	D4
1	Nd	Nd	Nd	7.51	9.10	8.12
2	9.50	8.45	7.54	Nd	Nd	Nd
3	8.20	7.20	7.05	9.26	11.16	7.29
4				8.20	9.08	9.49
5	11.28	10.37	9.38			
6	7.05	7.56	7.01	9.54	9.58	10.15
7	9.23	8.53	7.09	7.25	8.04	
8				11.54	8.16	9.51
9	7.52	10.00	8.00			
10	Nd	Nd	Nd			
<b>Mean</b>	<b>8.69</b>	<b>8.46</b>	<b>7.68</b>	<b>8.93</b>	<b>9.31</b>	<b>8.91</b>
<b>SEM</b>	<b>0.26</b>	<b>0.21</b>	<b>0.15</b>	<b>0.27</b>	<b>0.21</b>	<b>0.25</b>

Table 4-6: Individual DLMOff (Dec. h) for subjects for D2, 3 and 4 of the two study legs (light and no light treatment leg) (Nd = no data, grey boxes = subject did not participate in this leg).

(a)





(b)

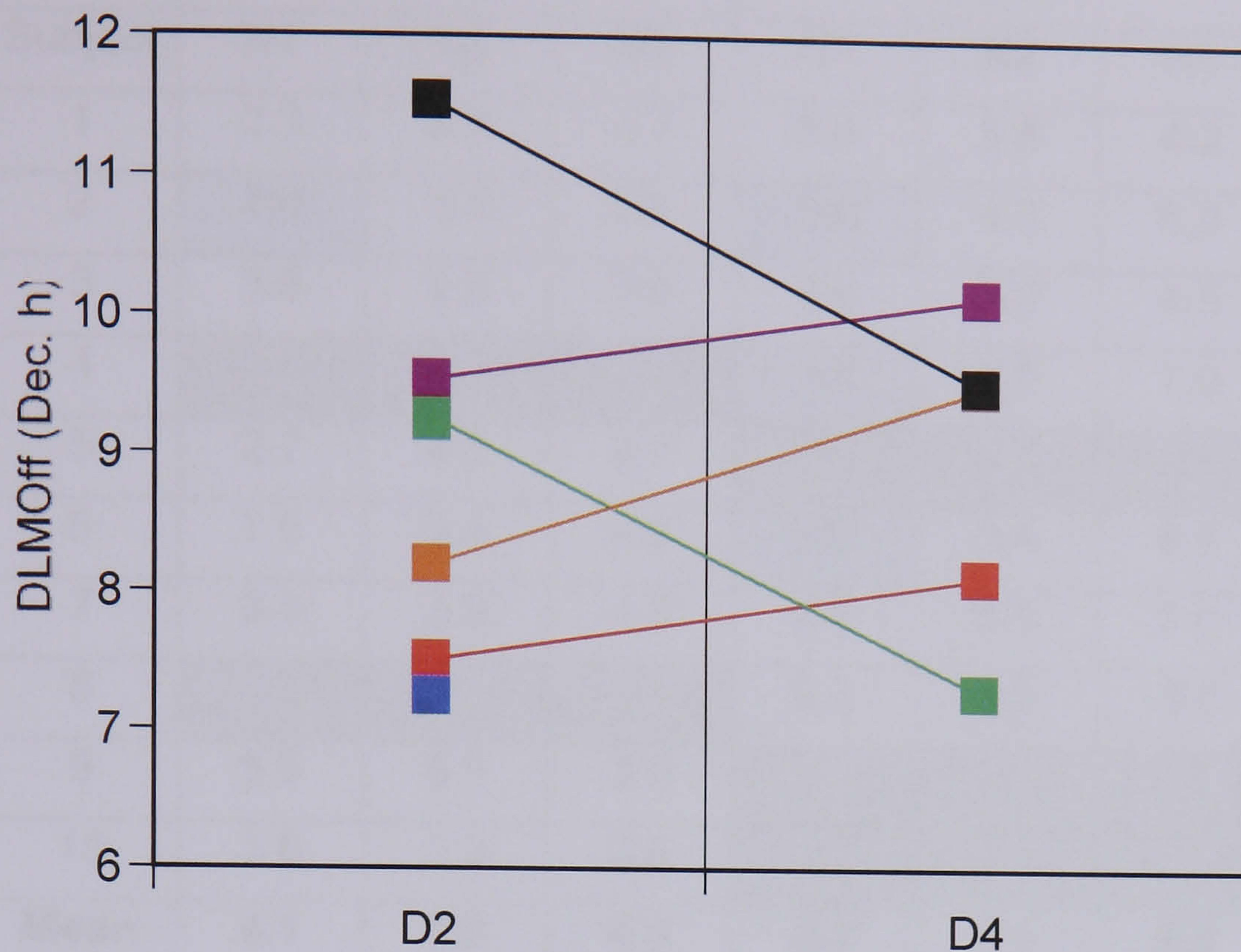


Figure 4-4: Plots individual data for DLMOff for D2 and D4, for the light treatment leg (a) and (b) control no light treatment leg.

#### 4.4.4. Urinary aMT6s acrophase

Individual aMT6s acrophases for each subject are shown in Table 4-7 for the light administration and the control no light administration legs of the study for N1-3. In the light administration leg three out of eight subjects phase advanced, however subjects 2, 3, 5, 6, 7 and 10 phase delayed (Figure 4-5a).

In the control no light treatment leg a significant phase delay ( $p=0.001$ , paired Students t-test) was observed in all subjects between the aMT6s acrophase on N2, ( $3.8 \pm 0.16h$ ) compared to N3 ( $5.9 \pm 0.47h$ ) (Figure 4-5b).



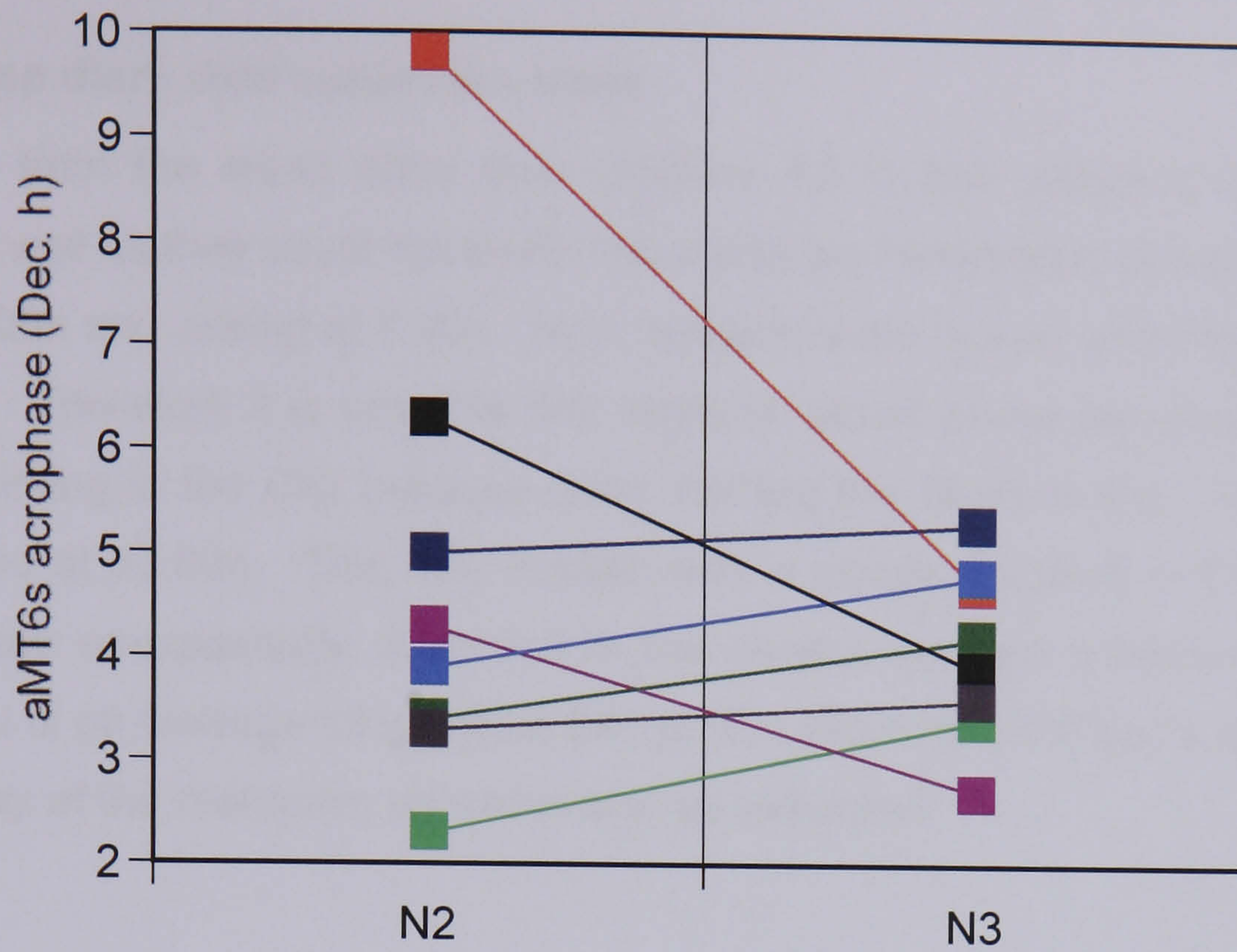
Subject	LIGHT			NO LIGHT		
	N1	N2	N3	N1	N2	N3
1	2.3	9.8	4.7	3.4	3.6	4.2
2	Nd	5.0	5.3	Nd	4.0	6.9
3	3.6	2.3	3.4	3.4	3.2	4.5
4				Nd	4.0	7.0
5	2.7	4.3	2.7			
6	7.5	3.4	4.2	Nd	3.4	6.5
7	3.9	3.9	4.8	4.8	4.5	7.0
8				9.0	3.8	5.0
9	5.8	6.3	3.9			
10	3.6	3.3	3.6			
<b>Mean</b>	<b>4.1</b>	<b>4.6</b>	<b>4.0</b>	<b>5.2</b>	<b>3.8</b>	<b>5.9</b>
<b>SEM</b>	<b>0.66</b>	<b>0.85</b>	<b>0.24</b>	<b>1.00</b>	<b>0.16</b>	<b>0.47</b>

Table 4-7: Individual aMT6s acrophases (Dec. h) for subjects for N1, N2 and N3 of the two study legs (light treatment (N=8) and no light treatment (N=7) (grey boxes = subject did not participate in this leg).

Figure 4-7 shows individual aMT6s acrophase data for light treatment and control no light administration legs.



(a)



(b)

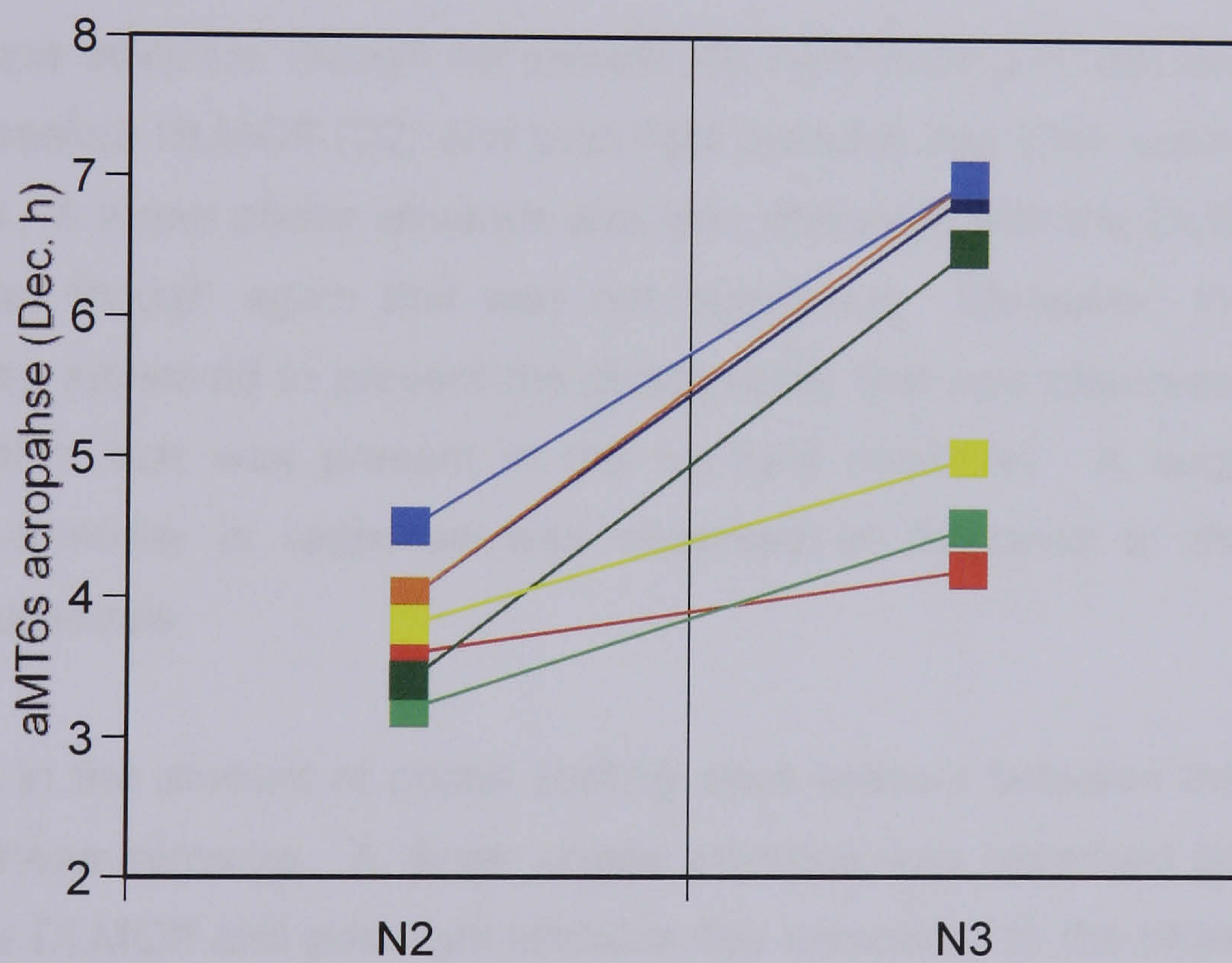


Figure 4-5: Plots of individual data for aMT6s acrophase on N2 and N3, for (a) the light administration leg and (b) control no light treatment leg.



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## 4.5. Discussion

### 4.5.1. Sleep diary data subjective sleep

It is clear from the sleep diary data (section 4.3.1) that subjects did not comply as well as they could have with the pre-study instructions of retiring to bed at 23.00h and arising at 7.00h. Most subjects went to bed later than was instructed. Therefore it is possible that subjects would phase advance their circadian timing in the CIU because upon starting the study in the CIU they went to bed at 23.00h. This may explain why a phase advance in DLMOff (D2-D4) was unexpectedly observed in the control no light treatment leg. Human tau is on average longer than 24h and in dim light (<10 lux) a natural phase delay of the melatonin rhythm would be expected.

### 4.5.2. Effect of light administration

A small phase advance, though not statistically significant ( $p=0.06$ ) was seen between baseline DLMOff (D2) and post light stimulus day (D4) illustrated in Figure 4-4a. A mean phase advance was also observed with the DLMO and aMT6s data, though again this was not significant. Moreover, the light treatment leg appeared to prevent the phase delay that was observed in the aMT6s rhythm that was present in the no light condition. A large inter individual variability in response was observed in response to the light between individuals.

Differences in the amount of phase shifting were present between the three melatonin measurements. A larger phase advance was observed between the baseline DLMOff and post light stimulus day compared to the phase shift in DLMO. This observation has been previously reported by Warman et al., 2003. A single light pulse as administered in the morning is more likely to affect the offset of melatonin secretion than the onset (Gordijn et al., 1999; Warman et al., 2003). This difference in phase shifts between DLMO and DLMOff has been previously attributed to the presence of two oscillators, morning (M) and evening (E), which through coupling form the circadian pacemaker. It has been proposed that the melatonin offset, a phase marker



for the M oscillator, is more sensitive to phase advancing light pulses whereas melatonin onset, a phase marker for the E oscillator, is more responsive to phase delaying light pulses (Illnerova and Vanecek, 1982).

The light treatment protocol was designed to phase advance the circadian system and hence its output markers (DLMO, DLMOff and aMT6s acrophase). Not all subjects shifted in the same direction (advance or delay). In some subjects phase delays were observed in both DLMO and DLMOff. Firstly this could be explained by subject's individual timing of their phase markers and highlights the importance of correctly timing the light exposure to cause a phase advance. Secondly perhaps the phase markers were not sufficient to resolve a small phase shift. Unfortunately completed data sets were not obtained from all subjects that participated in the study. It is thus difficult to draw any firm conclusions from the analysis of the saliva and urine samples.

#### **4.5.3. Effect of control “no light”**

In the control no light treatment leg a mean delay was seen in the aMT6s data and no phase shift was observed in DLMOff between baseline and post stimulus days. A phase delay was what one would expect to observe, as the human tau is on average just slightly longer than 24h (Middleton and Stone, 1996; Czeisler et al., 1999; Wright et al., 2001).

The results of the aMT6s data in the no light condition demonstrate the ability of the light box to phase shift, because the light treatment was able to counter the delay seen in the no-light condition.



#### **4.5.4. Limitations of the study**

##### **4.5.4.1. Measurement of salivary melatonin**

In a few of the subjects' salivary melatonin profiles it was impossible to determine a DLMO or DLMO<sub>off</sub> value as the plotted results for these subjects were very spiky. A clear onset or offset was not visible even with smoothing. This is the reason why some subjects had urinary aMT6s data and no DLMO and DLMO<sub>off</sub> values. This effect may have been influenced by the meals consumed but also if subjects had not rinsed their mouths out correctly. Secondly salivettes were used to collect the saliva samples, and it is now known that these produce some interference with the assay, as there is suppression of binding by unknown factors related to the salivette. It is difficult to know how much sample has been collected by the salivettes and they are expensive to use, thus in view of these various problems the use of salivettes has limitations.

#### **4.5.5. Timed light administration**

During the light administration session both phase advances and phase delays of the melatonin rhythms were observed. This may have been caused by the light not being administered at the correct circadian time to cause a maximum phase advance. As the timing of the melatonin rhythm was not assessed before the study, the timing of the morning light may not been optimal to cause a maximum phase advance the internal clock.

##### **4.5.5.1. Light exposure**

Light exposure the day before the CIU study could not be controlled, so the baseline N1 may have been influenced by environmental light exposure. As a result the N2 data was considered to be a more accurate representation of the melatonin onset time, and was used for statistical analysis. Other limitations of the study are that saliva samples were collected outside in the field. Bright natural light suppresses melatonin (Lewy et al., 1980), and subjects may have not have been wearing their sunglasses. It is also known



that posture, exercise (Buxton et al., 1997) and food affect melatonin production, and as a result field data collected from D5 was disregarded.

#### **4.5.5.2. Power of the study**

The present study was underpowered by the small sample size. Taking the observed DLMO<sub>off</sub> on the light administration leg as a measure of the phase shift and performing post hoc calculations revealed that the present study was underpowered with a power of 0.36 for 7 subjects included in the present study (Lenth, 2006; Power and Sample Size computer software). For the statistical power to reach 0.80 (80%), sample size calculations revealed that 12 subjects needed to be included in the study. Further work should aim to study more subjects in order to confirm the results.

#### **4.5.5.3. Protocol design**

A more suitable protocol, although more time consuming would have been to assess the subjects' circadian phase prior to the main study which has been done in previous studies (Thapan et al., 2001; Herljevic et al., 2005). Timing of the light administration could be individualised to ensure that the light was given at the correct circadian time to give a phase advance.

#### **4.5.6. Applications**

Previous laboratory and field studies have used light to treat circadian rhythm misalignment caused, for example by jet lag and shift work and has utilised light up to 10,000 lux (section 1.8.3). It is known that the phase shifting effect of light on the internal clock is dose dependent, with a response curve where maximum shift occurs at approximately 3000 lux (Zeitler et al., 2000). Despite its limitations the present study has demonstrated that the Litebook<sup>®</sup> producing 3000 lux may have the ability to phase shift the timing of the internal circadian system. It is a drug free approach to adjusting the internal timing of the circadian clock.



#### **4.6. Conclusions**

In conclusion, the present study failed to show any significant shift in phase as measured by salivary melatonin DLMO and DLMOFF after administration of the Litebook<sup>®</sup> for one hour in the morning. However the delay in aMT6s acrophase in the control (no light) condition from N2 to N3 was prevented by light treatment. Studying more subjects is necessary to confirm the present findings. Comparison of the phase shifting effect of light on the individual aMT6s acrophase times and DLMO and DLMOFF showed a combination of phase advances and phase delays following light exposure.



## **5. INVESTIGATING THE USE OF LIGHT TREATMENT TO HASTEN CIRCADIAN ADAPTATION IN OFFSHORE SHIFT WORKERS**

### **5.1. Introduction**

Previous research (Barnes et al., 1998a; Gibbs et al., 2002) has shown that subjects working 18.00-06.00h shift schedules offshore adapt to a night shift. If shift workers fully adapt to a night shift offshore they will be out of synchrony in their home environment for the few first days, with consequent problems of poor night sleep, reduced daytime alertness and performance and possible digestive problems (section 1.8). Factors such as season, length and timing of the shift, sleep/wake pattern (as this indirectly affects light exposure) and light exposure may all affect circadian adaptation. The use of light treatment appropriately timed, at home, may alleviate/reduce the physiological and behavioural problems caused by circadian rhythm disturbance experienced following night shift work.

Light has been suggested as a counter measure against night work impairment of sleep and alertness (Czeisler et al., 1990). Intense artificial light can shift the phase of the human circadian timing system and has been successfully used to induce phase shifts in circadian rhythms and to improve sleep, performance and alertness (section 1.5.4.1). Bright light as a counter measure for circadian desynchrony has been used in field studies of shift workers, though the number of studies are limited (section 1.7.5).

### **5.2. Aims and hypothesis**

The aim of this study was to investigate circadian adaptation in offshore night shift workers returning to day life at home. The study assessed the ability of light treatment to shift the circadian rhythm of urinary aMT6s, to improve night sleep daytime alertness and mood compared to a “no light treatment” condition.



The null hypothesis stated that there will be no difference in the timing of the aMT6s circadian rhythm in the light and no light treatment conditions. Secondly there will be no difference in the objective and subjective sleep at home following the night shift between the light and the no light treatment conditions.

