Optimizing Circadian Rhythm and Characterizing Brain Function in Disorders of Consciousness

By

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Declaration

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

Date:

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Preface

The number of patients who survive severe brain injuries are increasing. While some of the survivors make a good progress, some may suffer from severe disorders of consciousness (DOC) which may last from weeks to months and in some cases for life-long. These patients show none or minimal sign of awareness of themselves or their environment but have presence of sleep-wake cycles as they open/close their eyes for periods of time. However, a handful of studies, which investigated sleep in DOC, indicated that these patients may have abnormal sleep patterns as well as circadian rhythms (Cologan et al., 2013, Bekinschtein et al., 2009b, Isono et al., 2002).

On the other hand, growing number of studies indicate that sleep is not a mere state of rest-time for humans but also a state where memory processing, learning and memory consolidation, brain plasticity and memory reconsolidation occur (Stickgold and Walker, 2007, Stickgold et al., 2001, Walker and Stickgold, 2006). Therefore, one may hypothesize that improving sleep patterns/ circadian rhythms of patients with DOC may help with recovery of consciousness. To our knowledge, there are no previous studies performed examining the influence of circadian rhythm optimization on brain functions of patients with DOC. Could such optimization be achieved by using simple and inexpensive measures such as light treatment, melatonin and caffeine administration which may eventually lead to improvement of sleep, aid to neuro-plasticity and rehabilitation process in patients with DOC?

Sleep that knits up the ravel'd sleeve of care,

The death of each day's life, sore labour's bath,

Balm of hurt minds, great nature's second course,

Chief nourisher in life's feast.

W. Shakespeare, *Macbeth, Act 2 Scene 2*

Abstract

Sleep is a physiological state where memory processing, learning and brain plasticity occur. Patients with prolonged disorders of consciousness (PDOC) show no or minimal signs of awareness of themselves or their environment but appear to have sleep-wake cycles.

The main aim of this thesis was to investigate effect of circadian rhythm and sleep optimization on brain functions of patients with PDOC.

In the first instance, sleep and circadian rhythms of patients with PDOC were investigated using polysomnography and saliva melatonin measurements. The investigations that were performed at the baseline suggested that both circadian rhythmicity and sleep were severely deranged in PDOC patients.

This was followed by the interventional stage of the research where an attempt was made to optimize circadian rhythm and sleep by giving blue light, caffeine and melatonin in a small cohort of patients. To measure the effects of the intervention, we used a variety of assessments: Coma Recovery Scale-Revised (CRS-R) to measure changes in awareness; PSG for assessment of sleep, melatonin for assessment of circadian rhythm; and, event-related potential measures including mismatch negativity (MMN) and subject's own name (SON) paradigms.

Our results showed that it is possible to improve sleep and circadian rhythms of patients with PDOC, and most importantly, this improvement leads to increase in Coma Recovery Scale-Revised scores. Individually, those patients who responded well to the intervention also showed improvements in their functional brain imaging assessments.

Impact statement

Acquired brain injury is a worldwide public health problem with a significant impact on people with brain injuries themselves, others involved in their care, and more widely in society. While the clinical presentations of the brain injured patients vary from post-concussion syndrome to vegetative state, the severely brain injured patients who present with disorders of consciousness are the most vulnerable. Their best interests need to be determined and their care provided by others. Furthermore, the scientific knowledge in this area is scarce and unravelling the mechanisms behind this most severe and bizarre neurological condition may help to develop treatment options and improve outcomes for many other diseases of the brain.

This research was driven by the health needs of the patients with PDOC. Our interventions were simple and inexpensive which could be implemented in a wide range of healthcare settings both nationally and internationally. The results of this research suggest that improving sleep and circadian rhythms of patients with PDOC enable them to be in a better aroused and optimized neural network state and therefore both improve diagnostic accuracy and outcome.

Publications associated with this thesis

- **Late Recovery of Awareness in Prolonged Disorders of Consciousness- A Cross-sectional cohort study.** Yelden K, Duport S, Kempny AM, James L, Farmer S, Leff AP, Playford ED Disability and Rehabilitation, 2017 June 1-6. DOI: 10.1080/09638288.2017.1339209

- **Patients with a severe prolonged Disorder of Consciousness can show classical EEG responses to their own name compared with others' names.** Agnieszka M. Kempny, Leon James, Kudret Yelden, Sophie Duport, Simon F. Farmer, E. Diane Playford, Alexander P. Leff. NeuroImage: Clinical Volume 19, 2018, Pages 311-319

- **Understanding the decision-making environment for people in minimally conscious state.** Yelden K, Sargent S, Samanta J. Neuropsychol Rehabil. 2017 Apr 11:1-12.

- **Functional near infrared spectroscopy as a probe of brain function in people with prolonged disorders of consciousness.** Kempny AM, James L, Yelden K, Duport S, Farmer S, Playford E D, Leff A P. Neuroimage Clin. 2016 Jul 27;12: 312-9

- **Grounded theory analysis of a focus group study: should minimally conscious people have a right to reassessment.** S Sargent, J Samanta, K Yelden. Sage Research Methods Cases Health. Jan 2017

- **Should people in the minimally conscious state have a (recognized) right to reassessment?** J Samanta, K Yelden, S Sargent. Contemporary Issues in Law- Assisted dying, 2016, Volume 14, Issue 1

- **A Rehabilitation Unit at Night: Environmental Characteristics of Patient Rooms** Yelden K, Duport S, Kempny A, Playford ED. Disability and Rehabilitation, 2015; 37(1): 91–96

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µV	MicroVolt
5HT	Serotonin
AASM	American Academy of Sleep Medicine
ACC	Anterior cingulate cortex
Ach	Acetylcholine
ARAS	Ascending reticular arousal system
BAEPs	Brainstem auditory evoked potentials
BI	Brain injury
BL	Baseline
BOLD	Blood-oxygen-level-dependent
BP	Blood pressure
CANH	Clinically assisted nutrition and hydration
CBT	Core body temperature
CRS	Coma Recovery Scale
CRS-R	Coma Recovery Scale- Revised
CT	Computerized tomography
CVA	Cerebrovascular accident
DAI	Diffuse axonal injury
DBT	Distal body temperature
DLMO	Dim light melatonin onset
DLMOff	Dim light melatonin offset
dlPFC	Dorsolateral prefrontal cortex
DMN	Default mode network
DOC	Disorders of consciousness
DRS	Disability rating scale
DTI	Diffusion tensor imaging
ECG	Electrocardiography
EEG	Electroencephalography

EMG	Electromyography
EOG	Electro-oculography
EPs	Evoked potentials
ERP	Event related potential
fMRI	Functional magnetic resonance imaging
GABA	Gaba amino butyric acid
Gal	Galanin
GCS	Glasgow coma scale
GOS	Glasgow outcome scale
HIST	Histamine
HR	Heart rate
Hz	Hertz
ICA	independent component analysis
ICD-10	International Classification of Diseases version 10
LC	Locus coeruleus
LDT	Laterodorsal tegmental nucleus
LEP	Laser evoked potentials
LTC	Long term care
MATADOC	Music therapy assessment tool for awareness in DOC
MCS	Minimally Conscious State
MeSH	Medical subject headings
MMN	Mismatch negativity
MPFC	Medial prefrontal cortices
MRI	Magnetic resonance imaging
ms	Millisecond
NA	Noradrenaline
NIRS	Near infrared spectroscopy
NMDA	N-methyl-D-aspartate
NREM	Non-rapid eye movement
NSAIDs	Non-steroid anti-Inflammatory drugs
PBT	Proximal body temperature

PCC	Posterior cingulate cortex
PDOC	Prolonged Disorders of Consciousness
PEG	Percutaneous enteral gastrostomy
PET	Positron emission tomography
PI	Post-intervention
PPT	Pedunculopontine tegmental nucleus
Pr	Precuneus
pRGCs	Photosensitive retinal ganglion cells
PSG	Polysomnography
RCP	Royal College of Physicians
RCT	Randomized controlled trial
REM	Rapid eye movement
RHN	Royal Hospital for Neuro-disability
rTMS	Repetitive transcranial magnetic stimulation
SAH	Subarachnoid haemorrhage
SCN	Suprachiasmatic nucleus
SE	Sleep efficiency
SEPs	Somatosensory evoked potentials
SMART	Sensory Modality Assessment and Rehabilitation Technique
SON	Subject's own name
SPECT	Single Photon Emission Tomography
SPM	Statistical Parametric Mapping
SSI	Scoring structure index
SSRI	Selective serotonin re-uptake inhibitor
SWA	Slow wave activity
SWS	Slow wave sleep
TBI	Traumatic brain injury
tDCS	Transcranial direct current stimulation
TMN	Tuberomammillary nucleus
TRT	Total recording time
TST	Total sleep time

TWT	Total wake time
UK	United Kingdom
UKROC	UK Rehabilitation Outcomes Collaborative
US	United States
UWS	Unresponsive wakefulness syndrome
VEPs	Visual evoked potentials
VLPO	Ventrolateral preoptic nucleus
VS	Vegetative state
WASO	Wake after sleep onset
WHIM	Wessex Head Injury Matrix
WHO	World Health Organization

Chapter 1 INTRODUCTION: PROLONGED DISORDERS OF CONSCIOUSNESS

With the improvements in health services the number of patients who survive severe brain injuries such as traumatic brain injury, stroke and subarachnoid haemorrhage are increasing (Kelly et al., 2015, Ganesh et al., 2016, Gerber et al., 2013). While some severe brain injury survivors make good progress, some enter a prolonged disorders of consciousness (PDOC) state which includes vegetative state/ unresponsive wakefulness syndrome (VS/ UWS) and minimally conscious states (MCS) (Giacino et al., 2002, Jennett and Plum, 1972). These conditions are known to have a very poor clinical outcome and despite extensive research are still poorly understood (Steppacher et al., 2014, Royal College of Physicians of London, Bernat, 2006, Giacino et al., 2014). To be able to understand the pathophysiology and clinical features of prolonged disorders of consciousness and to be able to design treatment protocols for this peculiar condition, an understanding of consciousness as well as knowledge of the structural and functional systems of the brain that contributes to consciousness are needed.

1.1 CONSCIOUSNESS

The Oxford dictionary defines consciousness as the state of being aware of and responsive to one's surroundings (University). In medical terms, it can simply be defined as a state where the following two components are intact; arousal/ wakefulness and awareness. Impairment of either or both may result in disorders of consciousness. Therefore, these two components of consciousness will be discussed below.

1.1.1 Arousal

The ascending pathways from monoaminergic cell groups in the brain stem and hypothalamus to the cerebral cortex and thalamus increase wakefulness and vigilance, as well as the responsiveness of cortical and thalamic neurons to sensory stimuli, a state known as arousal (Fellinger et al.).

After exiting the brainstem, the ascending arousal system (ARAS) divides into two major branches at the junction of the midbrain and diencephalon. One branch enters thalamus, where it activates and modulates thalamic relay nuclei as well as intra-laminar and related nuclei with extensive diffuse cortical projections. The other branch travels through the lateral hypothalamic area and is joined by the ascending output from the hypothalamic and basal forebrain cell groups, all of which diffusely innervate the cerebral cortex.

Injury to either branch of the ascending arousal system whether it is through thalamus or hypothalamus can impair consciousness. For example, a lesion of the posterior lateral hypothalamus interrupts the pathway through hypothalamus and causes profound slowing of the electroencephalogram and behavioural un-arousability even if the pathway through thalamus is intact. Injury to thalamus or its reticular input prevents the brain achieving a desynchronized or wakeful state. The neurotransmitters acetylcholine, norepinephrine, serotonin, and dopamine play a role in the regulation of the ARAS where alertness is maintained (Saper et al., 2001, Saper et al., 2005). (see figure 1-1) A key component of this ascending arousal system is the cholinergic neurons in the pedunculopontine (PPT) and laterodorsal (LDT) tegmental nuclei. These cholinergic neurons play a key role within the ascending arousal system by providing excitatory signals from the upper brainstem to the thalamic relay nuclei and the reticular nucleus; consequently, improving thalamocortical transmission. (shown with blue lines in figure 1-1)

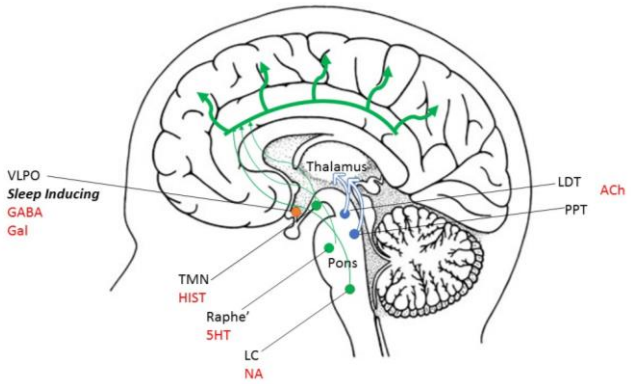


Figure 1-1: Schematic diagram of ascending reticular activating system illustrating key nuclei and the neurotransmitters that take in the regulation of the arousal. (This image was drawn based on the information and illustrations from Saper et al. Hypothalamic regulation of sleep and circadian rhythms. Nature. 2005;437:1257–1263)

ACh= Acetylcholine, NA= Noradrenaline, HIST= Histamine, 5HT= Serotonin, Gal= Galanin, LDT=Laterodorsal tegmental nucleus, PPT= Pedunculopontine tegmental nucleus, TMN= tuberomammillary nucleus, LC= locus coeruleus

1.1.2 Awareness

Awareness on the other hand is regulated by a group of complex network systems. Recently published research studies indicated that awareness is generated by three main networks: internal awareness network, external awareness network and thalamocortical network.