### **5.3. Methods**

#### **5.3.1. Pre-study**

Ethical permission was obtained from the University of Surrey Ethics Committee (Ethics number: ACE/2002/95SBL5). Before starting the study, written informed consent was obtained from all volunteers. Subjects completed the HÖ questionnaire prior to starting the study (*Appendix A*). Subjects had to be working a 2 or 3-week night shift, not be taking antidepressants,  $\beta$ -blockers, sleeping medication or any medication that is known to affect melatonin production (such as barbiturates, antipsychotics, benzodiazepines etc).

#### **5.3.2. Study design**

Subjects were studied for the last 7 days offshore of a 2 or 3-week 19.00-07.00h night shift or a 18.00-06.00h night shift and for 14 days upon returning home onshore, or in some circumstances when working a day shift offshore following a night shift.

Subjects completed daily sleep diaries (*Appendix B*) for 21 days (last 7 days offshore and 14 days at home). Daily mood was also assessed for 21 days by completing a questionnaire within an hour of awakening (section 2.3). Subjects wore an Actiwatch-L for 21 days. Light exposure and activity were recorded by the Actiwatch-L in one-minute epochs. Subjects were asked to keep the Actiwatch-L exposed all the time, i.e. not covered by clothing.



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Subjects also collected sequential urines approximately 4 hourly during the day and an overnight collection, for the last 3 days offshore, the first 7 days onshore and day 12 onshore. For those working one week of nights followed by one week of days, urine collection occurred on the last 3 days of the night shift and the following 7 consecutive days afterwards.

### 5.3.3. Light treatment protocol

#### 5.3.3.1. 19.00-07.00h subjects

The study was a randomised crossover design with 2 legs, one where the subject received timed light treatment and wore sunglasses at appropriate times upon returning home in a light control and light treatment condition (Table 5-1), and the second leg where the sample collection protocol was the same but light treatment was not given and sunglasses were not worn.

Light treatment was administered by the use of a light box, Litebook<sup>®</sup> (Figure 4-1). Day 1 was the day the subjects returned onshore, and they were asked to wear specialised sunglasses (Litebook<sup>®</sup>) from the end of their night shift until 13.00h on Day 1. On Day 2 subjects wore sunglasses from wake up until 13.00h and then received light treatment by sitting in front of the Litebook<sup>®</sup> for 1h, according to instructions (*Appendix H*). For the following 3 days (Days 3-5) the light treatment was scheduled an hour earlier each day with subjects wearing sunglasses as before until the beginning of light treatment. The protocol is shown in Table 5-1.

Previous studies by Gibbs et al. (2002) showed that the peak of the aMT6s rhythm of subjects leaving the rig after working a 1 week 18.00-06.00h night shift was  $12.98 \pm 0.50$ h (mean  $\pm$  SEM). From the findings of these previous studies the light treatment protocol was designed. The timing of the light as presented in Table 5-1 was designed to cause a phase advance in the aMT6s rhythm to help hasten readaptation back to active day life.



	<b>Wear sunglasses constantly FROM → TO</b>	<b>Light box treatment FROM → TO</b>
<b>Day 1</b> – travel home day	end of shift → 13.00 h	NO LIGHT TREATMENT
<b>Day 2</b> - at home	waking → 13.00 h	13.00 h → 14.00 h
<b>Day 3</b> - at home	waking → 12.00 h	12.00 h → 13.00 h
<b>Day 4</b> - at home	waking → 11.00 h	11.00 h → 12.00 h
<b>Day 5</b> - at home	waking → 10.00 h	10.00 h → 11.00 h

Table 5-1: Light treatment protocol used for the first days onshore to phase advance the circadian system to hasten adaptation back to day life.

### 5.3.3.2. 18.00-06.00h subjects

Due to the results from the 19.00-07.00h study (section 5.4.1.1) it was decided that the light treatment should be individually timed to phase advance the circadian system. This study was not a crossover design as subjects completed the no light treatment leg first to allow aMT6s offshore to be analysed, and then in the second leg the light treatment was appropriately timed to phase advance the circadian system if the aMT6s acrophase was before 15.00h and to phase delay the circadian system if the aMT6s peak was later than 15.00h. Subjects whose aMT6s acrophase was between 11.00 to 15.00h followed the protocol as described in Table 5-1. Subjects whose aMT6s was between 15.00-17.00h started their light treatment on day2 at 14.00h for one hour, and those who aMT6s acrophase was between 17.00-19.00h started their light treatment at 16.00h for one hour. For these subjects the timing of the light treatment was delayed by one hour each day.

### 5.3.4. Subjects

#### 5.3.4.1. 19.00-07.00h subjects (summer)

Eight men aged  $46 \pm 11$  years (mean  $\pm$  SD), BMI  $28.1 \pm 2.5$  kg/m<sup>2</sup>, HÖ  $57 \pm 8$ , working a 19.00–07.00h shift schedule offshore, on an oilrig platform in the North Sea at 58°N for 14 or 21 consecutive nights were recruited during the



summer months (May-August, 2005). All subjects returned home onshore after finishing their night shift.

#### **5.3.4.2. 18.00-06.00h subjects (winter)**

Eight men aged  $47 \pm 7$  years (mean  $\pm$  SD), BMI  $28.1 \pm 2.1$  kg/m<sup>2</sup>, HÖ  $61 \pm 4$ , working a 18.00-06.00h shift schedule offshore in the North Sea at 59°N for 14 consecutive nights were recruited during the winter months (October-March). Two of the subjects worked one week of nights followed by one week of days offshore (CT-18 and CT-19), the 6 others returned home onshore after finishing their night shift.

### **5.4. Results**

No significant difference was observed between the two shift schedules (18.00-06.00h and 19.00-07.00h) in terms of age, BMI and HÖ score.

#### **5.4.1. No light treatment leg 19.00-07.00h**

##### **5.4.1.1. aMT6s analysis**

Urinary aMT6s was measured by RIA. Subjects' aMT6s acrophases were determined by cosinor analysis and are shown in Table 5-2 and Figure 5.1 for the last 3 days offshore and 7 days upon returning home onshore.



		Subject Number					
		CT-01	CT-02	CT-05	CT-06	CT-07	CT-09
		aMT6s acrophase (Dec. h)					
Offshore	-3	4.5	Nd	12.4	12.9	Nd	Nd
	-2	4.6	12.8	13.3	8.9	12.3	16.4
	-1	3.9	Nd	17.1	Nd	18.6	13.7
Onshore	1	4.2	19.1	Nd	13.7	15.1	19.9
	2	1.7	12.9	19.8	Nd	12.3	19.2
	3	4.4	17.1	23.5	0.8	7.3	20.7
	4	4.8	9.4	24.3	3.8	Nd	22.2
	5	4.2	5.8	3.2	22.6	4.1	23.0
	6	4.8	8.2	5.3	3.4	3.5	22.5
	7	Nd	9.1	4.1	Nd	3.7	Nd
	12	3.6	1.4	4.6	0.2	Nd	3.7

Table 5-2: aMT6s acrophase (N=6) of subjects working a 19.00-07.00h shift schedule for the last 3 days offshore (days-3 to -1) and the first 7 days upon returning home onshore (days 1-7). CT-04 only completed the light treatment leg, and CT-08 completed the no light treatment leg, but did not collect any urine to calculate the timing of aMT6s. Nd = no data, due to subjects collecting an insufficient number of samples over the day to calculate an aMT6s acrophase.



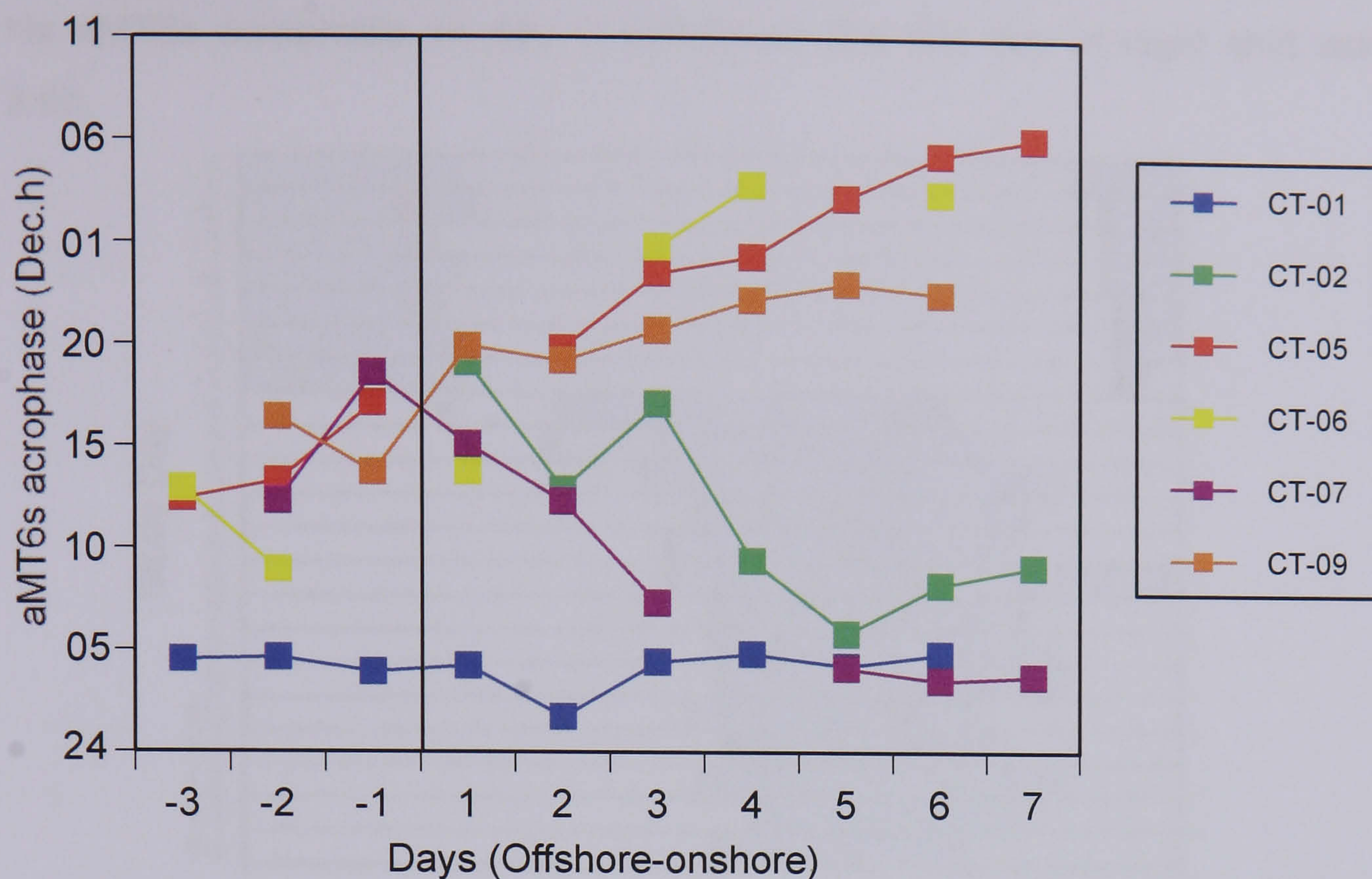


Figure 5-1: aMT6s acrophase for 10 days, last 3 days offshore followed by 7 days onshore of those working 19.00-07.00h (N=6) during the no light treatment leg. The vertical line on the graph indicates the end of the night shift. Each subject is coded by colour.

Figure 5-1 shows the aMT6s acrophase times for 6 subjects for the last 3 days offshore and the first 7 days upon returning home. The graph highlights the individual differences in aMT6s acrophase timing. The aMT6s acrophase times of the last day of the night shift offshore (day -1) range from 3.9h to 18.6h. In addition, a large variation in the change in aMT6s timing onshore is seen when the aMT6s rhythm readapts back to active day life.

The results from the no light treatment leg show that 3 subjects (CT-05, CT-06, CT-09) working offshore adapt back to active day life by phase delaying, as the aMT6s acrophase gets later and later in timing until it has realigned back to being produced during the night (02.00-04.00h), an example of this is illustrated in Figure 5-2. The other 2 subjects (CT-02 and CT-07) working offshore adapt back to active day life by phase advancing their aMT6s rhythm, the aMT6s acrophase gets earlier and earlier each day until readaptation has occurred. Subject CT-01 did not adapt to the night shift as



his aMT6s acrophase on day -1 (offshore), the last day of night shift was 3.9h.

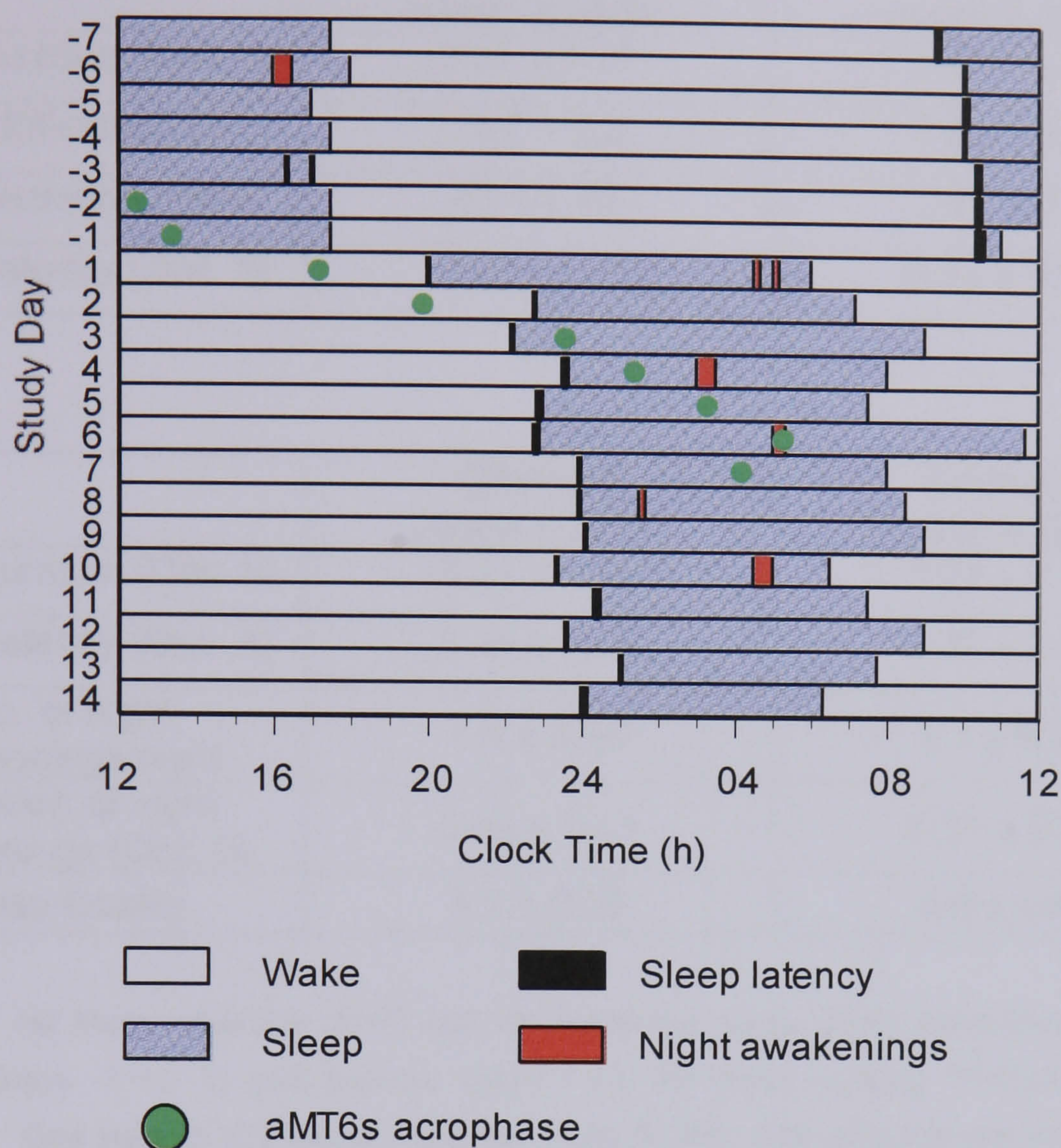


Figure 5-2: Sleep map of subject CT-05 (no light treatment leg, 19.00-07.00h shift) derived from sleep diary data, sleep periods are plotted in blue, wake in white, night awakenings are shown in red, and sleep latency in black, aMT6s acrophase times are shown by green circles.

The aMT6s acrophase (green circles) of CT-05, as shown in Figure 5-2, clearly phase delays upon returning home to home life onshore (day 1) as the timing of the calculated acrophase gets later and later.

#### 5.4.1.2. Objective and subjective sleep

Objective and subjective sleep parameters are shown in Table 5-3a and b, respectively, for the seven subjects working the 19.00-07.00h shift schedule.



(a)

	<b>Offshore</b> (mean $\pm$ SEM)	<b>Onshore</b> (mean $\pm$ SEM)
Sleep Duration (Dec. h)	5.31 $\pm$ 0.26	6.26 $\pm$ 0.22
Sleep Efficiency (%)	75.0 $\pm$ 2.3	79.0 $\pm$ 6.5
Fragmentation Index	42.4 $\pm$ 4.5	35.1 $\pm$ 2.9
Sleep Latency (Dec. h)	0.45 $\pm$ 0.06	0.42 $\pm$ 0.09

(b)

	<b>Offshore</b> (mean $\pm$ SEM)	<b>Onshore</b> (mean $\pm$ SEM)
Sleep Duration (Dec. h)	6.27 $\pm$ 0.07	7.25 $\pm$ 0.13
Sleep Latency (Dec. h)	0.20 $\pm$ 0.05	0.20 $\pm$ 0.06
No. of night awakenings/night	1.4 $\pm$ 0.26	1.1 $\pm$ 0.26
Duration of night awakenings (Dec. h)	0.35 $\pm$ 0.11	0.31 $\pm$ 0.10
Sleep Quality	5.1 $\pm$ 0.36	4.4 $\pm$ 0.42

Table 5-3 (a) Mean objective (N=7) and (b) subjective sleep (N=7) parameters for both offshore (days -7 to -1) and onshore (days 1-14) for those working 19.00-07.00h shift schedule. One subject (CT-04) working the 19.00-07.00h shift schedule did not complete the no light treatment leg.

No significant differences by Student's paired t-test were observed between the sleep offshore and sleep onshore in either objective or subjective parameters in the 19.00-07.00h shift schedule. Though in both objective and subjective sleep duration there was an increase observed in both of these measures, with objective sleep duration increasing from 5.31  $\pm$  0.26h offshore to 6.26  $\pm$  0.22h onshore, and subjective sleep from 6.27  $\pm$  0.07h offshore increasing to 7.25  $\pm$  0.13h onshore.



## 5.4.2. No light treatment 18.00-06.00h

### 5.4.2.1. aMT6s analysis

Subjects' aMT6s acrophases were determined by cosinor analysis and are shown in Table 5-4 and graphically in Figure 5-3 for the last 3 days offshore and 7 days upon returning home onshore.

		Subject Number							
Days		CT-12	CT-13	CT-14	CT-15	CT-16	CT-17	CT-18	CT-19
		aMT6s acrophase (Dec. h)							
Offshore	-3	Nd	Nd	12.5	Nd	Nd	Nd	Nd	Nd
	-2	Nd	17.9	13.0	12.1	Nd	16.0	11.2	12.9
	-1	12.5	14.9	16.9	11.1	14.5	17.2	14.6	13.4
Onshore	1	10.5	12.9	19.9	22.9	16.5	13.1	11.0	4.0
	2	14.0	17.7	20.8	9.6	Nd	18.5	14.3	12.9
	3	22.3	17.3	22.2	Nd	Nd	4.9	14.3	10.7
	4	9.1	21.1	23.9	21.3	1.0	23.5	17.6	13.5
	5	23.4	23.0	1.3	Nd	22.4	2.3	19.7	12.2
	6	Nd	Nd	2.0	21.1	0.6	Nd	Nd	11.0
	7	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	12	Nd	5.8	2.0	4.0	Nd	23.0	2.7	22.5

Table 5-4: aMT6s acrophase time (N=8) of subjects working 18.00-06.00h shift schedule for the last 3 days offshore (days -3 to -1) and the first 7 days upon returning home onshore (days 1-7). Nd = no data, due to subjects collecting an insufficient number of samples over the day to calculate an aMT6s acrophase.



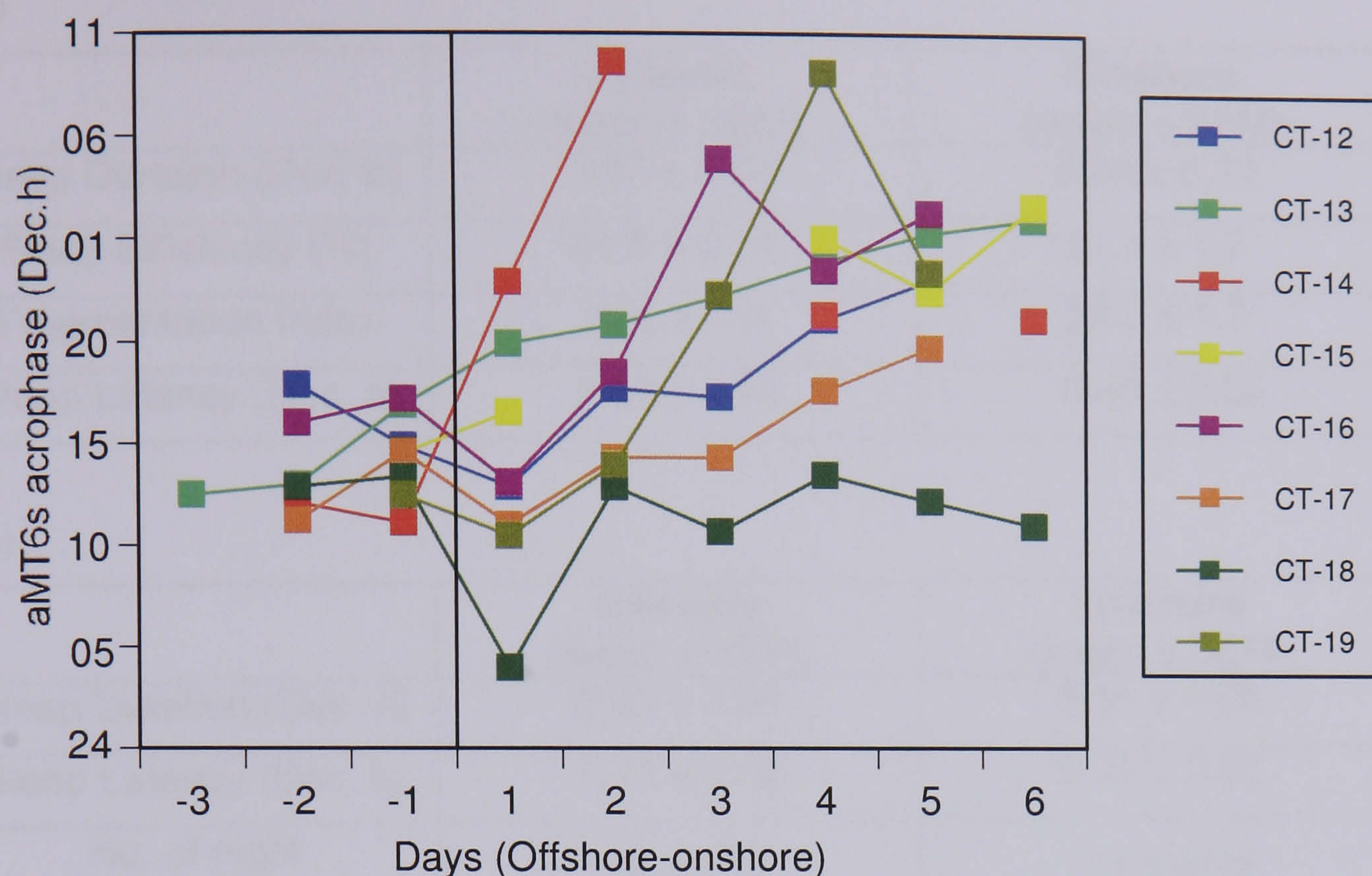


Figure 5-3: aMT6s acrophase for 10 days, the last 3 days offshore followed by 7 days onshore for those working 18.00-06.00h (N=8) during the no light treatment leg. The vertical line on the graph indicates the end of the night shift. Each subject is coded by colour.