The internal awareness network is also known as the Default Mode Network (DMN) and based on midline fronto-parietal networks. The internal awareness network includes midline structures posterior cingulate cortex (PCC)/ precuneus (Pr) and anterior cingulate (ACC)/medial prefrontal cortices (MPFC). (Figure 1-2) The external awareness network encompasses dorsolateral prefrontal cortices (DLPFC) and posterior parietal cortices (PPC) (Demertzi et al., 2013). (Figure 1-3) (Figures 1-2 and 1-3 are based on the drawings from Demertzi, Athena et al. “Consciousness supporting networks.” *Current Opinion in Neurobiology* 23 (2013): 239-244.)

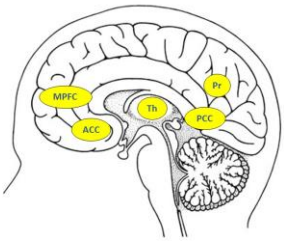


Figure 1-2: Brain areas which are part of internal awareness network (Demertzi et al. 2013)

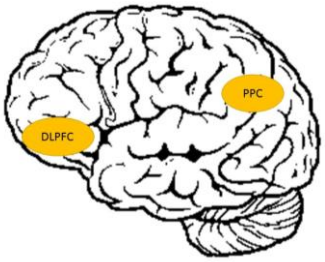


Figure 1-3: Brain areas which are part of external awareness network (Demertzi et al. 2013)

The cortico-thalamo-cortical network which has millions of connections between the thalamus and cortices also plays an important role in generation of consciousness. By using positron emission tomography (PET) scans Thibaut et al. showed that in VS/UWS there is a metabolic dysfunction in the thalamus, external and internal awareness networks. The degree of metabolic dysfunction was to a lesser extent in MCS patients, mainly showing dysfunction of the internal awareness networks. Moreover, it was shown that behavioural assessment scores correlated with activity in the extrinsic network and part of the intrinsic network (Thibaut et al., 2012).

Naro et. al. considered use of repetitive transcranial magnetic stimulation (rTMS) paradigms targeting structures belonging to default mode network (the anterior cingulate cortex – ACC) and external awareness network (the dorsolateral prefrontal cortex – dlPFC). They were able to differentiate patients with MCS from those with UWS at a group level, with the remarkable exception of two persons with UWS which then raised the question of misdiagnosis of these two subjects and the possibility of using rTMS technique to detect covert signs of awareness (Naro et al., 2017).

Despite the constantly growing knowledge base on brain networks which are responsible for generation of consciousness, and the increasing possibility of

use of neuro-imaging techniques for diagnosis of disoders of consciousness, all current definitions and diagnostic criteria remain based on bedside clinical assessment methods.

1.2 DEFINITIONS AND DIAGNOSTIC CRITERIA FOR PROLONGED DISORDERS OF CONSCIOUSNESS

Severe disorders of consciousness include coma, vegetative state (VS) and minimally conscious state (MCS).

Coma is a state of unarousable unconsciousness due to dysfunction of the brain's ascending reticular activating system (ARAS), which is responsible for arousal and the maintenance of wakefulness (Laureys and Tononi, 2009). Coma results from global brain dysfunction. A patient who is in a state of coma fails to open eyes to stimulation, presents motor responses no better than simple withdrawal type of movements and a verbal response no better than simple vocalization of non-word sounds (Plum and Posner, 1966). This state must last at least one hour to be differentiated from similar but transient states such as syncope or delirium. Coma may last up to two to four weeks. Emergence from coma is followed by outcomes that range from the vegetative state to complete recovery. The severity of damage to the cerebral cortex, the thalamus or their integrated function, determines the outcome from coma.

Structural brain lesions, metabolic/ nutritional/ toxic encephalopathies, systemic and central nervous system infections, hyperthermia and hypothermia and trauma are common causes of coma. Regardless of the aetiology, patients initially present in a state of coma following a brain injury. Spontaneous eye opening occurs after recovery of brain-stem reticular formation, which takes about one week after non-traumatic brain injury and about two to three weeks after traumatic brain injury. Once the eyes are open patients may recover consciousness to a degree or remain in a vegetative state, which is then diagnosed by the presence of reflex responses as well as preservation of autonomic functions but lack of any evidence of awareness.

The VS was first coined in the early 1970s by Jenner and Plum and the diagnostic criteria for MCS were first published in 2002 (Giacino et al., 2002). Other terms such as the “apallic syndrome” have been used previously and more recently “unresponsive wakefulness syndrome” as a less emotive synonym for the VS (Laureys et al., 2010).

The vegetative state results from profound damage to cerebral hemispheres and in most cases to thalami, with relative preservation of the brain stem, and is defined as a state of wakefulness without awareness. In other words, it is a state where there is dissociation between arousal and awareness. Three main patterns of brain damage were found to be associated with vegetative state: widespread destruction of the cortical ribbon, widespread damage to white matter tracts, and damage to the thalamus (Kinney and Samuels, 1994). However, as outlined in the awareness section above, in light of recent research, disorders of consciousness are thought to be the result of damage to internal awareness, external awareness and thalamo-cortical networks. The severity of damage to these networks determines the level of consciousness present in disorders of consciousness. Therefore, these conditions are considered to be part of a continuum which explains the variability in the observed behavioural signs of overt consciousness.

The difference between vegetative and minimally conscious state depends on the detection of reproducible and purposeful behaviours. There is no evidence of sustained, reproducible or purposeful behavioural responses in VS. However, there is inconsistent but clearly discernible behavioural signs of awareness such as visual tracking, simple command following, gestural yes/no responses are present in patients in minimally conscious state.

Table 1-1: Clinical features of coma, VS and MCS

Condition	Consciousness		Motor behaviour characteristics
	Sleep-wake cycles	Awareness	
Coma	No	No	No purposeful behaviour
Vegetative state	Yes	No	No purposeful behaviour
Minimally conscious state	Yes	Partial, fluctuating	Inconsistent but reproducible purposeful behaviour

Disorders of consciousness are now considered as a continuum, ranging from VS to high minimally conscious state (Bekinschtein et al., 2005). It was suggested that MCS can be further divided into low and high MCS categories due to the wide range of behaviours observed within the same diagnostic category. Low MCS is characterized by the presence of single low complexity behaviour such as visual pursuit or localization following noxious stimulation. High MCS on the other hand is characterized by the presence of clearly discernible evidence of one or more of the following behaviours: simple command following, gestural or verbal yes/no responses, intelligible verbalization or contingent behavioural responses (Giacino and Kalmar, 2005). The distinction between MCS and VS is important as it may influence treatment, as well as the way family and societies perceive the condition.

The incidence and prevalence of the vegetative state and minimally conscious state are not certain as both conditions are not formal diagnoses under International Classification of Diseases (ICD 10, World Health Organization)

and hence national statistics are not available. Difficulties with reaching a certain diagnosis which are due to either shortfall of assessment methods or inadequate time spared for observations, and effect of medications, contribute to the fact that no clear epidemiological data are available for these conditions (Strens et al., 2004). There are also significant national variations which are related to socio-economical as well as cultural factors. The incidence of vegetative state varies between 5 and 25 per million population. In the United States (US), the prevalence of VS in adults is reported to be between 40 and 168 per million population according to the published data. Although the figure may be lower in the United Kingdom (UK) and other European countries; accurate figures are not available.

Based on numbers of patients with PDOC in nursing homes, it was estimated that there are 4,000 to 16,000 patients in VS, with three times as many in MCS in the UK (2015, March 2015). It was suggested that the UK Rehabilitation Outcomes Collaborative (UKROC) incorporates a PDOC database which includes a minimum clinical dataset and longitudinal outcome data for patients in PDOC (Royal College of Physicians of London). At the time of writing this thesis work on creating PDOC database was ongoing. Even with the creation of a database, knowledge of the number of people in different disorders of consciousness states with certainty will be difficult due to the challenges in differentiating vegetative and minimally conscious states. The next section of this thesis outlines clinical features of PDOC and how observed behaviours can make the differential diagnosis problematic.

1.3 DIAGNOSIS AND CLINICAL FEATURES OF PROLONGED DISORDERS OF CONSCIOUSNESS

Currently, diagnosis of vegetative state requires demonstrating lack of behaviours that reflect cortical activity. In practice, this is difficult as some reflexive behaviours observed may be mistaken as conscious behaviours. For example, behaviours like the eyes and head turning briefly towards strong visual or auditory stimuli does not require cortical integration. Similarly,

touching a patient may produce a general startle reaction called startle myoclonus. All these responses may be mistaken with behaviours of cortical activity. While patients with higher level of responses habituate to sudden stimuli and produce less and less response, patients in vegetative state do not habituate. Similarly, brief fixation and even brief following of an object can occur in vegetative state. It is clear that, diagnosis of vegetative state is challenging for healthcare professionals and difficult to accept for patients' relatives especially in the presence of non-cortical responses such as the above. Significant effort, therefore, has been put into establishing clear diagnostic criteria for vegetative state.

Consistent and sustained visual pursuit is considered as one of the first observable signs of recovery in patients who go on to regain consciousness (Jennett et al., 2002). Some consider that those show this phenomenon, are no longer in vegetative state. However, occasionally patients who gained sustained visual pursuit develop no other behavioural evidence of consciousness. In 1996, an International Working Party suggested that isolated visual tracking could not be considered as evidence of emergence from vegetative state (Andrews, 1996). Hence, extreme caution is required when there is sustained visual pursuit, consistent and reproducible visual fixation, or response to threatening gestures in making a diagnosis of vegetative state. It was shown that assessment of visual pursuit with a mirror is superior to that with moving person or object (Thonnard et al., 2014, Vanhaudenhuyse et al., 2008).

Other clinical features of vegetative state which can lead to confusion of healthcare professionals and relatives are as follows;

- Increased muscle tone of the limbs and spontaneous movements
- Grasp reflex
- Facial grimacing and even groan to painful stimuli
- Smiling, frowning, occasionally laughing or weeping

In 1994, the Task Force Criteria (1994) has become the benchmark for American practice. In 1996, the Royal College of Physicians of London (RCP) (1996) produced a statement in response to a request from a parliamentary committee following *Bland* –the first case where withdrawal of artificial nutrition and hydration was considered by courts in the UK [Airedale NHS Trust v Bland (1993) AC 789 1 All ER 821]. See table 1-2 and 1-3.

Table 1-2: Statement of the Multi-Society Task Force 1994

Definition
The vegetative state is a condition of complete unawareness of the self and the environment accompanied by sleep-wake cycles with either complete or partial preservation of hypothalamic and brain stem autonomic functions.
Criteria
No evidence of awareness of themselves or their environment; they are incapable of interacting with others.
No evidence of sustained reproducible, purposeful or voluntary behavioural responses to visual, auditory, tactile or noxious stimuli.
No evidence of language comprehension or expression.
Intermittent wakefulness –sleep-wake cycles.
Sufficient preserved hypothalamic and autonomic functions to survive.
Bowel and bladder incontinence.
Variably preserved cranial nerve reflexes (pupillary, oculocephalic, corneal, vestibule-ocular, gag), and spinal reflexes.

Table 1-3: Royal College of Physicians of London 1996

Three clinical criteria all to be fulfilled
No evidence of awareness of self or environment. No volitional response to visual, auditory, tactile or noxious stimuli. No evidence of language comprehension or expression.
Cycles of eye closure and opening simulating sleep and waking.
Sufficiently preserved hypothalamic and brain stem function to maintain respiration and circulation.
Other clinical features
Incontinence of bladder and bowel. Spontaneous blinking and usually retained pupillary and corneal responses. Conjugate or dysconjugate tonic response to ice-water caloric testing.
No nystagmus to caloric testing. No visual fixation, tracking of moving objects with eyes or response to menace.
May be occasional movements of head and eyes towards sound or movement, and of trunk and limbs in purposeless way. May have startle myoclonus. May smile; may grimace to pain. May have roving eye movements.

Minimally conscious state was only described in 2002. Therefore, neither 1994 Multi-society Task Force not 1996 RCP guidelines covered this condition (Giacino et al., 2002). The latest guidelines for VS and MCS was published in 2013 by the Royal College of Physicians. The diagnostic criteria for VS and MCS have been summarized in table 1-4. The UK guidelines were due to be updated and new clinical guidelines for the US were being written at the time of writing this thesis.

Table 1-4: Diagnostic Criteria for Prolonged disorders of consciousness states (RCP Guidelines 2013)

	Diagnostic Criteria and Compatible/ Incompatible Features
Vegetative State	Essential Criteria: - No evidence of awareness of self or environment or the ability to interact with others - No evidence of sustained purposeful or voluntary behaviours, either spontaneously or in response to visual, auditory, tactile or noxious stimuli - No evidence of language, comprehension or meaningful expression. Usually present: - Cycles of eye closure and eye opening, giving appearance of sleep-wake cycle - Spontaneous respiration and circulation. Compatible features: - Spontaneous movements (chewing, smiles, grimaces, shedding tears, grunting or groaning sounds - Reflexive movements (brainstem, corneal, oral/facial, grasp reflexes) - Generalized arousal response to various stimuli - Eyes may turn fleetingly towards sound or visual target, react to menace. - Compatible but atypical features include isolated fragments of behaviour (i.e. utterance of a single inappropriate word). Incompatible features: - Evidence of discriminative perception, - Purposeful actions, - Anticipatory actions, - Communicative acts.