Seven subjects phase delayed the timing of their aMT6s rhythm. One subject, CT-18, does not appear to change phase working the 18.00-06.00h shift schedule.

#### 5.4.2.2. Objective and subjective sleep

Objective and subjective sleep parameters are shown in Table 5-5 a and b respectively for the eight subjects.



(a)

	<b>Offshore</b> (mean $\pm$ SEM)	<b>Onshore</b> (mean $\pm$ SEM)
Sleep Duration (Dec. h)	5.87 $\pm$ 0.08	6.26 $\pm$ 0.22
Sleep Efficiency (%)	84.8 $\pm$ 0.73	82.3 $\pm$ 1.2
Fragmentation Index	29.5 $\pm$ 1.5	28.7 $\pm$ 1.7
Sleep Latency (Dec .h)	0.34 $\pm$ 0.09	0.40 $\pm$ 0.06

(b)

	<b>Offshore</b> (mean $\pm$ SEM)	<b>Onshore</b> (mean $\pm$ SEM)
Sleep Duration (Dec. h)	6.85 $\pm$ 0.06	6.91 $\pm$ 0.08
Sleep Latency (Dec. h)	0.17 $\pm$ 0.02	0.17 $\pm$ 0.02
No. of night awakenings/night	1.2 $\pm$ 0.13	1.1 $\pm$ 0.15
Duration of night awakenings (Dec. h)	0.17 $\pm$ 0.03	0.15 $\pm$ 0.03
Sleep Quality	4.3 $\pm$ 0.19	4.7 $\pm$ 0.21

Table 5-5a and b: Mean objective (N=8) and subjective sleep (N=8) parameters for both offshore (days -7 to -1) and onshore (days 1-14) for those working 18.00-06.00h shift schedule.

No significant differences were observed in the sleep parameters shown in Table 5-5a and 5-5b between offshore and onshore in either objective or subjective measures for those working the 18.00-06.00h.

### 5.4.3. Light treatment leg 19.00-07.00h subjects

#### 5.4.3.1. aMT6s analysis

Subjects' aMT6s acrophases were determined by RIA and are shown in Table 5-6 and graphically in Figure 5-4 for the last 3 days offshore and 7 days upon returning home onshore.



	Days	Subject Number							
		CT-01	CT-02	CT-04	CT-05	CT-06	CT-07	CT-08	CT-09
		aMT6s acrophase (Dec. h)							
Offshore	-3	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	-2	6.5	5.6	13.9	17.3	16.3	14.3	13.6	14.7
	-1	6.6	4.9	13.4	17.6	11.7	12.1	14.5	14.4
Onshore	1	9.3	7.7	8.9	17.9	17.2	Nd	16.8	19.4
	2	7.9	5.2	12.3	20.9	Nd	12.6	18.6	15.1
	3	7.9	9.0	10.5	0.6	Nd	0.9	19.7	13.5
	4	8.3	7.4	6.5	3.0	Nd	Nd	3.5	21.3
	5	6.6	7.8	5.5	3.1	3.9	Nd	22.6	1.3
	6	5.2	6.2	9.0	0.6	3.1	2.3	Nd	2.6
	7	4.6	8.8	6.9	Nd	2.4	2.4	Nd	1.3
	12	4.5	8.9	Nd	Nd	1.9	Nd	Nd	2.5

Table 5-6: aMT6s acrophase (N=8) of subjects working the 19.00-07.00h shift schedule for the last 3 days offshore (days -3 to -1) and the first 7 days upon returning home onshore (days 1-7). Nd = no data, due to subjects collecting an insufficient number of samples over the day to calculate an aMT6s acrophase.

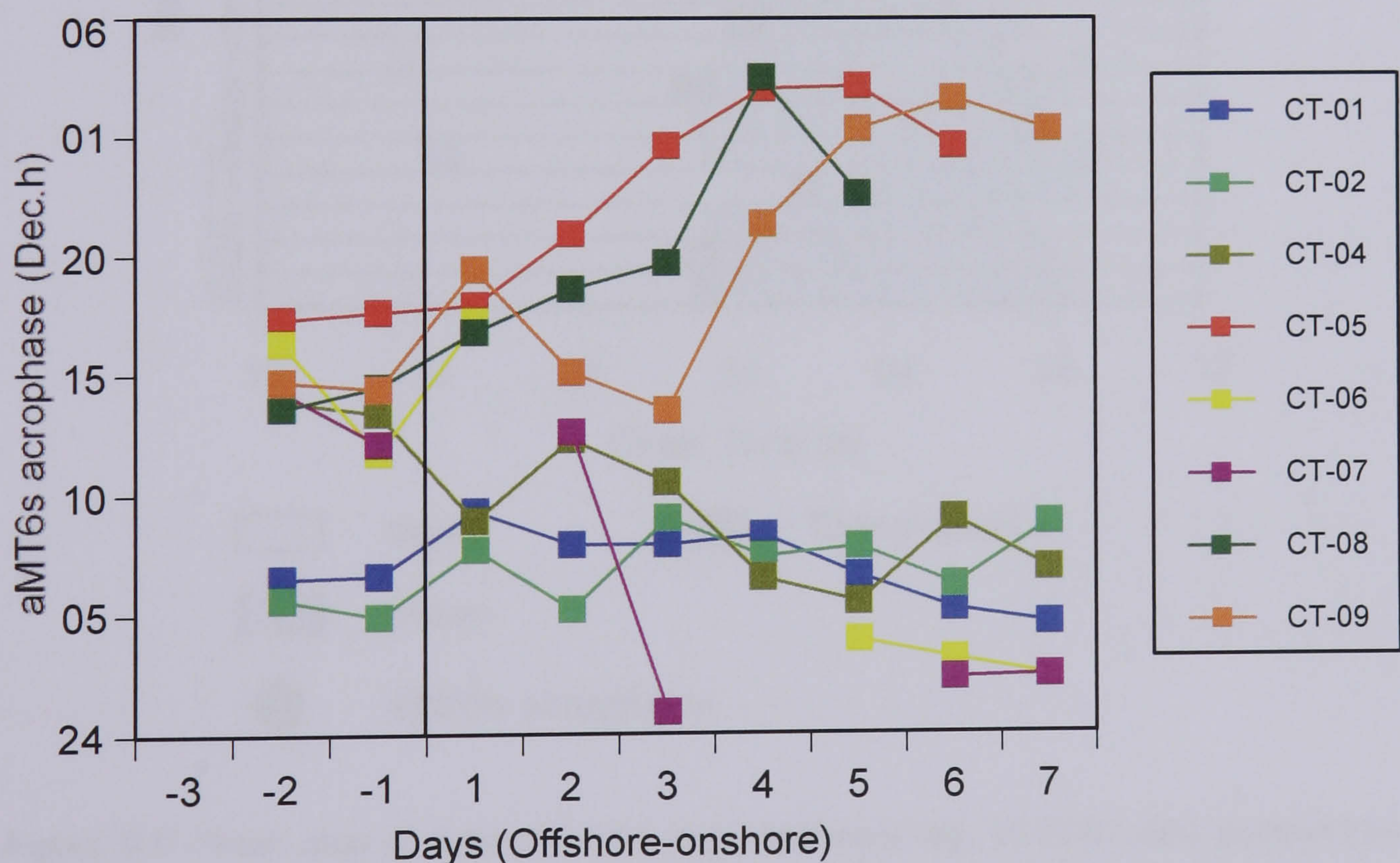


Figure 5-4: aMT6s acrophase for 10 days, last 3 days offshore (Days -3 to -1) followed by 7 days onshore (Days 1 to 7) of those working 19.00-07.00h (N=8) during the light treatment



leg. The vertical line on the graph indicates the end of the night shift. Each subject is coded by colour.

Figure 5-4 shows the aMT6s acrophase times for the 8 subjects for the last 2 days offshore and 7 days upon returning home. Three subjects adapt back to home life onshore by phase delaying their aMT6s rhythm (subjects CT-05, CT-08 and CT-09). Three subjects adapted back to home life by phase advancing their aMT6s rhythm (CT-04 and CT-07, CT-06) an example of this is illustrated in Figure 5-5. The other 2 subjects (CT-01 and CT-02) did not fully adapt to night shift offshore, hence the aMT6s acrophase did not change phase upon returning home onshore.

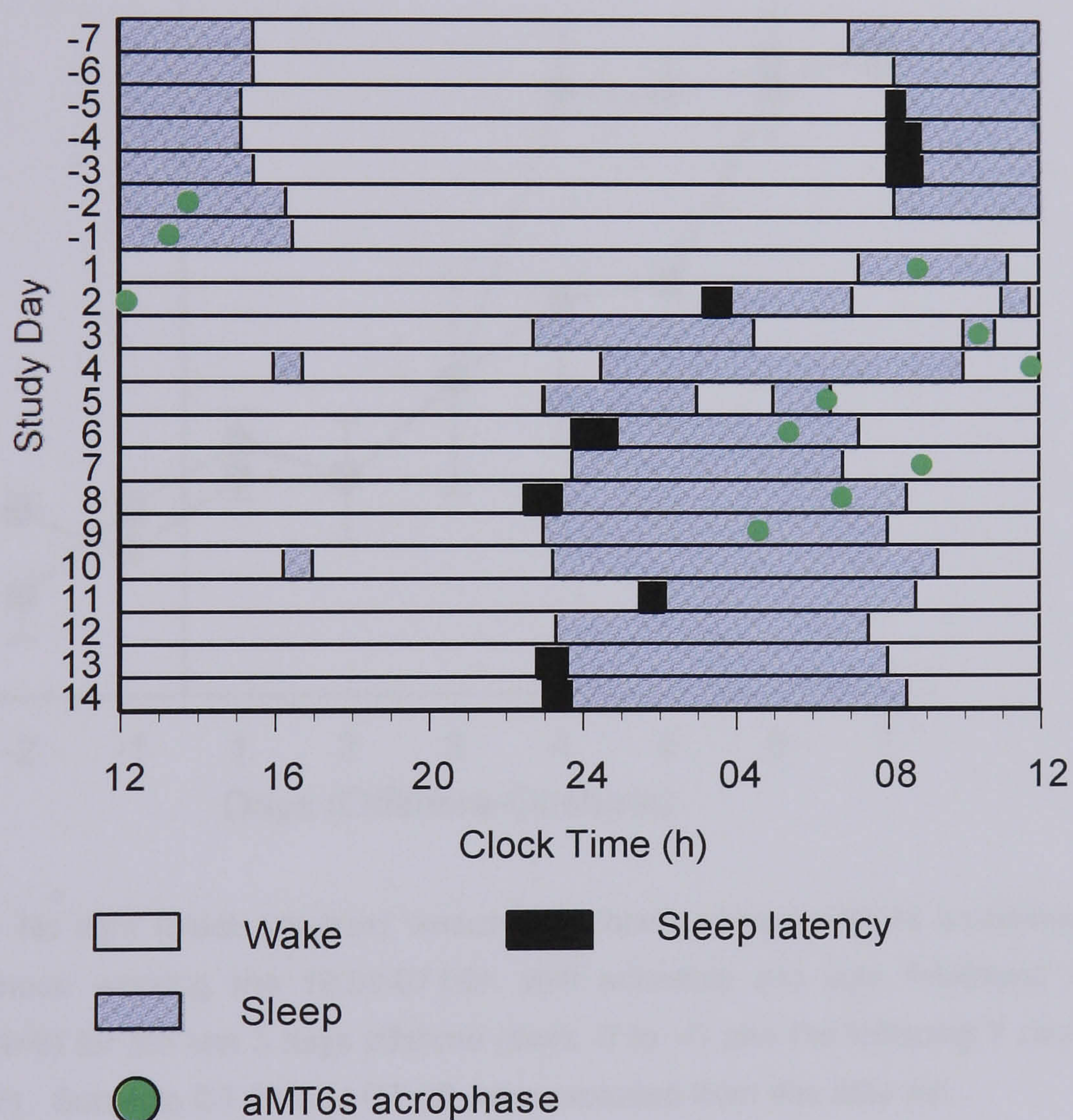


Figure 5-5: Sleep map of subject CT-04 (light treatment leg, 19.00-07.00h) derived from sleep diary data, sleep periods are plotted in blue, wake in white, aMT6s acrophase times are shown by green circles. This subject unfortunately did not record night awakenings in his sleep diary.



The aMT6s acrophase (green circles) of CT-04, as shown in Figure 5-5, clearly phase advances upon returning to home life onshore (day 1) as the timing of the calculated acrophase gets earlier and earlier.

#### 5.4.4. No light treatment versus light treatment for the 19.00-07.00h night shift

The average aMT6s acrophase for each day is plotted in Figure 5-6, excluding CT-01 and CT-02 who did not adapt to the night shift for those working the 19.00-07.00h shift schedule.

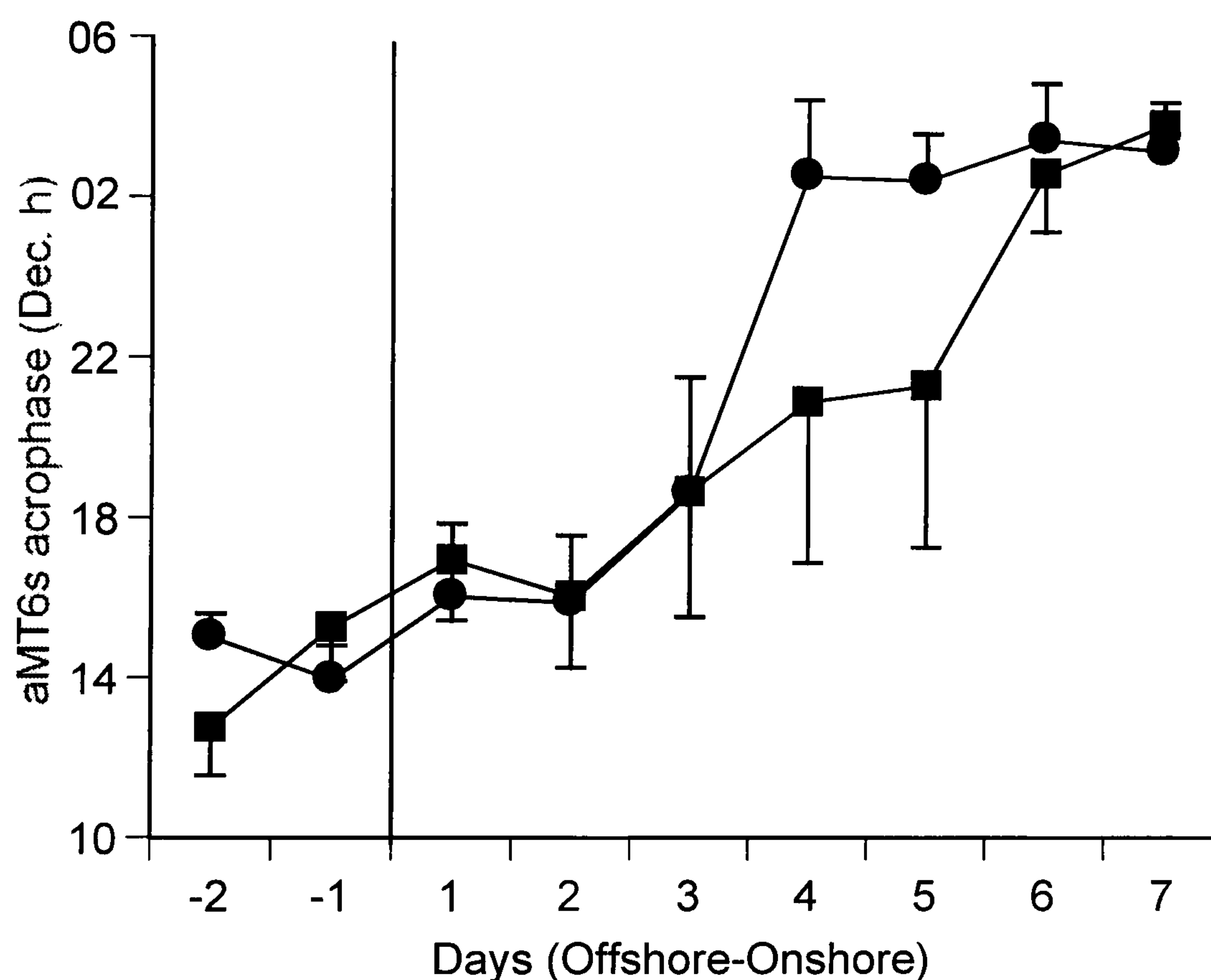


Figure 5-6: No light (black squares) versus light (black circles) aMT6s acrophase mean  $\pm$  SEM for those working the 19.00-07.00h shift schedule (no light treatment N=5, light treatment N=6) for the last 3 days offshore (days -3 to -1) and the following 7 days onshore (days 1 to 7). Subjects CT-01 and CT-02 were excluded from this data set.

The mean rate of adaptation between the light and no light treatment leg was calculated using the following equations: (mean aMT6s acrophase on day 5 – mean aMT6s acrophase last day (day -1) offshore) / number of days. No significant differences by Student's paired t-test were observed in the rate of



adaptation between light and no light treatment leg for those subjects who had data for both legs. The mean rate of adaptation in the no light treatment leg (N=5) was  $1.20 \pm 0.04$ h/day (mean  $\pm$  SEM). (CT-06 had no acrophase for the last day offshore, so the mean of the two preceding days was used for the calculation). For the light treatment leg (N=5) the mean rate of adaptation was  $2.50 \pm 0.28$ h/day.

#### 5.4.5. Light treatment leg 18.00-06.00h subjects

##### 5.4.5.1. aMT6s analysis

Subjects' aMT6s acrophases were determined by cosinor analysis and are shown in Table 5-7 and graphically in Figure 5.4 for the last 3 days offshore and 7 days upon returning home onshore.

		Subject Number								
		Days	CT-12	CT-13	CT-14	CT-15	CT-16	CT-17	CT-18	CT-19
Offshore		aMT6s acrophase (Dec. h)								
	-3	Nd	18.9	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	-2	Nd	15.5	12.1	13.4	Nd	16.9	11.4	19.3	
	-1	Nd	18.7	15.5	16.1	14.6	14.5	13.1	11.5	
Onshore / day shift	1	Nd	16.8	23.3	18.1	20.3	Nd	9.6	10.1	
	2	Nd	18.3	Nd	3.3	16.2	14.7	13.4	12.2	
	3	Nd	22.8	Nd	9.6	2.4	19.0	16.3	12.5	
	4	Nd	23.9	3.7	Nd	Nd	8.5	13.9	15.0	
	5	Nd	1.2	6.2	1.9	4.3	17.4	16.0	10.7	
	6	Nd	Nd	8.7	Nd	Nd	1.7	Nd	16.7	
	7	Nd	Nd	8.6	Nd	Nd	7.0	Nd	Nd	
	12	Nd	0.6	Nd	2.5	4.3	7.9	Nd	16.3	

Table 5-7: aMT6s acrophase (Dec. h) (N=7) of subjects working 18.00-06.00h shift schedule for the last 3 days offshore (days -3 to -1) and the first 7 days upon returning home onshore (days 1-7). Nd = no data, no urines were collected by subject CT-12.