Minimally Conscious State	Limited but clearly discernible evidence of self- or environmental awareness must be demonstrated on an inconsistent but reproducible or sustained basis by one or more of the behaviours below: - Following simple commands - Gestural or verbal 'yes /no' responses (regardless of accuracy) - Intelligible verbalisation - Purposeful or discriminating behaviours - Evidence of behaviours that is only explicable through some awareness being present: - Episodes of crying, smiling or laughter in response to linguistic or visual content of emotional topics or stimuli - Vocalization or gestures in response to the linguistic content of comments or questions - Reaching for objects in a manner that demonstrates a clear relationship between object location and direction of reach - Touching or holding objects in a manner that accommodates the size and shape of the object - Sustained pursuit eye movement or sustained fixation - Localizing or discriminating behaviours to people or objects.
Emergence from Minimally Conscious State	Reliable and consistent responses need to be demonstrated which include; - Functional use of objects - Consistent discriminatory choice-making - Functional interactive communication

RCP guidelines also lists the following preconditions for diagnosis of VS and MCS:

- The cause of the condition should be established as far as possible,
- The possibility of reversible causes must be excluded,
- Assessments should be made under appropriate conditions by a trained assessor using structured assessment tools such as Coma Recovery Scale- Revised (CRS-R), Wessex Head Injury Matrix (WHIM) or Sensory Modality Assessment and Rehabilitation Technique (SMART).

Barker re-iterates the importance of answering three main questions during assessment of a patient presenting with loss of awareness (Barker, 2005).

- Are there any focal deficits which help localize the main site of injury and which may be complicating the interpretation of patient's responses?
- Is the patient in a persistent vegetative or minimally conscious state?
- Is the patient suffering from locked-in syndrome?

Table 1-5: Clinical features of coma, VS, MCS and Locked-in syndrome

Condition	Coma	VS	MCS	Locked-in syndrome
Awareness	Absent	Absent	Present	Present
Sleep-wake cycle	Absent	Present	Present	Present
Response to noxious stimuli	+/-	+/-	Present	Present in eyes only
Motor function	No purposeful movement	No purposeful movement	Some consistent or inconsistent verbal or motor responses	Volitional vertical eye movements or eyeblink preserved
Respiratory function	Variable	Preserved	Preserved	Preserved
EEG Activity	Typically slow wave	Typically slow wave	Insufficient data	Typically normal
Prognosis	Recovery, VS, death within weeks	Poor	Variable	Full recovery unlikely

It is clear that, clinical diagnosis of vegetative state is rather difficult and misdiagnosis rate is high. Therefore, there has been great effort to identify and establish electro-physiological and radiological investigations that would support the clinical decision-making process.

Several research studies have shown that it is possible to detect the presence of higher level cerebral functions in such patients, by utilizing electrophysiological methods and/or functional neuroimaging techniques in cases where clinical assessments are unable to detect any behavioural sign of awareness (Cruse et al., 2011, Bekinschtein et al., 2011, Bruno et al.,

2011b, Monti et al., 2010). Even though there have been exciting and promising developments in research, currently diagnosis of vegetative state and minimally conscious states remain on clinical grounds alone.

1.4 CLINICAL ASSESSMENTS TOOLS IN DIAGNOSING DISORDERS OF CONSCIOUSNESS

Due to high percentages of clinical misdiagnosis of vegetative state- up to 43%, (Andrews et al., 1996, Schnakers et al., 2009) it has been suggested that diagnosis should be made only after an adequate period of observation and based on findings of formal and structured assessment tools such as Sensory Modality Assessment Rehabilitation Technique (SMART) (Gill-Thwaites, 1997) or Coma Recovery Scale- Revised (CRS-R) (Giacino et al., 2004). Wessex Head Injury Matrix (WHIM) can also be used to track changes in patient's consciousness levels (Shiel et al., 2000). RCP guideline recommends one or more of the above assessment tools in reaching diagnosis in PDOC.

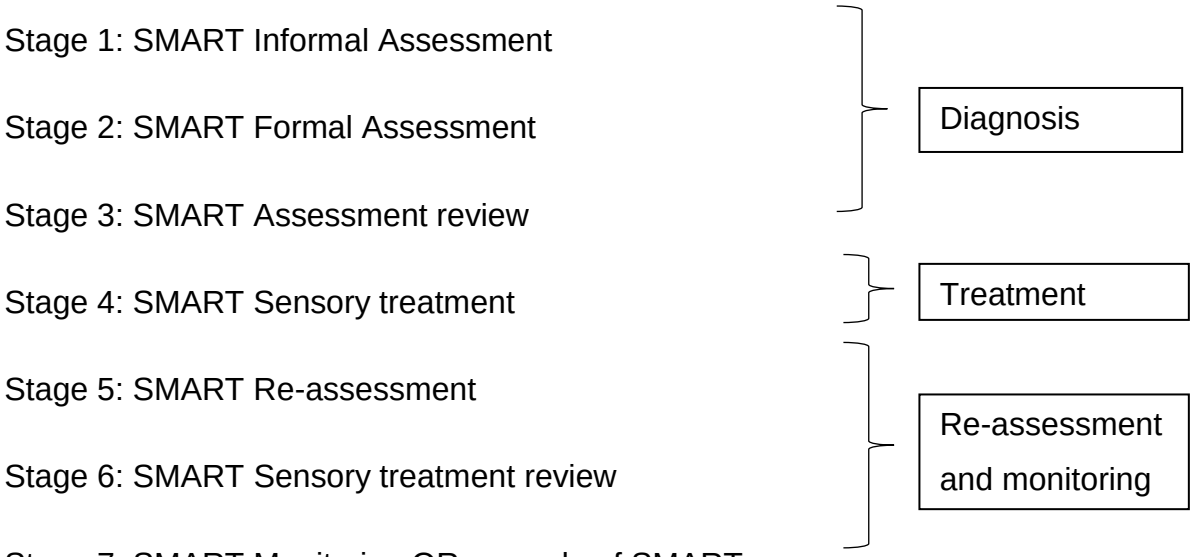
Factors that may temporarily reduce consciousness, such as metabolic disturbances, medication side effects and concurrent infections, require due attention. While obtaining collateral information from care givers, therapists and relatives can be very useful in reaching diagnosis, possible misinterpretation of reflex activity by family should be kept in mind. At the stage of history taking particular attention should be paid to type of insult, site of injury, time since injury, age of patient, time of day and previous activities in that day, and medication. During the neurological examination; the size and responsiveness of pupils, eye movements, oculo-cephalic movements, visual tracking, and response to painful stimuli should be recorded.