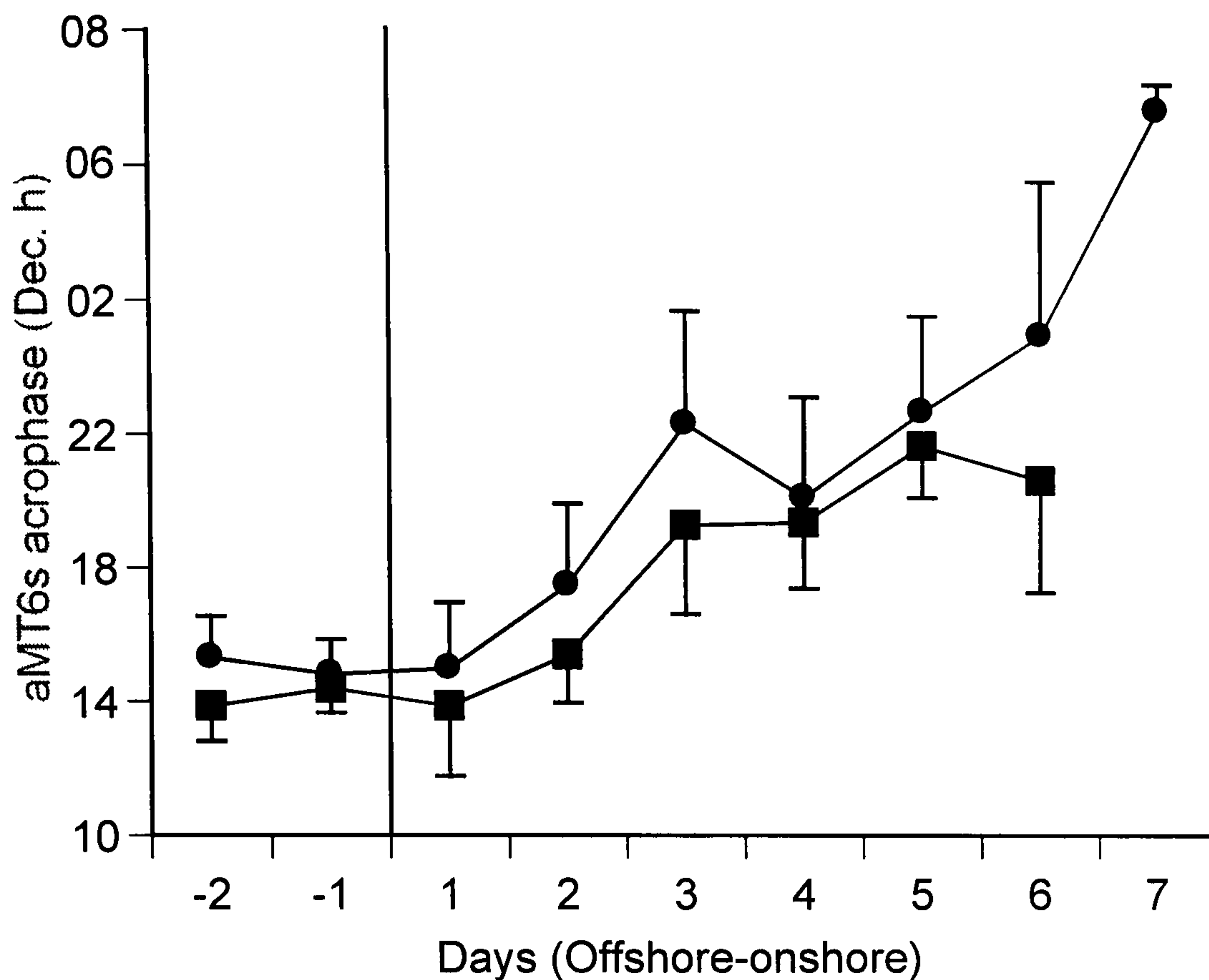


Figure 5-8: No light (black squares) versus light (black circles) aMT6s acrophase (mean  $\pm$  SEM) for those working the 18.00-06.00h shift schedule (no light treatment N=8, light treatment N=7) for the last 3 days offshore (days -2 to -1) and the following 7 days after the night shift (days 1 to 7). The vertical line on the graph indicates the end of the night shift.

From inspection of Figure 5-8, it can be seen that there is no difference in aMT6s acrophase with or without light treatment from day -2 offshore to onshore day 2. From days 3-7 the light treatment leg acrophase appears to move at a greater rate back to its normal peak time (approximately 04.00h), when it is being produced during the night.

However there were no differences observed in the rate of adaptation between the light and no light treatment legs. The mean rate of adaptation in the no light treatment leg was  $1.46 \pm 0.09$ h/day (N=8), compared to the light treatment leg where the mean rate of adaptation was  $1.58 \pm 0.42$ h/day (N=7).



### 5.4.7. Circadian timing of light

In order to interpret the results the circadian time of when the first light pulse was delivered has been calculated for each individual. The peak of aMT6s is known to occur 2 hours after the peak of plasma melatonin (Bojkowski et al., 1987). The melatonin acrophase for each subject (aMT6s acrophase time minus 2 hours) was calculated for the last day offshore on the light treatment leg (day -1). The melatonin acrophase for each subject is listed in Table 5-8.

Subject Number	Melatonin acrophase (Dec. h)
CT-01	4.6
CT-02	2.9
CT-04	11.4
CT-05	15.6
CT-06	9.7
CT-07	10.1
CT-09	12.4
CT-13	16.7
CT-14	13.5
CT-15	14.1
CT-16	12.6
CT-17	12.5
CT-18	11.1
CT-19	9.5

Table 5-8: Calculated melatonin acrophase from aMT6s acrophases from the last day offshore (day -1). Subjects CT-01 to CT-09 worked the 19.00-07.00h shift schedule whilst subjects CT-13 to CT-19 worked the 18.00-06.00h shift schedule.

In Figures 5-7 and 5-8, each black bar represents a subject's melatonin acrophase for day -1 offshore as shown in Table 5-8. Along the x-axis is clock time. A typical melatonin profile is also shown; this profile has a melatonin acrophase of 13.00h. If subjects received light at 13.00h on day 2, it would be predicted that those subjects with bars on the left-hand side of the



typical melatonin profile would have received light timed to phase delay their melatonin rhythm. Whilst those subjects with black bars on the right-hand side would have received light timed to phase advance their melatonin rhythm.

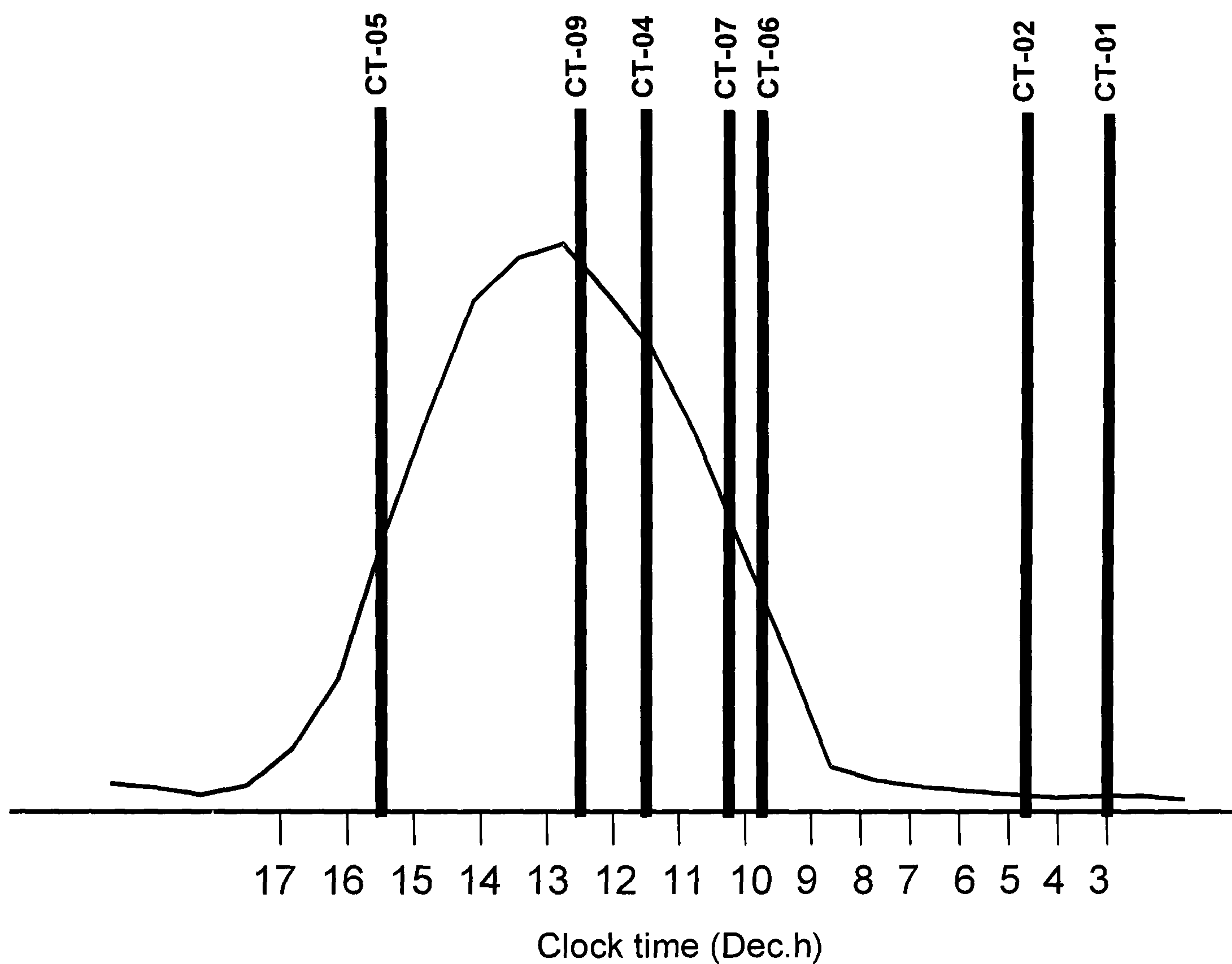


Figure 5-9: A typical melatonin profile with an acrophase of 13.00h is shown. Subjects' (N=7) melatonin acrophases for the last day offshore (day -1) for those working the 19.00-07.00h shift schedule are illustrated by black bars. Subjects with bars on the left-hand side of the typical melatonin profile would have received light timed to phase delay their melatonin rhythm, whilst those on the right-hand side would have received light timed to phase advance their melatonin rhythm. Subjects CT-01 and CT-02 who were unadapted to the night shift are excluded.



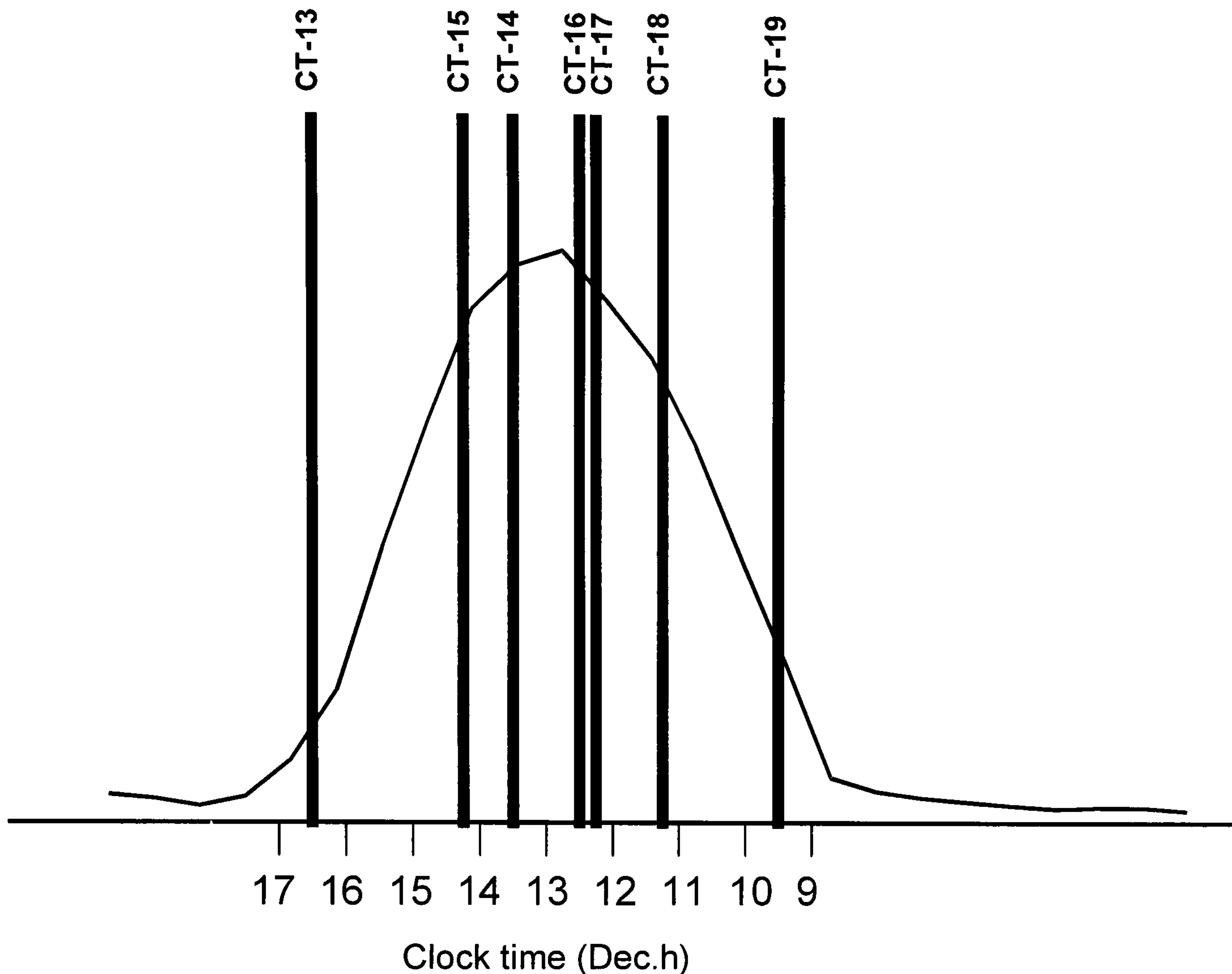


Figure 5-10: A typical melatonin profile with an acrophase of 13.00h is shown. Subject's (N=7) melatonin profiles for the last day offshore (day -1) for those working the 18.00-06.00h shift schedule are illustrated by black bars. Subjects with bars on the left-hand side of the typical melatonin profile would have received light timed to phase delay their melatonin rhythm, whilst those on the right-hand side would have received light timed to phase advance their melatonin rhythm.

From Figure 5-9 and 5-10 subjects CT-04, CT-06, CT-07, CT-09, CT-14, CT-16, CT-17, CT-18 and CT-19 received light approximately correctly timed to phase advance their melatonin rhythm. CT-04 received light at the correct time, but unfortunately did not complete the no light treatment leg. CT-06 appeared to receive light at the correct time but upon closer inspection of the data this subject looks as if he was free-running as the aMT6s acrophase was constantly shifting. The sleep parameters both objective and subjective for these subjects are described and discussed in the following section.



### 5.4.8. Sleep during light and no light treatment

#### 5.4.8.1. Objective sleep

Due to the small numbers in each shift schedule, the two shift schedules were combined. Days 6-14 were chosen for these analyses as this allows a direct comparison between the light and no light treatment leg. Days 2-5 could have been affected directly by the light. The data of the two shift schedules were grouped together and analysis by paired t-test was performed. Individual mean sleep duration for days 6-14 for light and no light treatment legs is shown in Table 5-9.

Subject Number	Sleep Duration (Dec. h)		Sleep Efficiency (%)		Fragmentation Index		Sleep Latency (Dec. h)	
	No Light	Light	No Light	Light	No Light	Light	No Light	Light
CT-07	5.23	7.30	68.7	78.4	56.5	51.9	0.35	0.33
CT-09	5.59	6.70	80.2	81.6	30.6	32.7	0.32	0.10
CT-12	4.52	7.50	86.7	92.8	44.0	34.4	0.06	0.06
CT-14	6.22	5.88	89.6	85.2	25.8	27.8	0.19	0.26
CT-15	5.57	7.32	84.8	94.4	28.0	23.9	0.21	0.00
CT-16	6.76	6.48	75.4	76.8	34.3	29.0	0.68	0.51
CT-18	3.65	4.75	83.6	81.4	28.7	37.8	0.17	0.15
CT-19	5.96	5.84	79.9	85.7	30.8	20.4	0.42	0.37
<b>Mean</b>	<b>5.44</b>	<b>6.47</b>	<b>81.1</b>	<b>84.5</b>	<b>34.8</b>	<b>32.2</b>	<b>0.30</b>	<b>0.22</b>
<b>± SEM</b>	<b>0.35</b>	<b>0.33</b>	<b>2.2</b>	<b>2.5</b>	<b>2.5</b>	<b>3.5</b>	<b>0.06</b>	<b>0.06</b>

Table 5-9: Mean individual actigraphic sleep parameters (N=8) for the light and no light treatment leg for days 6-14 onshore.

Mean actigraphic sleep duration after the light treatment (days 6-14) was significantly longer ( $6.47 \pm 0.33$ h, mean  $\pm$  SEM) compared to the no light condition ( $5.44 \pm 0.35$ h; paired Student's t-test,  $p=0.04$ ). No statistical differences were observed in the other sleep parameters shown in Table 5-9. However after the light treatment there was a trend for increased sleep



efficiency ( $84.5 \pm 2.5\%$ ) compared to the no light condition ( $81.1 \pm 2.2\%$ ). Mean actigraphic fragmentation index after the light treatment was less ( $32.2 \pm 3.4$ ) compared to the no light condition ( $34.8 \pm 0.35$ ). Mean actigraphic sleep latency after the light treatment was decreased ( $0.22 \pm 0.06\text{h}$ ) compared to the no light condition ( $0.30 \pm 0.06\text{h}$ ).

#### 5.4.9. Subjective sleep and mood assessment (sleep diaries)

Mean individual sleep parameters are shown in Table 5-10.

Subject Number	Sleep Duration (Dec. h)		Sleep Latency (Dec. h)		No. of night awakenings		Night awakenings (Dec. h)		Sleep Quality	
	No Light	Light	No Light	Light	No Light	Light	No Light	Light	No Light	Light
CT-07	6.74	9.42	0.08	0.10	0.52	0.08	0.08	0.52	4.4	2.8
CT-09	6.60	6.60	0.08	0.18	1.73	0.11	0.11	1.73	4.8	5.0
CT-14	6.67	8.59	0.17	0.24	0.16	0.07	0.07	0.16	2.3	4.3
CT-15	7.15	7.39	0.18	0.11	0.17	0.08	0.08	0.17	3.1	3.1
CT-16	7.46	8.33	0.09	0.08	0.02	0.04	0.04	0.02	3.2	8.1
CT-17	8.19	8.59	0.06	0.06	0.16	0.07	0.07	0.16	5.0	4.8
CT-18	7.01	5.42	0.13	0.12	0.15	0.25	0.25	0.15	6.8	5.5
CT-19	6.87	6.68	0.07	0.04	0.17	0.10	0.10	0.17	3.2	3.4
<b>Mean</b>	<b>7.09</b>	<b>7.63</b>	<b>0.11</b>	<b>0.12</b>	<b>0.39</b>	<b>0.10</b>	<b>0.10</b>	<b>0.39</b>	<b>4.1</b>	<b>4.6</b>
<b>± SEM</b>	<b>0.19</b>	<b>0.47</b>	<b>0.02</b>	<b>0.02</b>	<b>0.20</b>	<b>0.02</b>	<b>0.02</b>	<b>0.20</b>	<b>0.5</b>	<b>0.6</b>

Table 5-10: Mean individual subjective sleep parameters (N=8) for the light and the no light treatment legs for days 6-14 onshore.

No statistical difference was observed in the mean sleep duration, sleep latency and duration of night awakenings between the light and no light treatment leg. Individual mean sleep duration for days 6-14 for light and no light treatment leg is shown in Table 5-10. Individual mean sleep latency during no light treatment leg was  $0.12 \pm 0.02\text{h}$ , versus light treatment leg  $0.11 \pm 0.02\text{h}$ . Mean duration of night awakenings after the light treatment (days 6-



14) was decreased ( $0.10 \pm 0.02\text{h}$ ) compared to the no light condition ( $0.39 \pm 0.20\text{h}$ ).

No significant differences were seen in measurements of alertness (section 2.3) between the no light and light treatment legs (days 6-14). For the no light treatment leg the average alertness on a scale of 1-10, with 10 being most alert, was  $6.7 \pm 0.3$  compared to the light treatment leg which was  $6.6 \pm 0.4$ . Also no significant differences in mood on a scale on 1 to 10, 1 being sad and 10 being happy, were observed. For the no light treatment leg, the average mood was  $7.0 \pm 0.1$  compared to  $6.9 \pm 0.1$  on the light treatment leg.

## **5.5. Discussion**

With increasing economic and social demands, we are rapidly evolving into the 24-h society where shift and night work and irregular working hours are becoming more common in many countries (Harma and Ilmarinen, 1999). This project assessed individual circadian phase of night shift workers offshore in order to time light treatment appropriately upon returning home onshore to aid adaptation. With those working 19.00-07.00h, at the end of the night shift the timing of the subjects' aMT6s rhythm was approximately 1-3h later than previously reported by Barnes et al. (1998a) for a 18.00-06.00h shift.

Even with pre-assessment of individual response to night shift, it is difficult to predict the right timing of the light treatment. The protocol was initially timed to phase advance. Most subjects (N=10) received light treatment within a phase advance window. However given the large individual variability in response to night shift, others (N=4) did not receive correctly timed light.



### 5.5.1. aMT6s acrophase

If light was correctly timed, then a significant difference ( $p=0.04$ ) in actigraphic sleep duration was observed between the light and no light treatment legs. A study by Martin and Eastman (1998) clearly demonstrated the importance of circadian phase position and timing of light for shifting the body clock to readapt to the new sleep-wake cycle. The results from this study also support this observation.

Greater individual differences are seen in the timing of the subjects' aMT6s acrophases after working the 19.00-07.00h night shift schedule compared to the 18.00-06.00h shift schedule. These inter-individual differences in the aMT6s acrophase offshore seem to be more pronounced than those previously observed for the 18.00-06.00h shift schedule (Barnes et al., 1998a) where a quite consistent acrophase was reported at the end of a 2-week night shift. This may be due to the timing of the shift schedule, or factors such as season and light exposure could be affecting aMT6s adaptation offshore.

The findings show that half the subjects studied on the no light treatment leg adapt back to home life onshore by phase delaying. This is the most natural way to readapt back following night shift work. This may be due to the timing of the aMT6s acrophase at the end of the night shift being a factor determining in which direction the subject shifts. The other half of the subjects adapted back to home life by phase advancing, which is the faster way to adapt back to active day life. Though not statistically tested, there may be a predictive trend for subjects with an initial later timing of the aMT6s rhythm on the last day offshore (day -1) to adapt back to home life onshore by phase delaying the timing of the aMT6s rhythm, whilst those with an earlier timing of the aMT6s rhythm would tend to adapt back to home life by phase advancing their aMT6s rhythm, as previously observed during a change from night shift (18.00-06.00h) to dayshift offshore (Gibbs et al., 2007).



### **5.5.2. Sleep**

Significant improvement in objective sleep duration was observed in the light treatment condition in comparison to the no light treatment leg. Differences were also observed in other aspects of sleep but these were not significant.

Objective and subjective sleep in those who received light at the correct time (N=8) may have improved because of more rapid circadian adaptation (although the differences were not statistically significant in this small number of subjects). Similar results were reported by Dawson and Campbell (1991) who investigated the use of light treatment for those who were working night shifts. Those who were exposed to a 4h light pulse averaged 62 minutes more sleep than the control group with no light. Significant differences between objective sleep were reported, but there was no significant increase in the sleep efficiency leg in comparison to the no light control, therefore though the sleep was of longer duration, the sleep quality was not better. Other studies have reported similar findings; Ross et al. (1995) used light treatment in a field study in subjects who were working night shifts in Antarctica. They reported improvement in sleep latency with bright light treatment. Lastly Bjorvatn et al. (1999) investigated offshore shift workers working 2 weeks of nights, and reported that bright light treatment (~10,000 lux) which was scheduled individually to phase delay the circadian rhythm reduced self-rated sleepiness.

### **5.5.3. Limitations of the study**

#### **5.5.3.1. Expectations of the study**

The subjects recruited in this field study were motivated to try out the bright light upon returning home hoping that it would reduce their complaints of feeling 'jet-lagged' upon returning home onshore. This may have led them to provide more positive scores following bright light treatment. By contrast statistical significance between the light and the no light condition was observed in the objective measures e.g. actigraphy and not in the subjective



measures, such as sleep diaries and mood assessment. This finding argues against a “placebo” effect of light.

As with any field study, certain conditions such as natural light exposure cannot be controlled. There was no control of the seasonal and daily changes in outdoor levels of light; though participating subjects completed both legs in the same season, either summer or winter. Subjects were asked when participating in the light treatment leg to wear the sunglasses provided at certain times; however there was no way of checking that this had actually occurred.

#### **5.5.3.2. Individual differences**

Individual differences in sleep-wake cycles have been widely reported (Mongrain et al., 2004). Differences between individuals in the timing of the aMT6s rhythm whilst offshore were observed, with a larger variation in aMT6s in the 19.00-07.00h shift schedule compared to the 18.00-06.00h shift schedule

#### **5.5.3.3. Light treatment protocol**

This study demonstrated that appropriately timed light can be used to hasten the adaptation to day life at home, as evidenced by improved sleep. In both shift schedules rate of adaptation was faster (though not significantly so) back to day life after working the night shift, in the light treatment leg compared to the no light treatment. There is the possibility to either phase advance the circadian system, if light exposure is timed on the declining phase of the melatonin rhythm. There is also the possibility to phase delay the circadian system if light exposure is timed on the rising phase of the melatonin rhythm (section 1.6.1). Thus if bright light exposure occurs at the ‘wrong’ time, this may lengthen the time in which it takes to readapt back to day life, though if subjects are very delayed it may be better to phase delay the melatonin rhythm rather than impose phase advances.



There was a clear beneficial effect of light treatment on sleep parameters, however due to the study design using both light and sunglasses it cannot be determined if it was light treatment, the avoidance of light or a combination of the two which was responsible for the observed effect. This ambiguity could only be resolved if a third leg was carried out with subjects just wearing sunglasses.

#### **5.5.3.4. Adaptation and seasonal differences**

Though it is hard to establish due to the two study groups working two different time schedules offshore (18.00-06.00h and 19.00-07.00h) it could be predicted that there would be seasonal differences in adaptation back to home life. In theory it should be quicker to adapt in the summer months due to higher intensity of the natural light exposure. However, in summer the likelihood of light exposure at a time conflicting with desired phase change is greater than in winter.

#### **5.5.4. Future work**

More recently the use of light and melatonin separately and in combination for adaptation offshore and onshore has been successfully demonstrated by Bjorvatn et al. (2007). This was a study of those working one week of nights (19.00-07.00h) followed by one week of days (07.00-19.00h). It would be interesting to look at subjects who work two weeks of nights on a similar regime, as these subjects are more likely, or have an increased opportunity to adapt to the night shift. Therefore this population of subjects are more likely to have greater problems returning home. The current work has investigated the aMT6s rhythm, and how this changes with light treatment, other researchers have only looked at the effects of light treatment and/or melatonin on sleep (Bjorvatn et al., 1998; Bjorvatn et al., 1999; Bjorvatn et al., 2006; Bjorvatn et al., 2007).



## **5.6. Conclusions**

The underlying cause of many of the health effects that are associated with working night shifts is thought to be desynchronisation of the circadian system with detrimental effects on sleep. If light treatment, appropriately timed, can hasten circadian readaptation, the time in which the body is in misalignment will be reduced. This study has demonstrated that appropriately timed light administered to hasten adaptation to day life after working night shift significantly improved actigraphic sleep duration, together with non-significant but beneficial effects on some other aspects of objective and subjective sleep. To confirm these observations, it would be highly desirable to conduct a study with larger numbers of subjects with a similar design.



## **6. THE EFFECT OF LIGHT ON MELATONIN SUPPRESSION**

### **6.1. Introduction**

Attempted suppression of human melatonin production at night by light was first reported in 1978 by Arendt and Wetterberg. In 1980 Lewy and colleagues using much brighter light than previously examined, described complete suppression with 2500 lux but not with 500 lux. Subsequently Bojkowski et al. (1987) showed that dim 'domestic intensity' light of 300 lux as well as bright light of 2500 lux could suppress both melatonin and aMT6s in humans. In a very thorough re-examination, Zeitzer et al. (2000) reported a complete dose response of melatonin suppression to light in young healthy volunteers. The response to light had a non-linear relationship to illuminance, with the maximal sensitivity of melatonin suppression being at approximately 3000 lux. More importantly the minimum intensity for detectable melatonin suppression was found to be about 50 lux.

### **6.2. Aims and hypotheses**

There is very little data on either light exposure or melatonin suppression on the night shift or any other shifts. In view of possible health implications (Davis et al., 2001; Schernhammer et al., 2001), I undertook an analysis of total aMT6s production when adapted to a night shift offshore and during the first 3 days at home, or on the day shift. The null hypotheses stated that there would be no effect of light exposure on aMT6s production.

### **6.3. Methods**

#### **6.3.1. Subjects**

Eight male subjects, mean age  $43 \pm 7$  years, (mean  $\pm$  SD), with a BMI of  $27.9 \pm 2.6$  kg/m<sup>2</sup> working a 19.00-07.00h 2 or 3 week night shift in summer,



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latitude 58°N were recruited. All subjects returned onshore after the completion of their night shift.

A further seven male subjects, mean age  $47 \pm 9$  years with a BMI of  $27.8 \pm 2.1$  kg/m<sup>2</sup>, working a 18.00-06.00h shift schedule in winter, latitude 59°N, were recruited. Five of these worked a 2 week night shift; whilst two subjects (CT-18 and CT-19) worked 7 nights followed by 7 days.

### 6.3.2. Urinary 6-sulphatoxymelatonin collection

Subjects were asked to collect sequential urines, approximately 4 hourly during the day and an overnight collection, for the last 3 days offshore, the first 3 days upon returning home onshore, or going onto the day shift offshore (*Appendix G*). aMT6s production was measured by RIA (section 2.2). Total 24 h production of aMT6s was calculated and then compared between the last 3 days of the night shift and the following 3 days. aMT6s suppression was calculated using the following equation:

$$\% \text{ aMT6s suppression} = 100 - (\text{offshore aMT6s 24 h production} / \text{onshore aMT6s 24h production}).$$

### 6.3.3. Light exposure

Subjects were asked to wear an Actiwatch-L on their non-dominant wrist for the last 3 days offshore and for the following 3 days to measure light exposure. Measurements of light were taken in one minute epochs and later analysed over the 24h day (section 2.3). Total 24h light exposure was calculated for each day and then averaged over the last 3 days offshore and the first 3 days onshore/working day shift. The light exposure on the last 3 days of the night shift and the following 3 days were then compared by paired Student's t-test.



## 6.4. Results

### 6.4.1. Subjects working 19.00-07.00h shift schedule in the summer

#### 6.4.1.1. 24h total aMT6s production

Individual mean total 24h production for the last 3 days of the night shift and the 3 days after the night shift are shown in Table 6-1.

Subject Number	Offshore aMT6s 24h production (ng/24h)	Onshore aMT6s 24h production (ng/24h)	aMT6s suppression (%)
CT-04	8527	4383	48.6
CT-05	4804	3598	25.1
CT-06	2515	808	67.9
CT-07	2162	1829	15.4
CT-08	2938	1089	62.9
CT-09	3107	3444	-10.9
NN18	9720	5166	46.9
NC23	4261	4027	5.5
<b>Mean ± SEM</b>	<b>4754 ± 1078</b>	<b>3043 ± 607</b>	<b>32.7 ± 10.0</b>

Table 6-1: Average individual aMT6s production over 24h for the last 3 days of the night shift and for the first 3 days at home onshore (N=8).

For subjects working the 19.00-07.00h shift schedule in the summer months, there was a significant suppression ( $p=0.03$ , paired Student's t-test) in the production of aMT6s over the 24h period for the first 3 days upon returning home onshore ( $3043.0 \pm 606.5$  ng/24h, mean  $\pm$  SEM) compared to ( $4754 \pm 1078$  ng/24h). This is illustrated in Figure 6-1. The overall mean melatonin suppression when comparing offshore and onshore values was  $32.7 \pm 10.0\%$



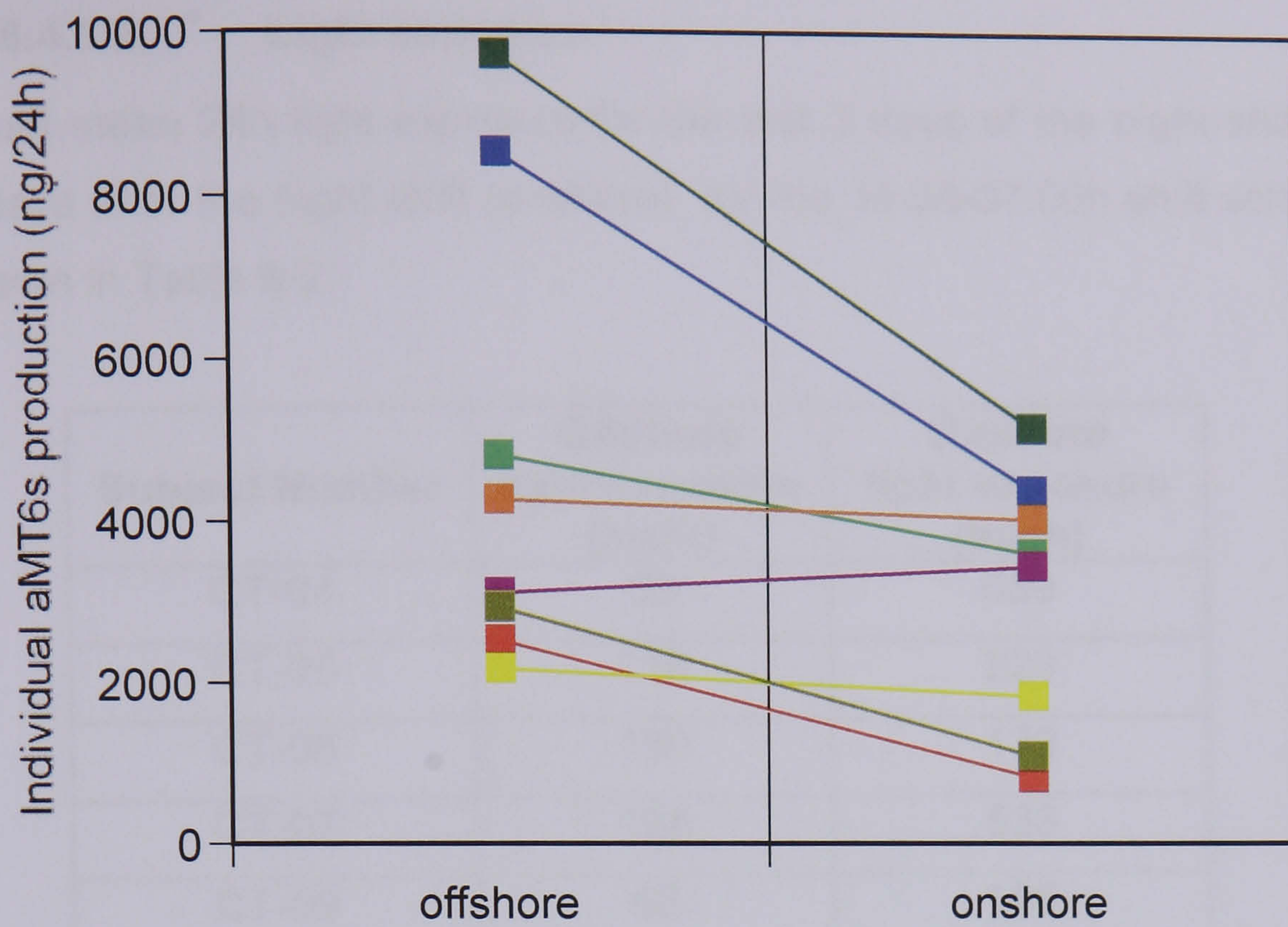


Figure 6-1: Average individual aMT6s production over 24h for the last 3 days of the 19.00-07.00h night shift and for the first 3 days at home onshore (N=8) in summer.

Seven of the eight subjects illustrated in Figure 6-1 showed a decrease in their 24h production of aMT6s upon returning home onshore, whilst one subjects showed a slight increase in aMT6s production upon returning home onshore.



### 6.4.1.2. Light exposure

Individual mean 24h light exposure for the last 3 days of the night shift and the 3 days after the night shift (onshore) for the 19.00-07.00h shift schedule are shown in Table 6-2.

<b>Subject Number</b>	<b>Offshore light exposure (lux/h)</b>	<b>Onshore light exposure (lux/h)</b>
CT-04	59	659
CT-05	118	229
CT-06	130	139
CT-07	105	533
CT-09	62	108
NC23	124	1151
NN18	84	728
<b>Mean ± SEM</b>	<b>98 ± 11</b>	<b>507 ± 143</b>

Table 6-2: Average individual light exposure per hour (lux/h) over the 24h day for the last 3 days of the night shift and for the first 3 days at home onshore (N=7) in summer.

Whilst offshore the average light exposure over 24h for the last 3 days offshore was  $98 \pm 11$  lux/h (mean  $\pm$  SEM) and this was significantly lower ( $p=0.03$ , paired Student's t-test) compared to the first 3 days onshore where light exposure was  $507 \pm 143$  lux/h. This is illustrated in Figure 6-2. CT-08 had no light data, and was therefore excluded from this section of analysis.



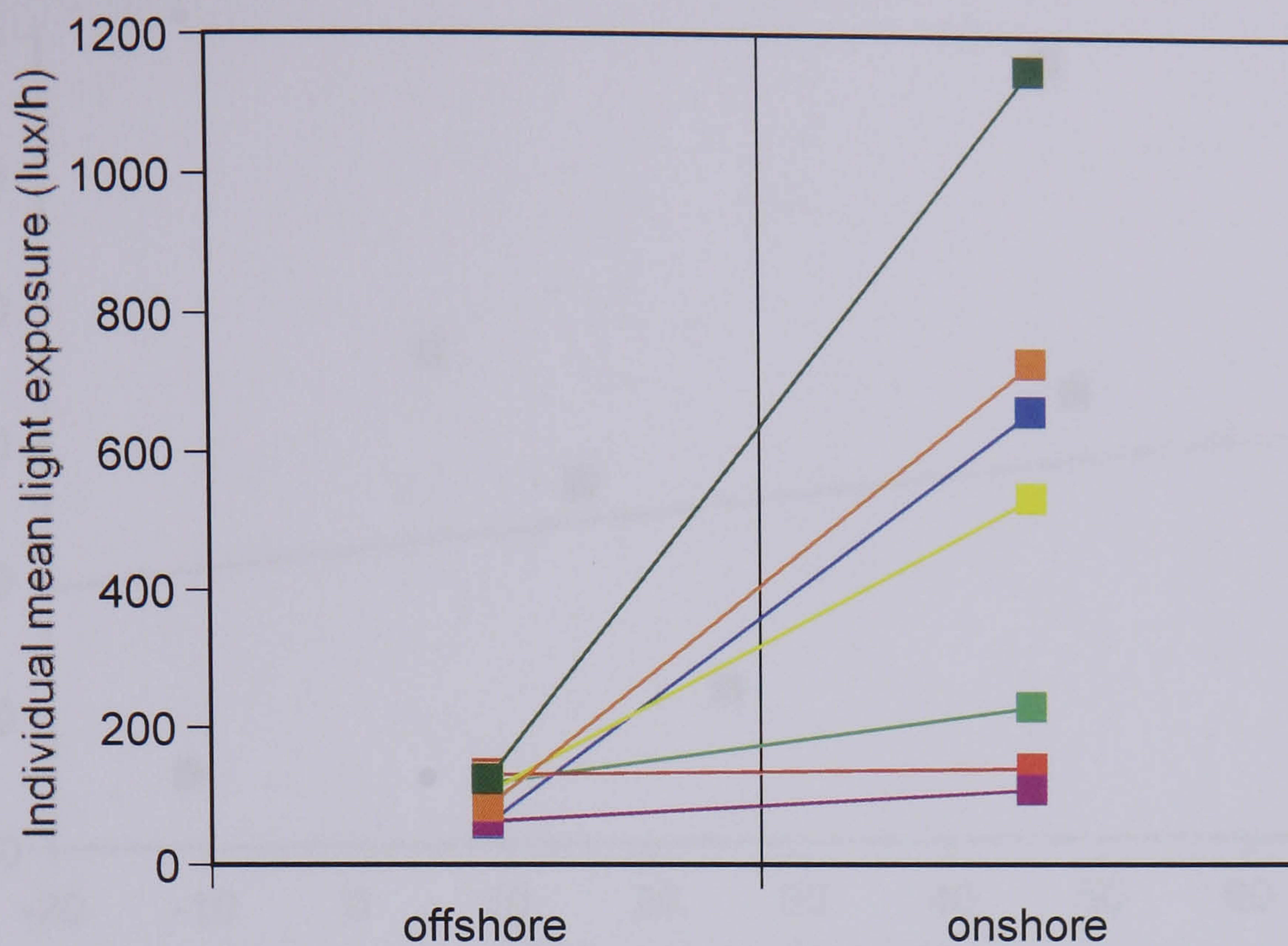


Figure 6-2: Individual mean light exposure over 24 hours for the last 3 days of the 19.00-07.00h night shift offshore and for the first 3 days onshore at home (N=7) in summer.

As expected all subjects (N=7) showed increased levels of light exposure upon returning home, as shown in Figure 6-2. Unexpectedly there was no significant correlation observed between the percentage of melatonin suppression upon returning home onshore and the amount of light exposure at home onshore. This is illustrated in Figure 6-3.



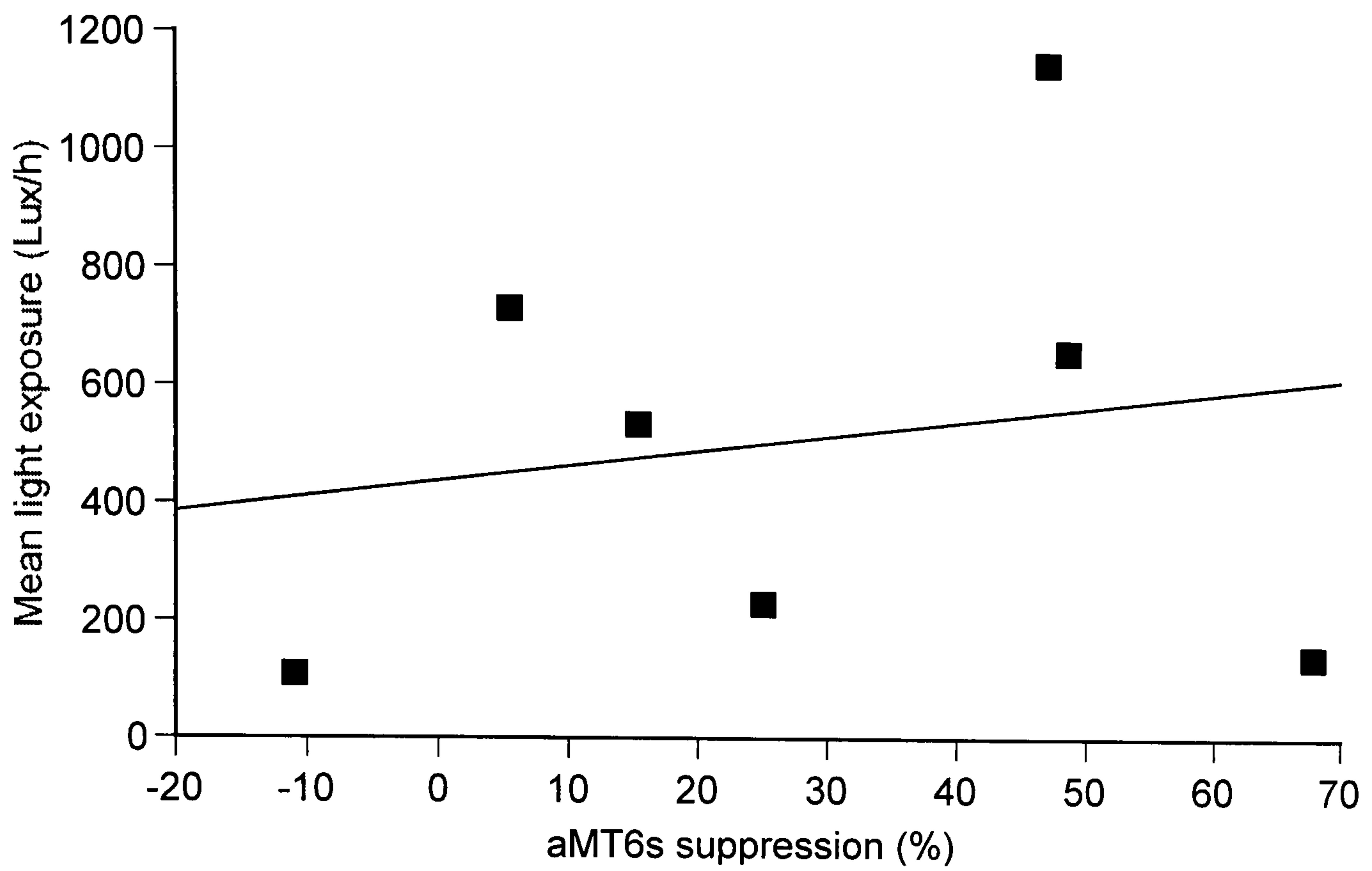


Figure 6-3: Correlation between mean light exposure onshore with aMT6s suppression.

#### 6.4.2. Subjects working 18.00-06.00h shift schedule in the winter

##### 6.4.2.1. 24h total aMT6s production

Individual mean 24h production for the last 3 days of the night shift and the 3 days after the night shift is shown in Table 6-3.