The most commonly used clinical assessments tools are SMART, WHIM and CRS-R. These three assessment tools will be reviewed here, and their pros and cons will be summarized as the subjects of this research were assessed with these tools at the time of their diagnosis and throughout the research project.

1.4.1 Sensory Modality Assessment Rehabilitation Technique (SMART)

SMART was designed by Gill-Thwaites and Munday in late 1990s as both an assessment and a treatment tool to identify signs of awareness in patients diagnosed in vegetative state (Gill-Thwaites, 1997). Later on it was shown that SMART is a very detailed assessment tool and is more effective than other assessment tools in distinguishing VS patients from those in MCS (Godbolt et al., 2012).

SMART Assessment is completed in seven stages;



The informal component of SMART includes a Communication and Lifestyle questionnaire as well as gathering information from family and staff (SMART “informs”) in order to establish premorbid interest/ dislikes, to involve relatives in the assessment process, to establish behaviours in different settings, to establish relative interpretation of behaviours, to identify frequency of behaviours and to allow comparison with formal assessment.

The formal components include SMART Behavioural Observation Assessment and SMART Sensory Assessment. Assessment phase of SMART is conducted over ten sessions which normally takes two to three weeks to

complete. Equal numbers of morning and afternoon sessions are allocated for SMART assessment and the assessor remains same.

Behavioural observation assessment is performed at the beginning of each assessment session and aims to establish patient's unique behavioural repertoire at rest, patient's arousal levels and reflexive/ spontaneous behaviours that occur without stimulus.

SMART sensory assessment is a structured and graded assessment of a patient's responses in eight domains; behavioural response to sensory stimuli across the five senses (visual, auditory, tactile, olfactory and gustatory) in addition to wakefulness/ arousal, motor and communicative responses. It uses 29 standardised techniques using standardised stimuli.

A 5-point hierarchical scale is employed for each assessment domains and patient responses are rated from no response (Level 1) through a differentiating response (Level 5). Emergence from VS is indicated if a consistent Level 5 response is observed in any modality except wakefulness, on at least five consecutive occasions.

SMART Hierarchical Scale for Sensory Modalities:

- Level 1: No response from any stimulus
- Level 2: Reflex (reflexive, generalized response to stimuli)
- Level 3: Withdrawal (turn head/ eyes away or withdraw limbs from stimuli)
- Level 4: Localizing (turn head or move upper limbs towards stimuli)
- Level 5: Differentiating (follow visual or auditory commands/ use objects appropriately)

Further categorization of responses is made as follows:

- Consistent response: a response occurs that in at least 5 consecutive assessment sessions

- Frequent inconsistent response: a response occurs 5 times or more but not consecutive
- Highest inconsistent response: highest response observed in the modality that occurs on 1-4 sessions over the assessment stage.

For example, responses to light might be recorded as:

SMART Level: 2

Category: consistent (number of occurrences consecutively: 6; Total number of occurrences: 8)

Direction/ side: left

Type of response: eye blink

Following the completion of assessment all information obtained through informal and formal components are reviewed and analysed. An indicative diagnosis is provided following review and analysis.

Table 1-6: SMART responses and corresponding indicative diagnosis

SMART Level	Frequency of responses	Type of response	Indicative diagnosis
1	Highest inconsistent, Frequent inconsistent, Consistent	No response Reflexive responses or non-meaningful facial expression Withdrawal response or specific facial (non-meaningful) expression to stimuli	VS
2			
3			
4	Highest inconsistent, Frequent inconsistent, Consistent	Localising to stimuli or Communicative facial expression	MCS- or MCS
5	Highest inconsistent, Frequent inconsistent, Consistent	Meaningful functional movement/ cause and effect or yes/no response or communicates needs	MCS or MCS+

Following the SMART assessment, treatment block is delivered in eight weeks period. It aims to explore impact of any pre-requisite factors, differences between formal and informal findings as well as between verified and unverified responses. It also aims to enhance level of responses and potential to use them at functional level.

Following the completion of SMART treatment, a re-assessment is conducted. SMART sensory treatment review is completed to review the patient's progress including responses recorded by the family and team members (informs). Results, conclusions and future directions are discussed with the family and the team members.

1.4.2 Wessex Head Injury Matrix (WHIM)

Wessex Head Injury Matrix was developed by Shiel et al. in 2000. The scale was developed by observing recovery of 88 patients with brain injury (age Mean=30 years, range 14-67; coma duration median= 6 days; duration of post-traumatic amnesia median= 30 days) (Shiel et al., 2000). 62 behaviours or steps relating to social behaviour, cognition, attention, and communication were included in the scale which is administered by observation, presenting patients with meaningful stimuli and recording their responses to stimuli. Types of behaviours are examined by WHIM are spontaneous behaviours, responses to naturally occurring stimuli such as tracking of source of sound and responses to presentation of standard stimuli such as performing a physical movement in response to command. WHIM mainly focuses on behaviours rather than diagnostic features and carries potential of being able to be used by all members of the multi-disciplinary treatment team. Therefore, it has a value on monitoring the changes during natural recovery as well as rehabilitation. WHIM requires simple test materials that include everyday items such as coin, key, magazine pictures. Administration starts at item 1 and continues until the occurrence of 10 consecutive failures.

Inter-rater reliability of WHIM was also examined with an independent sample of 25 patients. In this study two novice raters underwent brief training on administration of WHIM. Another inter-rater reliability study was conducted after providing raters with more detailed training session. The results of this study revealed that while inter-rater and test-retest reliabilities between novice raters were low (kappa = 025 to 0.84 for inter-rater reliability and – 0.66 to 0.12 for test–retest reliability), after a training session, both inter-rater and test-

retest reliabilities reached acceptable levels. Mean kappa scores were 0.86 for interrater reliability and 0.74 for test–retest reliability. Training of the assessor is required to ensure reliability.

Psychometric properties of WHIM was explored in detail by Majerus et al. using a 66 item version of WHIM in 23 patients who suffered from stroke or traumatic brain injury (Majerus, 2000). This study also indicated that WHIM is sensitive to subtle changes in patients with disorders of consciousness. Inter-rater agreement was excellent for 73% of the 66 items (K = 0.8–1), fair to good for 20% (K = 0.4–0.73) and poor for 7% (K = –0.1–0.07). Test–retest reliability which was assessed with Spearman rank order correlation between the WHIM scores obtained in the test and re-test sessions was 0.981 (P < .0001).