Subject Number	Offshore aMT6s 24h production (ng/24h)	Onshore/dayshift aMT6s 24h production (ng/24h)	aMT6s suppression (%)
CT-12	2255	2704	-19.9
CT-13	13268	14097	-6.2
CT-14	2738	2385	12.9
CT-16	358	113	68.5
CT-17	4075	1929	52.6
CT-18	5100	4266	16.4
CT-19	4591	4835	-5.3
<b>Mean</b>	<b>4626 ± 1563</b>	<b>4333 ± 1730</b>	<b>17.0 ± 32.5</b>

Table 6-3: Average individual aMT6s production (ng/24h) over 24h for the last 3 days of the 18.00-06.00h night shift and for the first 3 days at home onshore (N=7) and % aMT6s suppression. CT-18 and CT-19 worked one week of nights followed by one week of days.

Subjects working the 18.00-06.00h shift schedule in the winter months showed no significant suppression in the production of aMT6s over the 24h period for the first 3 days upon returning home onshore ( $4333 \pm 1730$  ng/24h, mean  $\pm$  SEM) compared to the last 3 days offshore ( $4653 \pm 1563$  ng/24h). These data are presented graphically in Figure 6-4. The overall mean aMT6s suppression when comparing offshore and onshore/day shift was  $17.0 \pm 32.5\%$



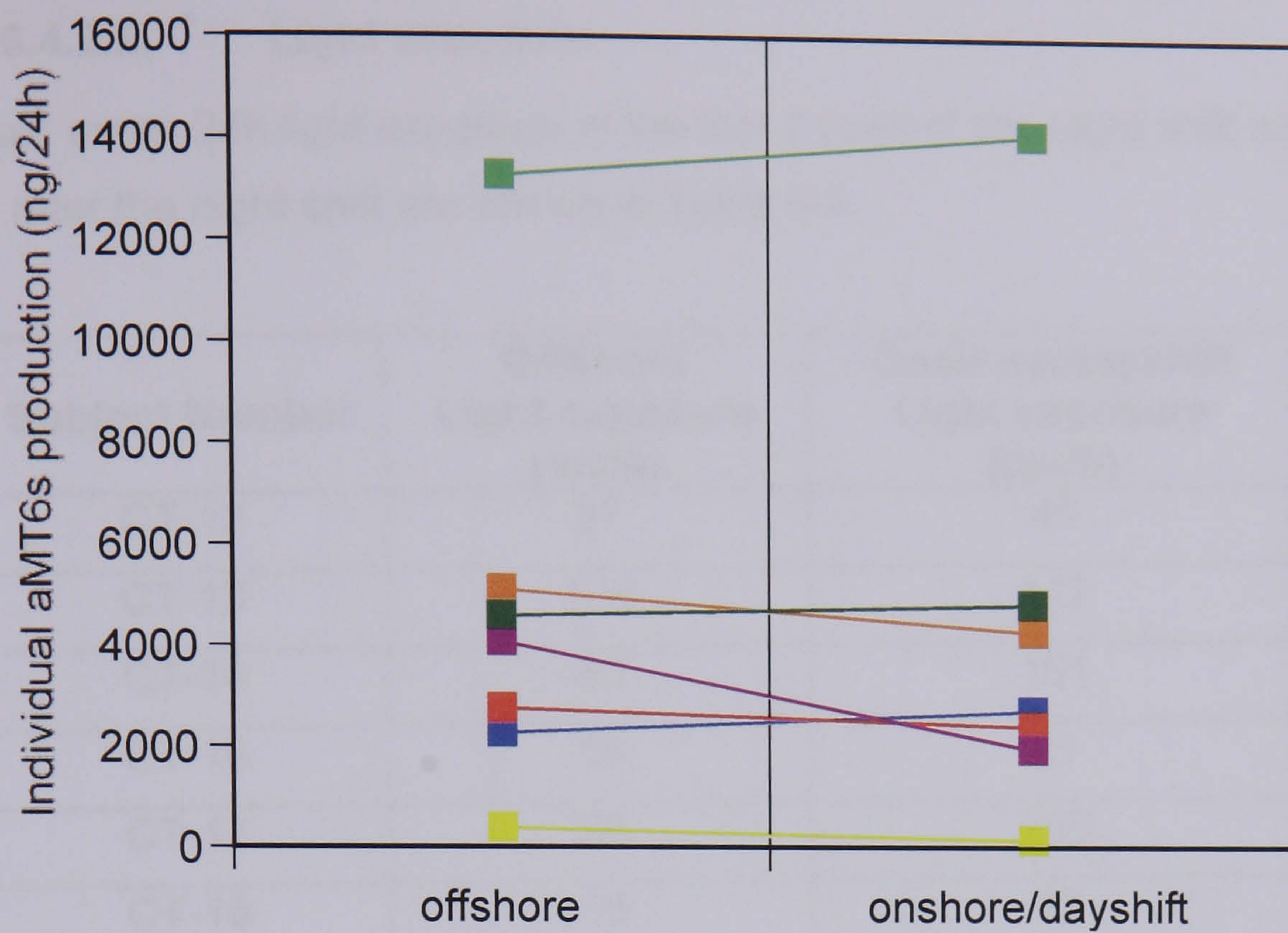


Figure 6-4: Average individual 24h production of aMT6s for the last 3 days of the 18.00-06.00h night shift and for the first 3 days at home onshore or on the day shift offshore (N=7) in winter.

Five of the seven subjects showed a small decrease in their production of aMT6s upon returning home onshore, whilst two subjects showed an increase in their aMT6s production over 24h.



### 6.4.2.2. Light exposure

Individual mean 24h light exposure of the last 3 days of the night shift and the 3 days after the night shift are shown in Table 6-4.

<b>Subject Number</b>	<b>Offshore Light exposure (lux/h)</b>	<b>Onshore/dayshift Light exposure (lux/h)</b>
CT-12	27	46
CT-13	108	112
CT-14	40	161
CT-16	55	21
CT-17	26	210
CT-18	15	12
CT-19	208	178
<b>Mean ± SEM</b>	<b>69 ± 26</b>	<b>105 ± 30</b>

Table 6-4: Average individual light exposure per hour over the 24h day for the last 3 days of the night shift and for the first 3 days at home onshore (N=7) in winter.

No significant differences were observed between the last 3 days offshore and the first 3 days at home onshore, concerning light exposure with those working the 18.00-06.00h shift schedule. Whilst offshore the average light exposure over the 24h for the last 3 days offshore was  $68.6 \pm 26.0$  lux, compared to the first 3 days onshore or on the day shift offshore, where light exposure was  $105.7 \pm 30.4$  lux, these data are presented graphically in Figure 6-5.



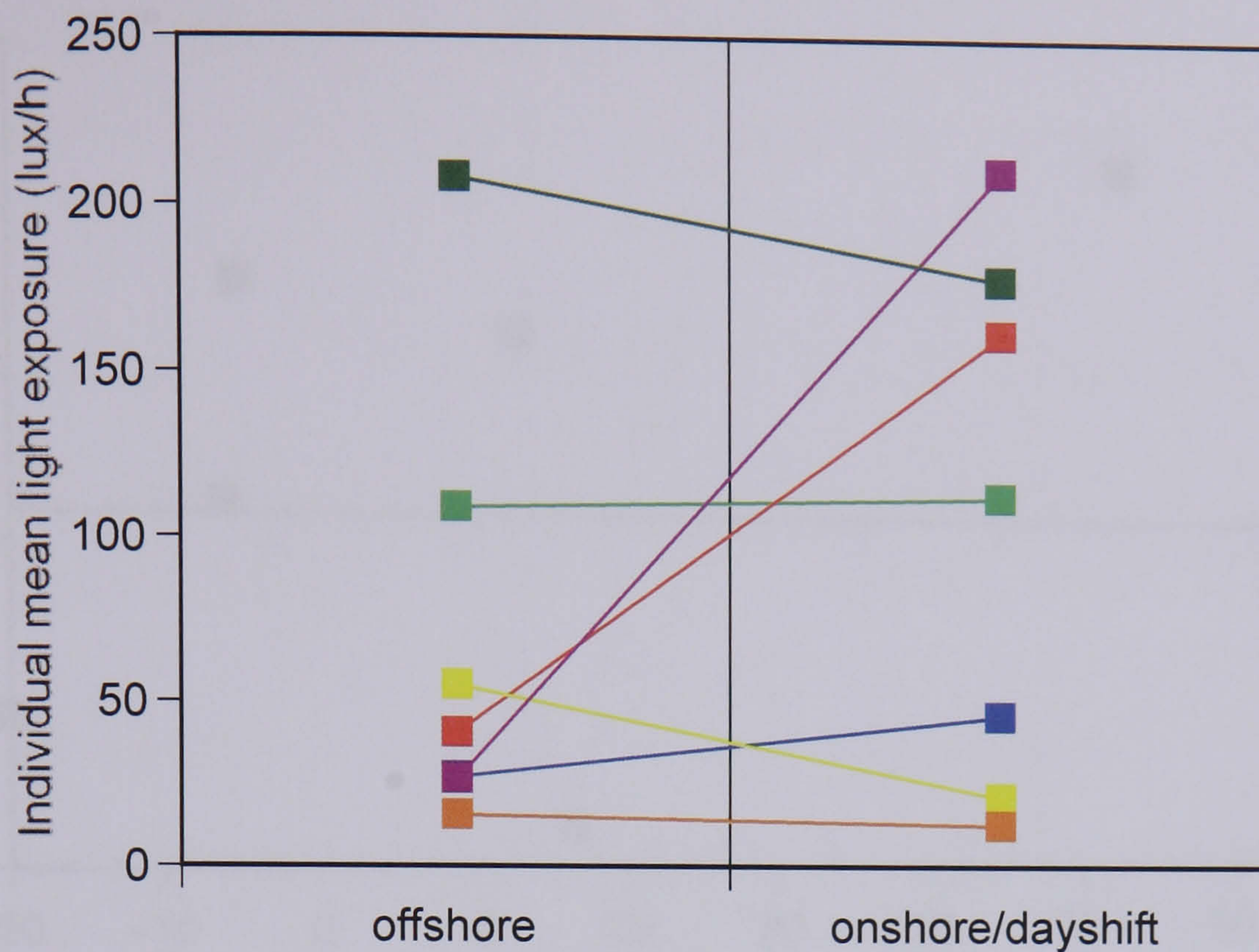


Figure 6-5: Individual light exposure per hour over 24 hours for the last 3 days of the night shift offshore and for the first 3 days onshore at home or on the dayshift offshore (N=7) in winter.

Five of the seven subjects as discussed in section 6.3.2.1 had an increased amount of light exposure upon returning home onshore, whilst the other two subjects had decreased amounts of light exposure upon returning home onshore.

No significant correlation observed between the percentage of melatonin suppression upon returning home onshore and the amount of light exposure at home onshore. This is illustrated in Figure 6-6.



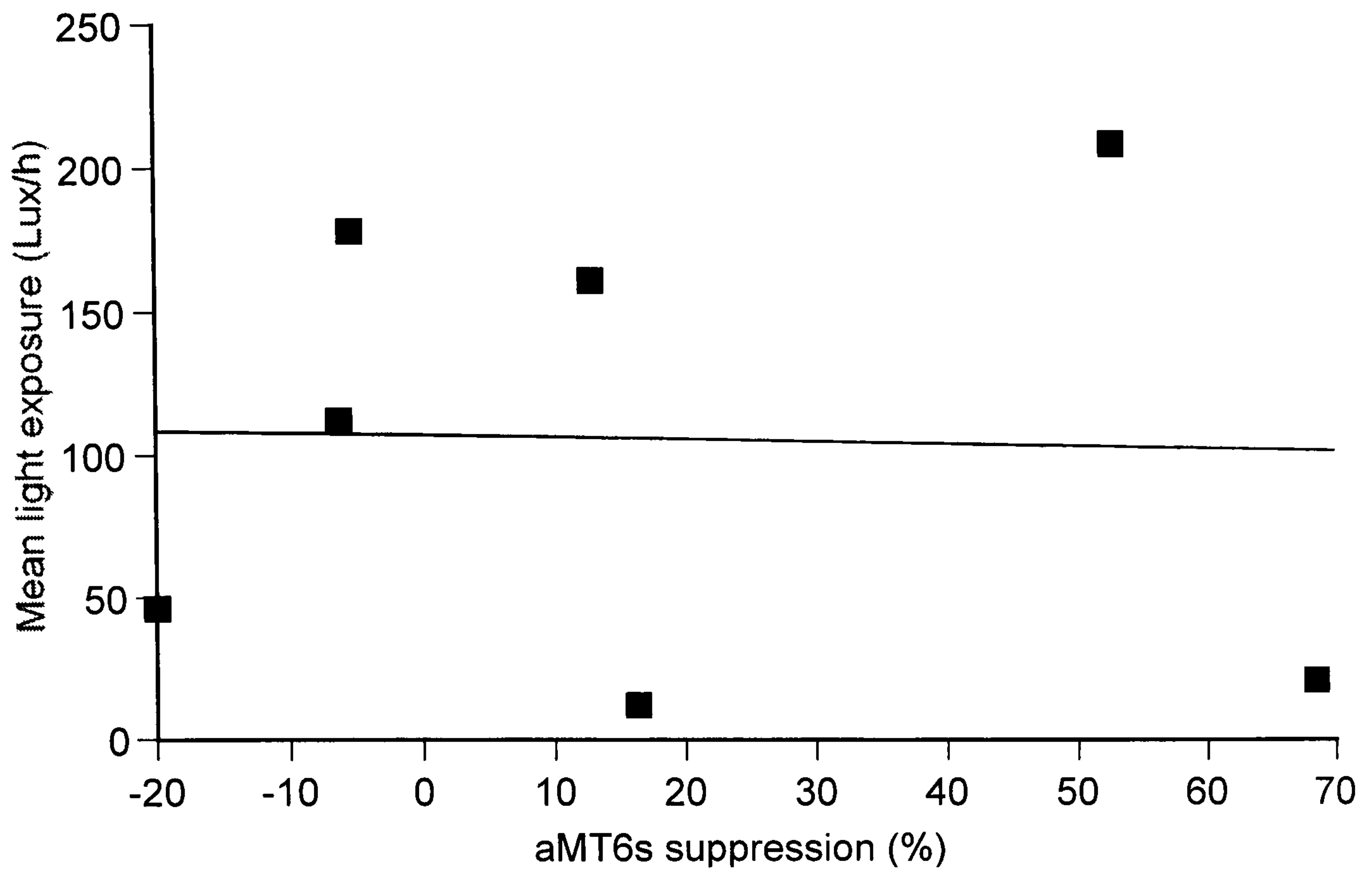


Figure 6-6: Correlation between mean light exposure onshore with aMT6s suppression.



### 6.4.3. Summer versus winter differences

As expected light exposure profiles change between seasons. These are shown in Table 6-5.

<b>Offshore</b>	<b>19.00-07.00h summer</b>	<b>18.00-06.00h winter</b>	<b>P-value</b>
Light exposure (lux/h)	98 ± 11	69 ± 26	NS
aMT6s production (ng/h)	4754 ± 1078	4626 ± 1730	NS

<b>Onshore/dayshift</b>	<b>19.00-07.00h summer</b>	<b>18.00-06.00h winter</b>	<b>P-value</b>
Light exposure (lux/h)	507 ± 143	106 ± 30	p=0.04
aMT6s production (ng/h)	3043 ± 607	4333 ± 1730	NS

Table 6-5: Mean aMT6s production (ng/24h) and mean light exposure (lux/h) for 19.00-07.00h shift schedule in the summer and 18.00-06.00h shift schedule in the winter, (NS = non significant).

No significant difference was observed in the light exposure between the two groups (summer versus winter) whilst the subjects were working offshore. Upon returning home onshore or working the day shift, significant differences (p=0.04) are observed between the two groups. Those working in the summer are exposed to an increased amount of light (507 ± 143 lux/h) over the 24 h day upon returning home or working the day shift in comparison to those who have been working in the winter (106 ± 30 lux/h).

## 6.5. Discussion

### 6.5.1. Subjects working 19.00-07.00h shift schedule in the summer

For subjects working the 19.00-07.00h shift schedule in the summer, a significant (p=0.03) reduction in the 24h production of aMT6s was observed between the last 3 days offshore and the following 3 days onshore. Whilst subjects are working the night shift offshore their 24h aMT6s production



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should not be affected by light as it is peaking whilst the subjects are sleeping (i.e if the subjects are adapted to the night shift) and therefore not exposed to bright light. When subjects return home onshore they are out of phase due to night shift adaptation (section 3.3). Therefore upon returning home, subjects are out of phase. They are exposed to a significantly higher amount of natural light ( $p=0.03$ ) when endogenous melatonin levels and aMT6s are at their highest. This explains the observed significant suppression of aMT6s.

### **6.5.2. Subjects working 18.00-06.00h shift schedule in the winter**

The differences observed in the 24h production of aMT6s and the average light exposure over the last 3 days of night shift offshore compared to the following 3 days onshore or on the day shift offshore in the winter 18.00-06.00h shift schedule are not as pronounced as those observed in the summer 19.00-07.00h shift schedule. The season (winter or summer) has a clear influence on the level of light exposure that the subjects are exposed to, and thus its suppressing effects are dependent upon season.

### **6.5.3. Individual differences**

From the results presented there is a wide variation in both total 24h aMT6s production and light exposure profiles between individual subjects. Individuals respond differently to light, and repeated exposure to bright light, will lessen the sensitivity of the circadian system (Hébert et al., 2002). Therefore the greatest effects of light will be observed in the days immediately following the night shift, as the circadian system has been sensitised by low prior light exposure (Owen and Arendt, 1992; Guillemette et al., 1998). Age also will have a direct influence on the ability of the light to suppress melatonin (Herljevic et al., 2005). There is also a huge variation in the timing of circadian phase when subjects finish their night shift. In consequence it is hard to predict when subjects will be exposed to natural light in relation to individual circadian phase and how light exposure will influence their circadian rhythms.



#### **6.5.4. The effect of light on the human circadian system**

Higher light levels occur in the summer months when returning home onshore or working the day shift. The increased amount of light therefore has a direct effect on the production of melatonin.

Jewett et al. (1991) reported that exposure of humans to fewer cycles of bright light, centred around the time at which the human circadian pacemaker is most sensitive to light-induced phase shifts, can markedly attenuate endogenous circadian amplitude and large phase shifts may be observed. Therefore in some circumstances abrupt changes in phase would be more likely to happen when a subject finishes a night shift. In some cases this can result in an apparent loss of a circadian rhythm, which is observed in some of the subjects upon returning home onshore after working a night shift. One of the most mysterious properties of circadian rhythms is the loss of robust circadian rhythms following exposure to a stimulus such as a pulse of bright light. One recent theory states that desynchronization of individual cellular clocks underlies this response (Ukai et al., 2007).

#### **6.6. Conclusion**

The data clearly demonstrate the effect of natural light exposure on suppressing the aMT6s rhythm of the offshore shift workers who are either returning home onshore after working a 2- or 3-week night shift or who are going on to working the day shift. This study highlights the fact that light does not only suppress melatonin production during the night, but if the melatonin rhythm is desynchronised with the light-dark cycle, it can be suppressed during the day (Gibbs et al., 2007). Season plays an important role as natural light exposure especially in the summer months is greater than that of winter and a greater amount of melatonin suppression is observed.



## **7. DISCUSSION**

### **7.1. Introduction**

In the oil and gas industry, shift work is key to the efficiency of the work place. A night and day work force enables optimal productivity of exploration and production activities. Shift work is undertaken by approximately 12-14% (HSE and Office of National Statistics) of the UK population; this is dependent on how the term 'shift work' is defined. Many health problems are associated with shift work such as sleep problems (section 1.9.4), increased risk of heart disease (section 1.9.9) and recently a link with cancer (section 1.9.7) has been proposed. Offshore oil and gas installations have some shift schedules which allow workers to adapt to the night shift. Certain shift schedules are better for sleep (Tucker et al., 1998) and circadian adaptation than others (Barnes et al., 1998a; Gibbs et al., 2002).

The aims of this project were to investigate circadian adaptation in offshore shift workers and the use of light treatment to hasten circadian adaptation upon returning to day life at home. This chapter will discuss the schedules studied and strategies for improving adaptation to day life after working a night shift. Suggestions for further study will be presented.

### **7.2. Shift schedule and its effects on circadian adaptation**

The 18.00-06.00h 2 week 12h night shift shows a better sleep quality in terms of sleep duration and sleep efficiency compared to the 19.00-07.00h 2 week 12h night shift. This may be in part due to the increased light exposure in the morning, as discussed in chapter 3. Light exposure seems to be of major importance to circadian adaptation, and therefore the consequences for day time sleep. The current data and previous studies demonstrate that subjects working offshore adapt to the night shift (Barnes et al., 1998a). As a result problems in terms of sleep occur upon returning home due to the misalignment of circadian rhythms and the inappropriate relationship of



endogenous melatonin to the light-dark cycle. The results discussed in chapter 3 showed that the 18.00-06.00h shift schedule is better than the 19.00-07.00h for circadian adaptation. However this may not be the case in winter, when those working the 19.00-07.00h shift would not be exposed to morning light countering adaptation. If time had allowed, it would have been interesting to see if there are any differences between sleep and circadian adaptation between these two shift schedules in the winter months.

### **7.3. Use of the Litebook in healthy young volunteers**

Previous studies have shown that light is able to shift the circadian clock (section 1.6.1). The phase shifting ability of the Litebook in healthy volunteers was investigated in chapter 4. Results showed that the portable light box, used to give a single light pulse in the early morning, was capable of inducing a small phase advance in the DLMOff, though this was not significant ( $p=0.07$ ). Though our study did not show a significant phase advance, a recent study by Paul et al. (2007) used the Litebook, in the evening and showed a significant phase delay compared with no-light control leg. A significant decrease in sleepiness with the Litebook compared to the control leg was also observed. From the Litebook study reported in chapter 4 it was considered that its properties would be able to help those who are working shift work offshore, if repeated treatments were given. The laboratory study was conducted on normal, young, healthy volunteers. It was considered that they might be more resistant to shifting the phase of their internal circadian rhythm compared to those subjects working offshore who are used to adapting to the night shift and readapting back to day life. Therefore the Litebook treatment may have more of an effect on those who are used to working shift work compared to the normal non-shift working population. In addition, in the real life situation numerous other factors will intervene, to reinforce but also possibly to counteract phase adaptation. Thus a laboratory simulation is of limited value in predicting field responses.



For example the field study population was of an older age range. A study by Herjevic et al. (2005) showed that melatonin was more readily suppressed in younger subjects than older subjects. The implication of this finding would be that older people would show less of a phase shift to light treatment than younger subjects (Klerman et al., 2001; Duffy et al., 2007).

#### **7.4. Use of the Litebook in offshore shift workers**

The assumption of a greater effect of the Litebook in the field than in the laboratory seemed to be correct as the study investigating the use of the light box in offshore shift workers working the night shift showed improved sleep when the light treatment was correctly timed. Sleep, the major complaint of night shift workers returning home, was improved. If the circadian clock can be shifted to hasten circadian adaptation the length of time where shift workers circadian rhythms are misaligned to the light dark cycle is reduced. It is hypothesised that this misalignment may be an underlying cause of the increased risk of working night shifts. Therefore shifting the clock both to adapt to night shift and back to day shift, could be seen as beneficial to the health concerns of the shift worker (Eastman et al., 1994; Eastman and Martin, 1999; Rajaratnam and Arendt, 2001).

The current research shows evidence for night shift adaptation offshore and the benefits of timed light treatment to in part aid circadian adaptation back to day life after working the night shift (Bjorvatn et al., 1999; Bjorvatn et al., 2007). The reduced time of misalignment between circadian rhythms may aid in reducing risk factors which are associated with working night shift work.

A recent hypothesis is that light at night and its ability to suppress melatonin is in part causal for the increased risk of breast cancer in shift workers (section 1.9.7). Light is known to be able to suppress nocturnal melatonin (section 1.5.4.1). Though in offshore night shift workers this does not occur as their melatonin rhythm is peaking during the day whilst they are sleeping.



This situation however is apparent when shift workers return home onshore especially in the summer months, when light exposure during the day causes suppression of their day time melatonin rhythm (present data and Gibbs et al., 2007).

## **7.5. Benefits of adaptation**

Spontaneous adaptation to the night shift only occurs in extreme environments such as offshore oil rigs or for example in Antarctica (Midwinter and Arendt, 1991; Ross et al., 1995; Barnes et al., 1998b; Gibbs et al., 2002). Adaptation to the night shift is clearly advantageous in terms of sleep. Those who were adapted to the night shift as discussed in chapter 3, had significantly longer sleep duration than those who were not adapted. In addition the timing of the circadian clock was of importance, since those working 18.00-06.00h had a shorter phase angle difference between sleep onset and the aMT6s acrophase, and slept significantly longer than those with a longer phase angle difference.

### **7.5.1. Advance v delay**

In principle there is no right or wrong direction of circadian phase shifting to enable adaptation to a night shift or back to day life. The circadian system can be either advanced (timing brought forwards in time), or delayed (timing brought backwards in time) to synchronise itself with a new time schedule. The response is affected by a combination of factors including individual circadian phase position, and the timing of the new sleep-wake schedule. Most rapid direction of readaptation to day life will depend on an individual's circadian phase at the end of the night shift. For example with a peak aMT6s at 13.00h, a 6-7h phase advance would theoretically be desirable compared to a 15-16h phase delay. Thus in theory to phase advance is the 'shorter' option to get re-entrained, but this is harder to achieve than a phase delay (Monk et al., 2000). This is because the endogenous period of the body



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clock is usually greater than 24h, so the body naturally phase delays (Middleton and Stone, 1996; Czeisler et al., 1999).

Combinations of timed light and scheduled darkness/sleep have been reported by Czeisler et al. (1990) and Eastman et al. (1994). A 'skeleton' light treatment was successful in adapting subjects back to day work in Antarctica (Midwinter and Arendt, 1991). Another method of shifting the timing of the internal circadian clock is by melatonin administration, examples include Burgess et al. (2002) and Crowley et al. (2003) who used melatonin to facilitate circadian adaptation of those working shift work. All of these studies have been successful in shifting the internal phase of those subjects investigated.

Studies by Bjorvatn et al. (1998) have used phase delaying strategies to hasten circadian adaptation. These studies have been successful, but they have only measured sleep, both subjective (sleep diaries) and objective (actigraphy). Bjorvatn et al. (1998) did not determine circadian phase during their studies unlike the current study reported in chapter 5.

#### **7.5.1.1. Diurnal preference**

The ability to shift the clock in a certain direction may be influenced by an individual's diurnal preference. Dumont et al. (2001) reported that "morningness" and "eveningness" were strongly associated with phase advance and phase delay respectively. This study highlights the importance of individual differences and therefore certain strategies implemented to help aid circadian adaptation to the night shift, and afterwards when readapting back to day life. It is the combination of individual preferences, unscheduled zeitgebers and irregularity of daily routines, that means that adaptation in any direction is difficult in a home and family environment. The more isolated and controlled environment offshore confers a huge advantage as adaptation strategies can be implemented more readily.



The clock gene *per3* has been associated with diurnal preference (Archer et al., 2003), and more recently those who are homozygous for the long repeat polymorphism *per3* 5/5, have been shown to cope less well with sleep deprivation in terms of cognitive performance than those who are homozygous with the short repeat of the polymorphism *per3* 4/4 (Viola et al., 2007). Firstly this polymorphism may affect diurnality (if subjects are morning types or evening types), and genotyping shift workers may be a good predictor of which way it might be best to shift the clock, for example to phase delay, or phase advance to adapt to the night shift or readapt back to day life. Secondly the implications of the study conducted by Viola et al. (2007) are that in the future, people might be genetically screened to see their suitability for coping with night work, though this in my view has serious ethical considerations.

#### **7.6. Strategies to aid shift work**

Adaptation to the night shift is appropriate for certain types of night shifts, but not for others. Those who are working offshore for 2 weeks of nights will favour adaptation, whilst those working one night of shift work for example will not. For those working a few consecutive nights partial adaptation would be appropriate. Therefore different strategies to coping with night shift will depend on the required outcome. The following section discusses some of the different options that can be undertaken.

There are strategies that can shift the internal clock, and therefore help adaptation to the night shift; these include melatonin and light treatment. Other strategies are taken to alleviate the symptoms of tiredness associated with shift work. These include the consumption of caffeine, modafinil and napping.



### **7.6.1. Light treatment**

Light treatment has been shown to be able to successfully shift the internal circadian clock; it has been practically demonstrated in both laboratory (Dawson et al., 1991; Revell et al., 2005) and field studies (Midwinter and Arendt, 1991; Ross et al., 1995; Bjorvatn et al., 1999; Burgess et al., 2002; Thorne et al., 2008).

### **7.6.2. Use of melatonin**

Bjorvatn et al. (2007) used light and melatonin in offshore shift workers. This study is the only study in offshore shift workers that has demonstrated the use of light and melatonin to hasten adaptation. Melatonin administration is a more convenient way to shift the clock and it is not a time consuming process as compared to light exposure. Therefore compliance to this treatment should be higher than to that of light treatment. However there are few guidelines as to the timing and dose of melatonin in field situations.

### **7.6.3. Napping**

Alertness and performance capabilities of night workers are often impaired on the job. Field studies of actual shift worker performance are rare because of methodological difficulties.

Taking a nap is a natural instinct to combat fatigue and tiredness. Napping as a strategy to temporarily relieve the urge for sleep has been investigated in those working shift work. Purnell et al. (2002) reported that the deterioration of performance associated with the first night shift can be counteracted by a 20 min nap taken in the workplace during the night shift. Sallinen et al. (1998) compared different lengths of naps (30, 50 minute naps at 01.00h and 04.00h). All naps improved visual performance tests later in the night. They concluded that a short nap, around 30 minutes, early in the night would promote alertness without impairment of the subsequent sleep. Dinges et al. (1987) found that the circadian placement of a nap was not



important in terms of beneficial effects on reaction performance and suggested that placement of the nap just prior to the night of sleep loss will maximally aid performance that night.

#### **7.6.4. Caffeine**

Caffeine is the most widely used drug in the world: it is found in coffee, tea and many soft drinks and is available in tablet form. Muehlbach and Walsh (1995) indicated that caffeine at commonly used doses consumed at the appropriate time, reduces sleepiness and performance decrements during night time work hours. Other studies have reported these findings, but often in a combination with naps or bright light (Babkoff et al., 2002; Schweitzer et al., 2006).

#### **7.6.5. Modafinil**

Modafinil was originally developed as a drug used to treat narcolepsy, due to its wake promoting effect (Black and Houghton, 2006). Modafinil has been marketed as a drug for improving alertness and reducing excessive daytime sleepiness. Czeisler et al. (2005) demonstrated that treatment with 200 mg of modafinil before the start of each night shift reduced the extreme sleepiness observed in patients with shift-work sleep disorder and resulted in a small but significant improvement in performance as compared with placebo.

### **7.7. Further work**

If time had permitted it would have been interesting to study subjects' aMT6s during the first week of their night shift to see if there was a trend between how subjects adapt to the night shift (either by advance or delay of aMT6s) and if this is related to how they readapt back to home life (either by advancing or delaying of aMT6s). Research by Gibbs et al. (2007) showed that circadian phase at the beginning of an offshore night shift is a



determining factor in the speed of circadian adaptation to nights and to the direction of adaptation (by advance or delay) to subsequent day shifts worked offshore. This knowledge could have helped to give subjects information to cope with both adaptation to the night shift and also adaptation back to day life upon returning to home onshore.

## **7.8. Conclusions**

The results presented in this thesis contribute towards an understanding of shift schedules and adaptation to night shift and readaptation back to day life. The combined outcomes of the presented and suggested studies should further advance knowledge, and offer some reduction in the consequences of night shift with regard to human health.



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## 9. APPENDICES

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

Time: .....

#### QUESTION 2

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

Time: .....



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**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 [     ]



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**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 [     ]



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**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 [     ]



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**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 two 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 [    ]



## SLEEP DIARY

**Subject code:**..... **Date:**.....

This form is to be completed everyday when you awake during the study (24 hour clock time). Please also fill in times and durations of any 'cat-naps' taken on the day of form completion.

What time did you go to bed?	
What time did you try starting to sleep at?	
How long did it take you to fall asleep (mins)?	
How many times did you wake up?	
How long were you awake for? (mins) Please estimate the time and duration of each night awakening	
What time did you wake up?	
What time did you get up?	
Would you describe this as a typical night?	

How would you rate your quality of sleep?

1      2      3      4      5      6      7      8      9

**Best sleep ever**

**Worst sleep ever**

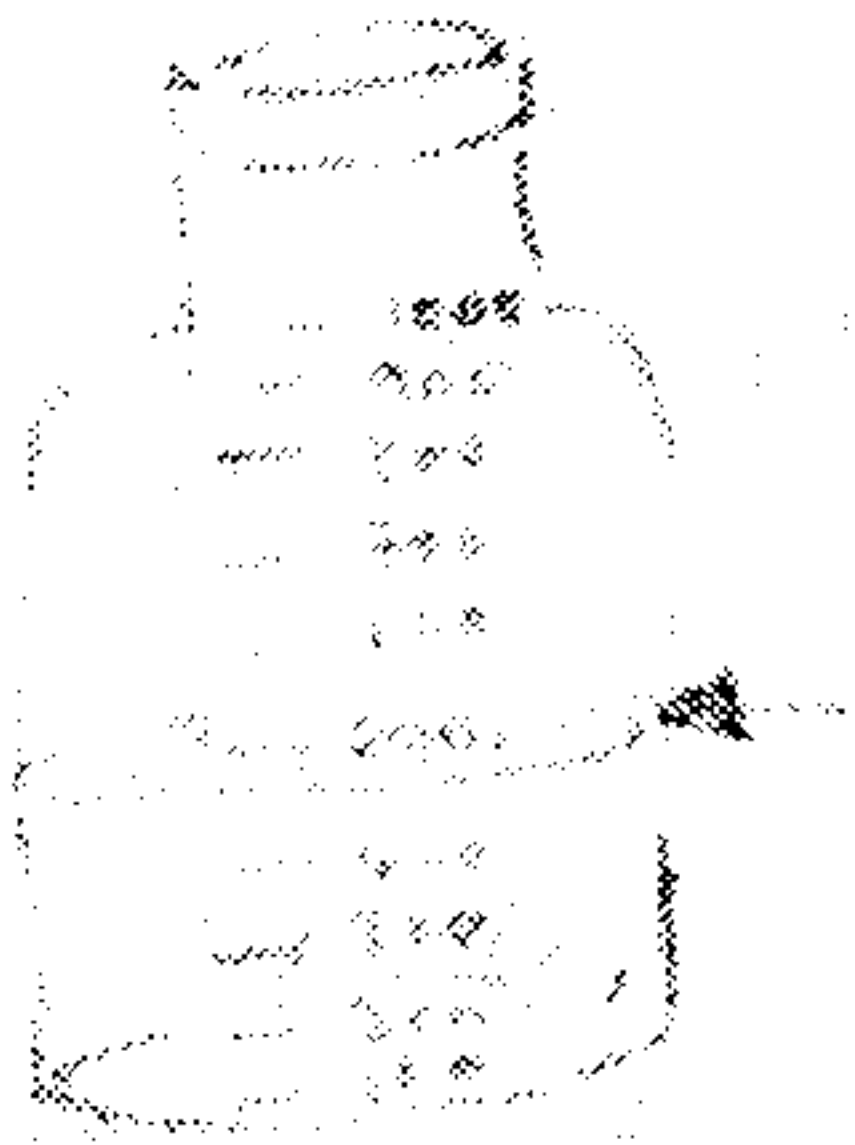
**Have you taken any 'cat-naps' (on this day of form completion)?**

If yes, at approximately what time and for how long?

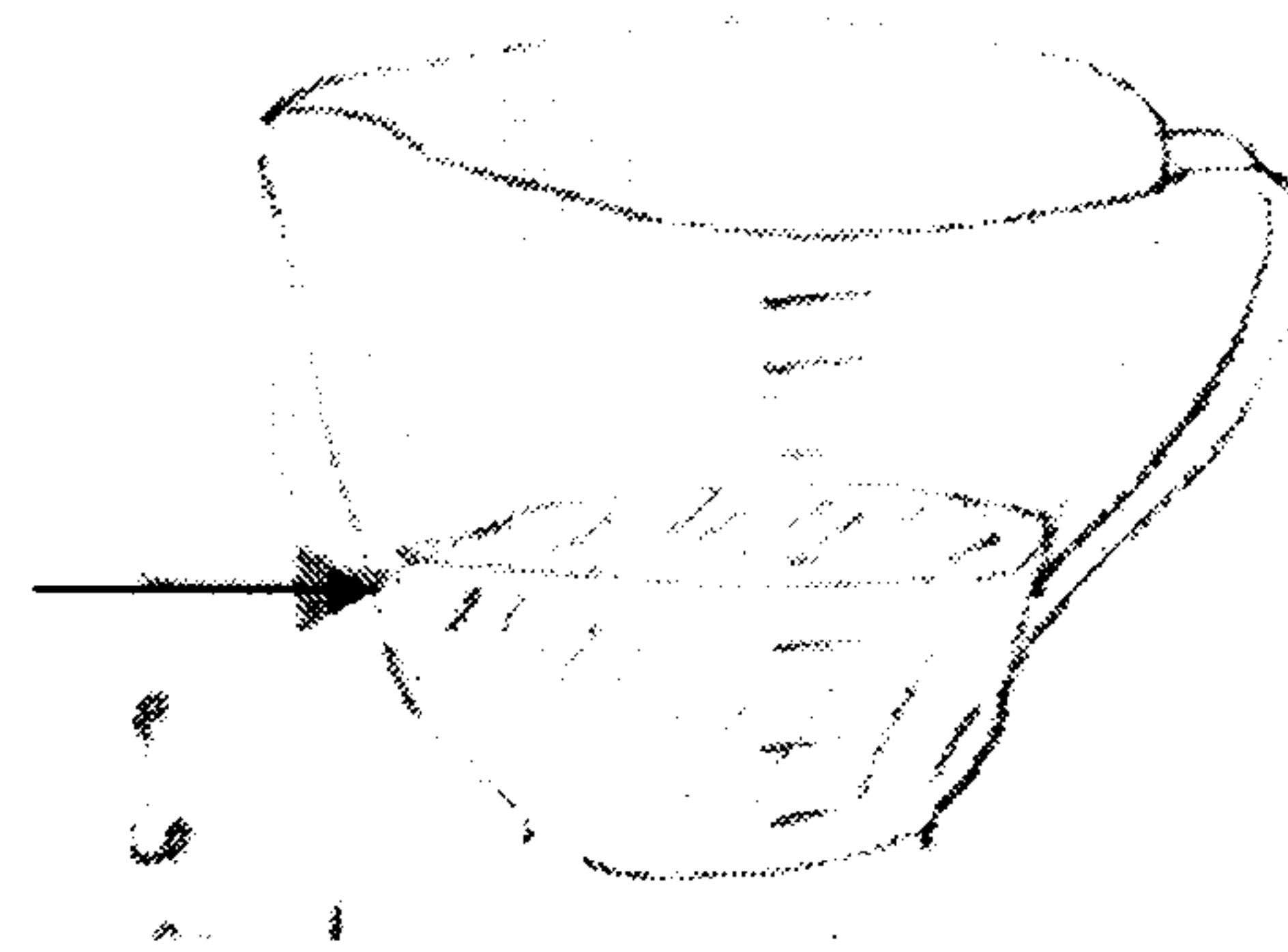


## SEQUENTIAL URINE SAMPLES

1. EACH TIME YOU PEE
2. Use calibrated jug (or bottle)

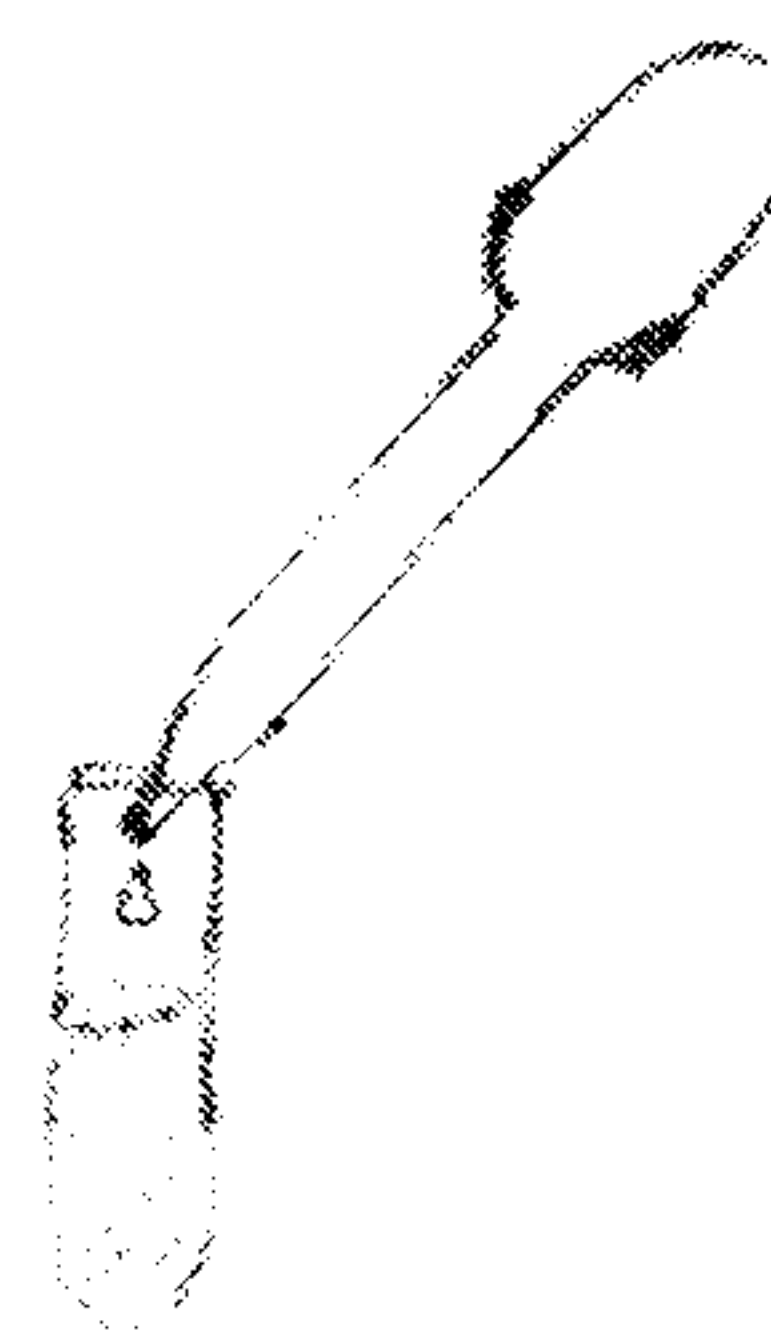
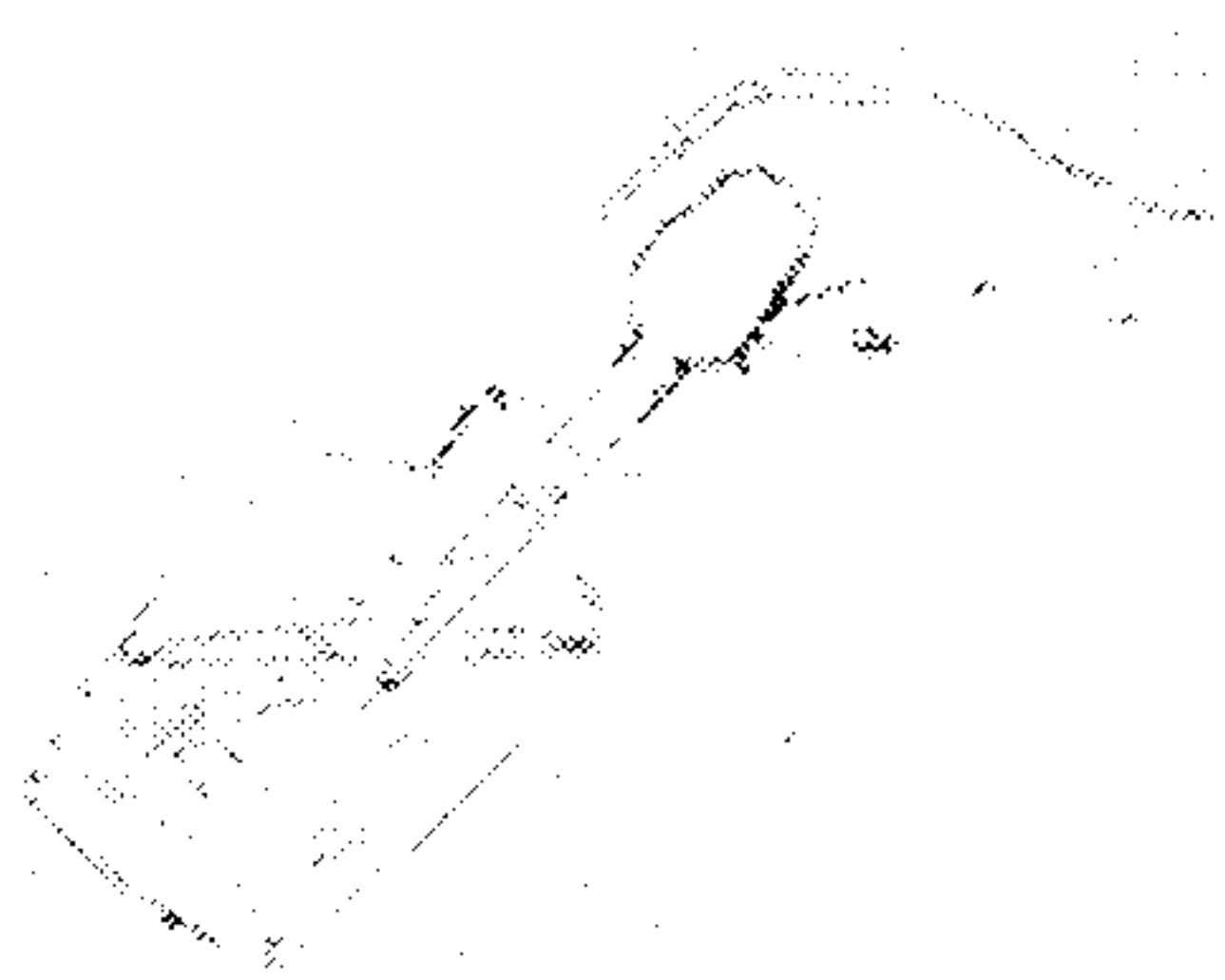