Recently published studies provided further insight into this tool where re-ordering of the hierarchical items is recommended (Turner-Stokes et al., 2015). Nevertheless, WHIM is a valuable assessment tool especially when it is used alongside another assessment tool- SMART (Morrissey et al., 2017). At the time of writing of this thesis, WHIM-2 was being developed in order to resolve the problems with ordering of hierarchical items of the scale.

1.4.3 Coma Recovery Scale- Revised (CRS-R)

JFK Coma Recovery Scale (CRS) was developed in 1991 by Giacino et. al. with the purpose of creating an assessment tool that could differentiate between patients in the vegetative state and those who are minimally conscious (Giacino et al., 1991). CRS was used for many years in clinical and research settings. Therefore, there was a considerable amount of feedback received from CRS users which led to further analysis of CRS and eventually the development of CRS-Revised (CRS-R).

CRS-R comprises six subscales: Auditory, Visual, Motor, Oro-motor/ Verbal, Communication, and Arousal. The responses are elicited with standardized instructions which are provided in the Administration and Scoring Guidelines manual. The lowest level responses within a subscale represent reflex activity

and the highest-level responses represent “cognitively mediated” behaviours. Administration of the scale requires some test materials such as comb, mirror, ball and time required for administration is 20 to 25 minutes. The total score ranges from 0 to 23 and scores can be converted to a classification of VS, MCS or emergence from MCS.

According to CRS-R, emergence from VS is denoted by the presence of the following behavioural responses:

- Reproducible movement to command,
- Visual fixation,
- Motor localization to noxious stimuli
- Intelligible verbalization
- Intentional (even if non-functional) communication.

Emergence from MCS requires additional criteria of:

- Functional object use, and/or
- Accurate/ functional communication.

CRS-R has excellent content validity and showed substantial evidence of good interrater reliability. It was suggested that CRS-R may be used to assess DOC with minor reservations (whereas SMART and WHIM with moderate reservations) (Seel et al., 2010).

1.5 REHABILITATION AND TREATMENT OPTIONS IN PDOC

Currently there is not a recommended treatment or a therapeutic intervention for PDOC. Several medications with neuro-stimulant properties (i.e. dopaminergic medications, GABA-ergic drugs, serotonin and/or noradrenalin reuptake inhibitors) were trialled and the outcomes mainly reported in the form of case reports or series of cases.

Amongst all the medications trialled Amantadine has the most rigorous scientific evidence suggesting it can be beneficial in precipitating recovery in VS and MCS. A multicentre, prospective, double-blind randomized, placebo-controlled study which involved 184 patients showed that recovery was significantly faster in the amantadine group than in the placebo group (Giacino et al., 2012).

Currently, there is not enough evidence to recommend a particular medication for treatment of PDOC, but clinicians often try different medications to improve arousal levels and aid to recovery of brain functions.

Neurostimulation with electrical and magnetic stimulation have attracted attention recently with some research indicating promising and some disappointing outcomes.

For example, a recently published study showed that neuromodulation with Transcranial Direct Current Stimulation (tDCS) which was repeated for 5 days in the left prefrontal area, improved the recovery of consciousness in some chronic patients in MCS, up to 1 week after the end of the stimulations (Thibaut et al., 2017). On the other hand, another study which used repeated tDCS over the left dorsolateral pre-frontal cortex did not exert remarkable short-term clinical and Electroencephalography (EEG) effects. Furthermore, clinical features and visual analysis of EEG did not distinguish patients who improved from those who didn't. It was concluded that further studies should ascertain whether tDCS might promote clinical recovery in the long-term period (Estraneo et al., 2017).

In another study, Repetitive Transcranial Magnetic Stimulation (rTMS) of the left dorsolateral prefrontal cortex for ten consecutive days was given to 16 patients with prolonged disorders of consciousness. Following treatment, a significant enhancement of CRS-R scores ($p=0.007$) were observed in all MCS patients (five out of five) and four out of eleven VS patients (Xia et al., 2017).

Although studies exploring use of methods like tDCS and rTMS suggest some promising outcomes, they remain to be research methods and further validation of these techniques with a cohort of larger sample size are needed.

1.6 PROGNOSIS IN PROLONGED DISORDERS OF CONSCIOUSNESS

RCP guidelines in 2013(Royal College of Physicians of London) covered the diagnostic criteria and definitions for VS, MCS and emergence from MCS in relation to passage of time since brain injury and prognostic predictors. According to the guidelines, if the disorders of consciousness (VS or MCS) last more than one month it is described as Prolonged Disorders of Consciousness (PDOC). If the vegetative state lasts more than 6 months following non-traumatic brain injury or more than 12 months following traumatic brain injury it is deemed to be permanent. The guidelines also say that after these time points, recovery is deemed 'highly improbable'. MCS, on the other hand is deemed permanent if it lasts more than 3-5 years- depending on clinical circumstances of a patient. At the time of publication, the RCP guidelines recommended consideration of application to courts for withdrawal of clinically assisted nutrition and hydration (CANH) when permanent VS or MCS is diagnosed. A recent ruling in the case of *M v. a Hospital [2017] EWCOP 19,* lifted this necessity and left decisions to withdraw CANH to the clinicians who need to obtain the agreement of the families and act in accordance with prevailing professional guidance.

Our current knowledge of long-term outcome in severe DOC is incomplete largely because once a diagnosis is made, patients are discharged to diverse care settings and their follow up rarely extends beyond 12 months after brain injury (Binkley, 1994, Steppacher et al., 2014, Yelden et al., 2017b). A recent study examined the long-term prognosis (for a mean of 25.7 months from onset of brain injury) in 50 patients with VS. This study reported that late recovery was detected in 25% of the patients; suggesting that late recovery of responsiveness may occur more frequently than previously appreciated

(Estraneo et al., 2010). This study also demonstrated a higher chance of recovery in the post-anoxic brain injury sub-group (21.4%) than in earlier published studies which were in the form of case reports (Arts et al., 1985, Sancisi et al., 2009, Childs and Mercer, 1996, McMillan and Herbert, 2004, Avesani et al., 2006, Sara et al., 2007, Estraneo et al., 2014). Luauté et al. showed that a third of patients in MCS with mixed aetiologies improved more than 1-year post ictus (Luauté et al., 2010). In this study however, Glasgow Outcome Scale was used as the main outcome measure with no specific attention to improvement of awareness/ responses to given stimuli.

In our recently published study, we were able to show that that late improvements in awareness are not exceptional even in non-traumatic PDOC (Yelden et al., 2017a). It highlights the importance of long term follow up of patients with PDOC, regardless of the aetiology, age and time passed since the brain injury. Long term follow-up will help clinicians to identify patients who may benefit from further assessment and rehabilitation. In this study, although only one patient achieved recovery of function, recovery of awareness may have important ethical implications especially where withdrawal of artificial nutrition and hydration is considered.