**1. Measure volume to the nearest 50ml**

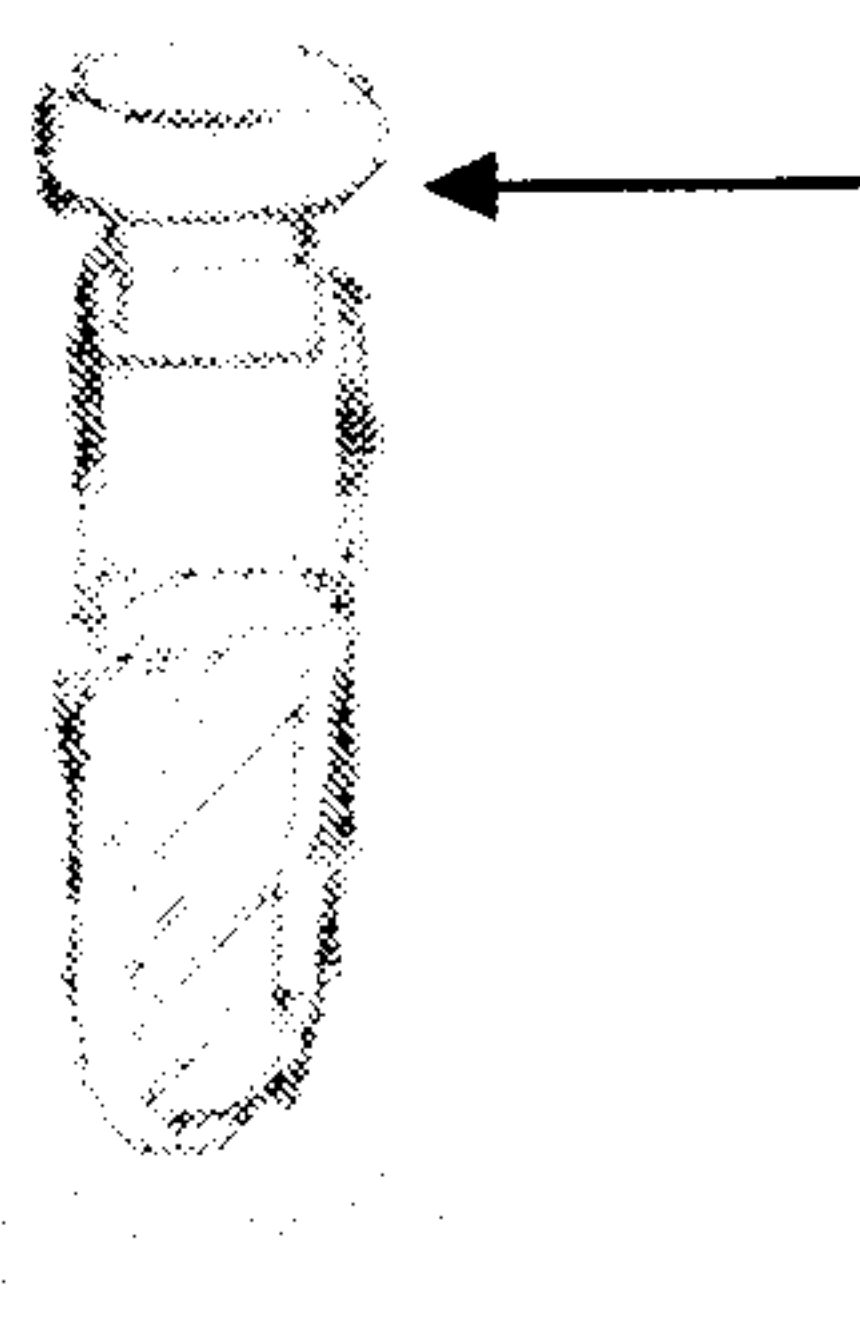


2. Record volume, time of pee and date on the tube provided

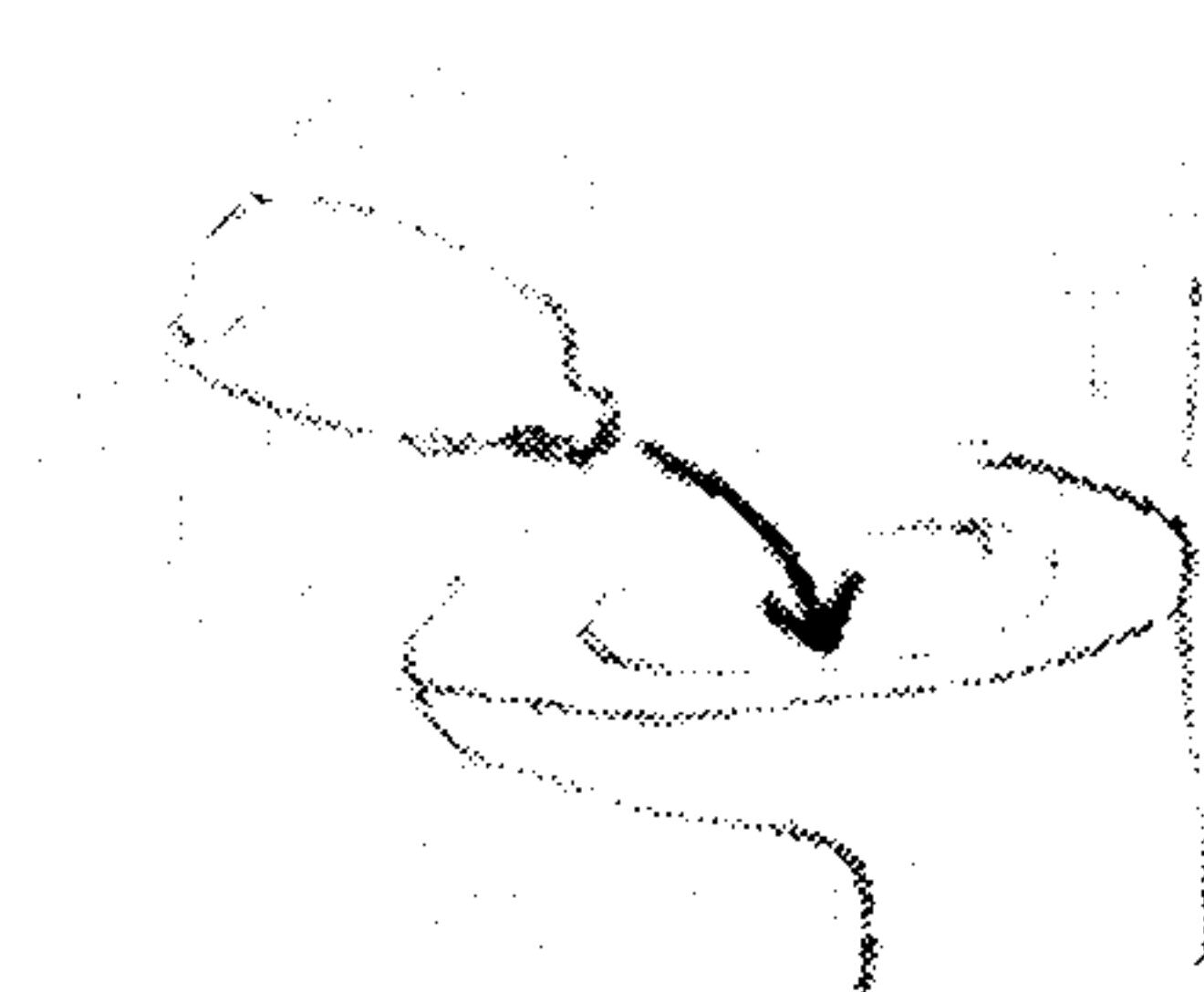
3. Use a dropper to take a small sample



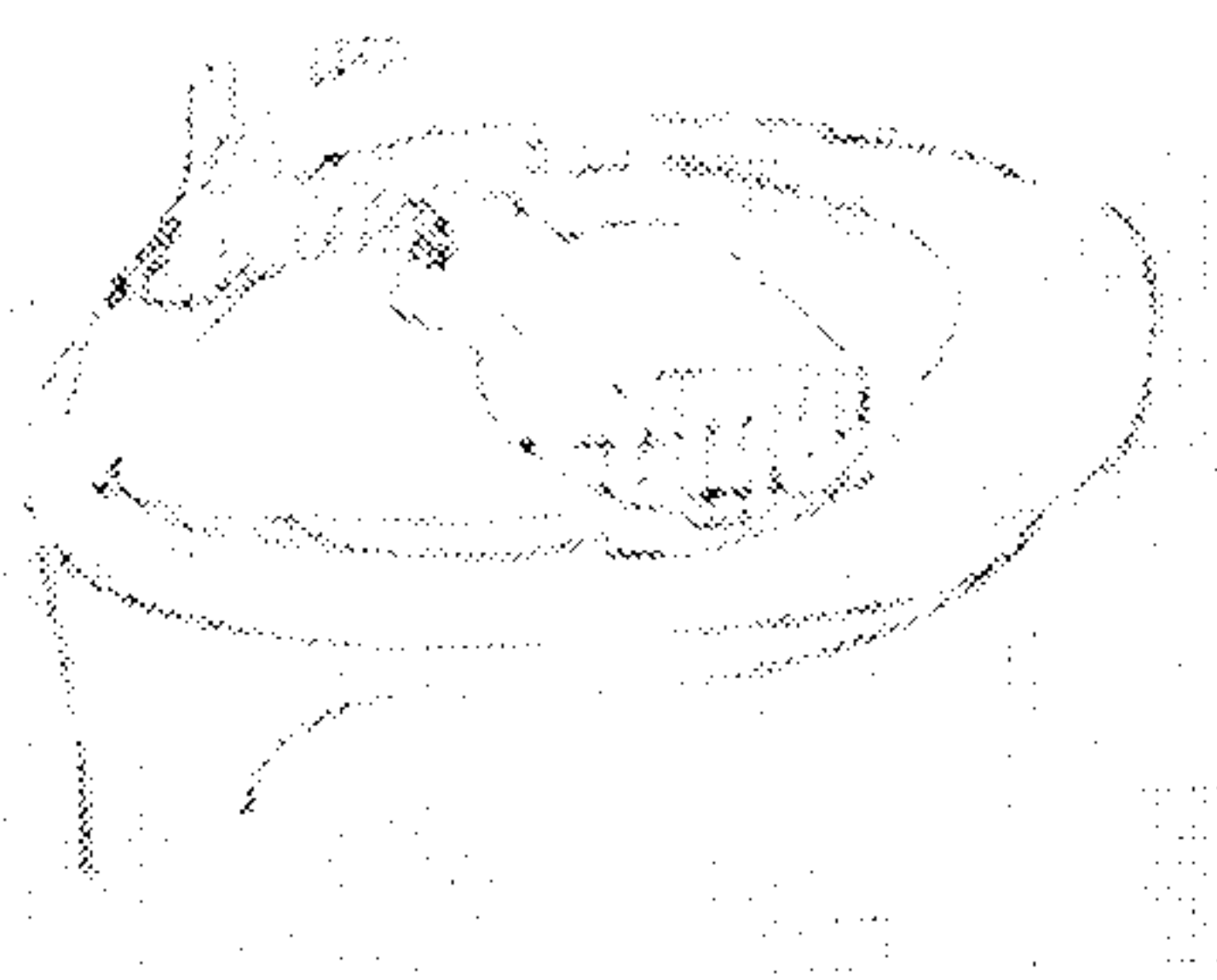
**4. Put sample in the labelled tube for the time period**



**5. Put stopper in tube and store in a fridge or preferably a freezer**



**6. Empty remaining urine down the loo**



**7. Rinse urine bottle or jug 3 times with tap water and use for the next collection. DO NOT USE DISINFECTANT OR BLEACH**



MEDICAL SCREENING QUESTIONNAIRE

DATE.....

SUBJECT CODE... ..

Name:

Date of Birth:

Address:

Ethnic Origin:

Telephone: Home  
 Work  
 Mobile

Email:

GP's name:

GP's address:

Approx. Body Weight (kg):

Height (cm):

Eye Colour:

1) Have you or a member of your family ever suffered from the following conditions? (Please circle YES/NO and if YES give details)

- |                               |          |
|-------------------------------|----------|
| a) High blood pressure        | YES NO   |
| b) Cardiovascular diseases    | YES / NO |
| c) Asthma                     | YES / NO |
| d) Psychiatric conditions     | YES / NO |
| e) Migraine                   | YES / NO |
| f) Endocrine disorders        | YES / NO |
| g) Haematological conditions  | YES / NO |
| h) Neurological conditions    | YES / NO |
| i) Epilepsy                   | YES NO   |
| j) Renal disease              | YES/ NO  |
| k) Gastrointestinal disease   | YES NO   |
| l) Hepatic disease            | YES NO   |
| m) Drug or Alcohol Dependence | YES NO   |



If YES to any of the above please give details

2) Do you ever feel faint or have dizzy spells? YES NO

3) Have you been in hospital at all in the last 2 years? YES NO

If YES please give details

.....  
.....

4) Do you anticipate any treatment in the next 2 – 3 months (e.g. surgery, dental treatments, physiotherapy) YES / NO

If YES please give details .....

.....

5) Have you suffered any major illnesses in the past 6 months? YES / NO

If YES please give details .....

.....

6) Are you undergoing treatment at the moment? YES NO

If YES please give details .....

.....

7) Are you taking regular medication at the moment or have you taken any medication in the last six weeks? YES NO

If YES please give details (Name, Dose, Frequency, When started and stopped)



- .....
- 8) Are you taking or have you ever taken any of the following medications?
- |  |          |
|--|----------|
| a) Anti depressants (including MAOI)                                   | YES / NO |
| b) Tranquilisers   | YES / NO |
| c) Sleeping tablets / Hypnotics (e.g. Benzodiazepines)                 | YES / NO |
| d) $\alpha$ blockers   | YES / NO |
| e) Anti hypertensives ( $\beta$ -blockers, $Ca^{2+}$ channel blockers) | YES / NO |
| f) NSAIDs  | YES / NO |
| g) Anti epileptics   | YES / NO |
| h) Vitamins B12 and B6   | YES / NO |
| i) Selective serotonin uptake inhibitors (e.g. Lithium, Prozac)        | YES / NO |
| j) Anti psychotics   | YES / NO |

If YES to any of the above please give the name of the medication and the dates when it was taken .....

.....

.....

- 9) Do you have any physical disabilities of any kind? YES / NO

If YES please give details.....

.....

- 10) Do you suffer from any allergies? YES / NO

If YES please give details.....

.....

- 11) What is your weekly intake of alcohol?

..... units per week

(One unit is half a pint of beer/ one glass of wine/ one measure of spirits)

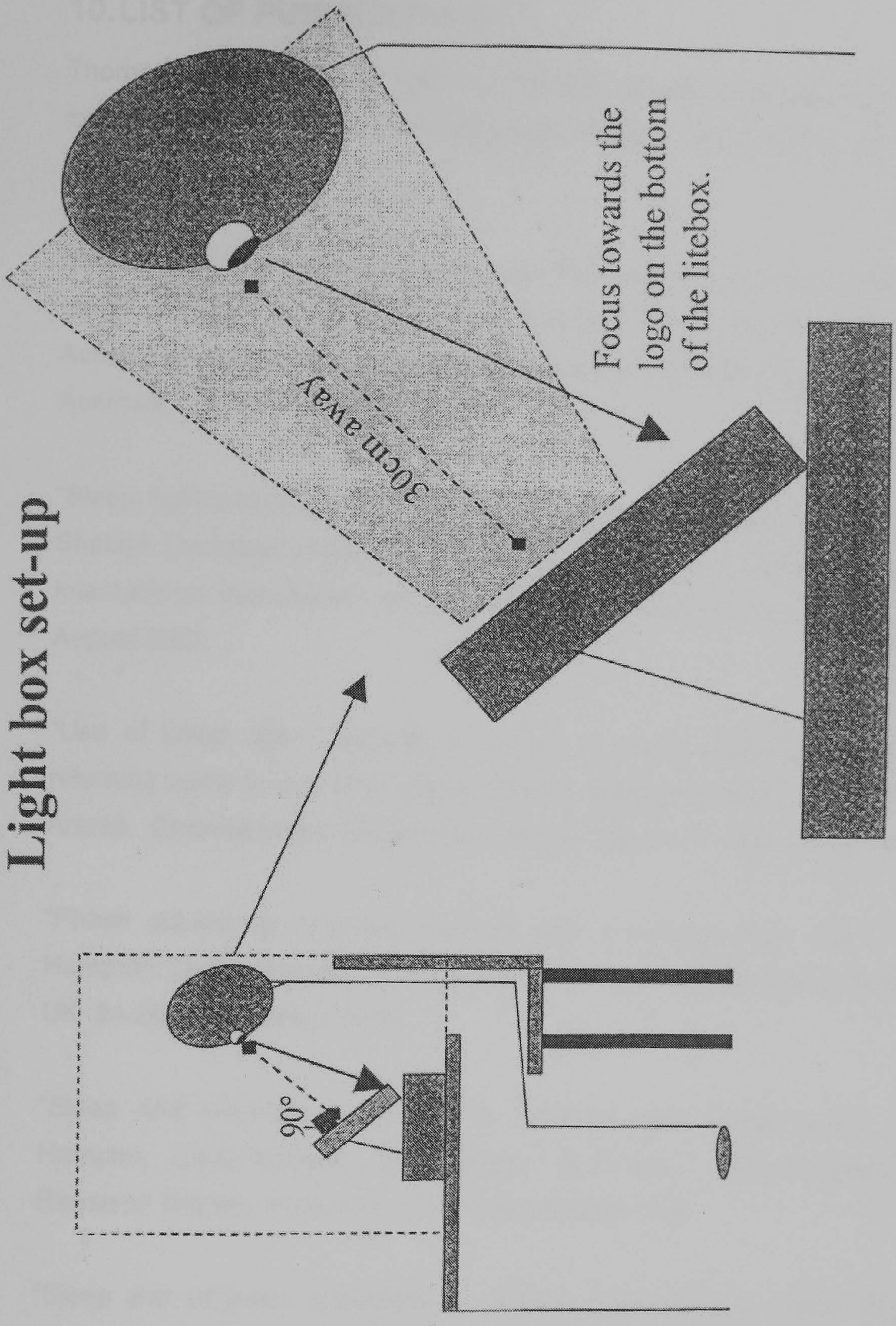
- 12) What is your average caffeine consumption per day?

.....

- 13) Are you a smoker or a non-smoker?



# Light box set-up



The light may seem too bright at first. It is 3000lux. On a sunny day you receive 10,000 lux. When you look away, you may see white dots. This is similar to when you walk into a dark room after a very sunny day and have difficulty seeing, so do not worry, it is not damaging your eyes and has been approved for safety.

If for any reason, any adverse side effects do occur other than the above mentioned during the light treatment, stop treatment and contact us a.s.a.p.



## **10. LIST OF PUBLICATIONS**

Thorne H, Hampton S, Morgan M, Skene D and Arendt J (2008). "Differences in sleep, light and circadian phase in offshore shiftworkers working 18.00-06.00h and 19.00-07.00h." *Chronobio. Int.* 25 (2) 225-235.

"Differences in sleep between two (18.00-06.00h and 19.00-07.00h) offshore shift schedules?" Helen Thorne, Shelagh Hampton, Linda Morgan, Debra Skene, and Josephine Arendt. Accepted for the 18th International Symposium on Shiftwork and Working Time, Yeppoon QLD, Australia, 28-31st August 2007.

"Sleep, light and circadian phase in offshore shiftworkers working 19.00-07.00h." Helen Thorne, Shelagh Hampton, Linda Morgan, Debra Skene, and Josephine Arendt. Accepted for the 18th International Symposium on Shiftwork and Working Time, Yeppoon QLD, Australia, 28-31st August 2007.

"Use of timed light treatment to hasten circadian adaptation of offshore nightshift workers returning home to day life." Helen Thorne, Shelagh Hampton, Linda Morgan, Debra Skene, Jo Arendt. Chronobiology Gordon Conference, Aussois, France 6-11th May 2007.

"Phase advancing circadian rhythms with a portable light box." Helen Thorne, Shelagh Hampton, Linda Morgan, Debra Skene, Jo Arendt. British Sleep Society meeting, Cambridge, UK 24-26th September 2006.

"Sleep and circadian adaptation in offshore night shiftworkers." Helen Thorne, Shelagh Hampton, Linda Morgan, Debra Skene, Jo Arendt. 18th Congress of the European Sleep Research Society, Innsbruck 12-16th September 2006.

"Sleep and circadian adaptation in offshore shiftworkers." Helen Thorne, Shelagh Hampton, Linda Morgan, Debra Skene, Jo Arendt. Festival of Research, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, UK 3rd July 2006.

"Sleep on oilrigs." Helen Thorne, Shelagh Hampton, Debra Skene, Linda Morgan, Jo Arendt. British Sleep Society meeting, Cambridge, UK 25-27th September 2005.