1.7 CHAPTER SUMMARY AND CONCLUSIONS

This chapter of the thesis illustrates that the concept of consciousness is difficult to define, measure and quantify which makes the understanding of disorders of consciousness difficult. Diagnosis and prognostication of vegetative and minimally conscious states are challenging for clinicians as well as neuro-scientists. Therefore, in the last decades there has been a desire to explore the use of different neurophysiological and neuro-imaging techniques to measure consciousness levels of people with disorders of consciousness as diagnostic and prognostic markers in addition to bedside clinical assessment tools. The next chapter will review the most commonly researched neuro-physiological techniques to assess consciousness in this patient group.

Chapter 2 INTRODUCTION: ASSESSING CONCIOUSNESS WITH ELECTROPHYSIOLOGICAL AND NEURO-IMAGING TECHNIQUES

As discussed in chapter 1, distinguishing between vegetative and minimally conscious states depends on discriminating reflexive and voluntary responses. Although bedside behavioural assessment tools aim to achieve this, they are often limited due to severe motor impairments, the possible presence of aphasia, confounding medical factors such as tracheostomy, as well as fluctuations of arousal levels. Neuro-imaging and neuro-physiological techniques may provide valuable information and may help to detect covert signs of awareness and any brain activity linked to consciousness. In most research projects, they are often used as additional means of obtaining objective measures of brain activity, in addition to outcomes of clinical assessment tools such as CRS-R and SMART. However, researchers need to choose the most appropriate technique, which can help to answer their research question in the most effective and practical way.

The aim of this chapter is to provide a general review of techniques that can help to assess changes in brain activity in PDOC, so that the most beneficial tool(s) can be identified for this research project.

These techniques may be divided into three broad categories based on methods used to trigger brain activation while the assessments are performed (table 2-1).

Table 2-1: Techniques to measure brain activity

What is measured?	Electrophysiological Techniques Examples	Neuro-imaging Techniques Examples
Spontaneous brain activity (no stimulation)	Electroencephalography (EEG) Sleep EEG/ Polysomnography (PSG)	Positron Emission Tomography (PET) scans Resting state Functional MRI (fMRI) to assess default mode network
Brain activity during passive paradigms (stimulation given)	EEG responses to deviant stimuli (event related potentials- [ERPs])	fMRI
Brain activity during active paradigms (stimulation and instructions given to perform a task)	Counting deviant stimuli during ERPs.	Motor imagery tasks during fMRI, Near Infrared Spectroscopy (NIRS)

The general principles and the advantages and disadvantages of using these techniques in disorders of consciousness patients will be summarized below.

2.1 ELECTROPHYSIOLOGICAL TECHNIQUES

2.1.1 Electroencephalography (EEG)

EEG measures the synaptic potentials from pyramidal cells of brain cortex using scalp electrodes. The EEG trace obtained represents potential differences between two electrodes. The routine EEG is usually performed over 20-30 minutes and can give useful information about brain's baseline activity and may help to identify any epileptiform activity, especially if it is performed repeatedly (Marsan and Zivin, 1970). EEG scalp electrodes are

applied according to 10-20 system which uses four anatomical landmarks (nasion, inion and the preauricular points bilaterally) from which measurements are made and electrodes are placed 10% or 20% of the distances (figure 2-1).

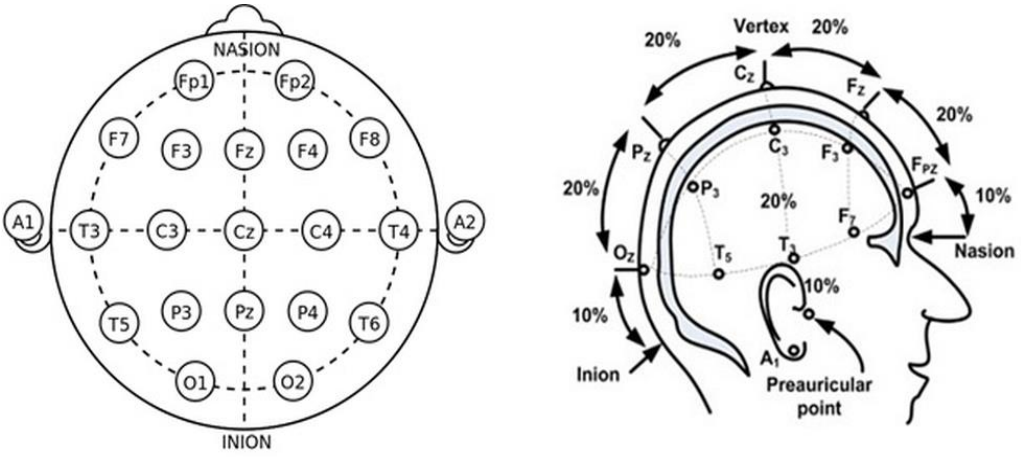
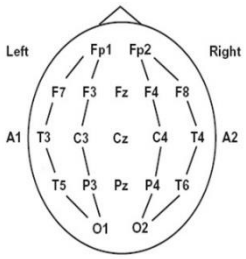
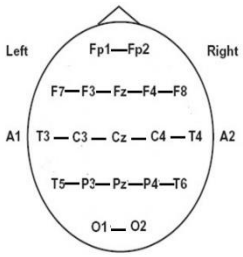
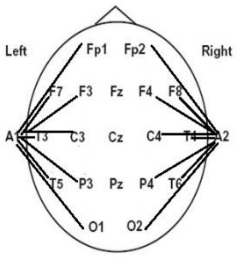


Figure 2-1: EEG electrode placement according to 10-20 system (redrawn from Norani et.al. 2010)

The EEG can be recorded using a bipolar (longitudinal or transverse) or referential montage methods (table 2.2).

Table 2-2: Most commonly used EEG montages (adapted from Behavioural Neurology and Neuropsychiatry, Cambridge University Press, 2013)

Longitudinal bipolar montage (double banana)	Transverse bipolar montage	Referential montage
		

Once recorded, interpretation and reporting of an EEG involves description of waveforms and discharges using following parameters (Lee-Chiong, 2006):

- Amplitude: how high the voltage is in microvolt (μV),
- Duration: how long the discharge is in milliseconds (ms) or seconds,
- Frequency: how frequently the identified waveform repeats itself per second in Hertz (Hz),
- Morphology: shape and structure of the discharge,
- Latency: the delay between an event and another event in milliseconds,
- Location: where the discharge happens,
- Reactivity: what affects the discharge.

During EEG recording the electrodes may capture other activity in addition to electrical activity of the brain. These additional activities captured on EEG are called artefacts and can be classified as physiological and extra-physiological artefacts. Physiological artefacts may be due to muscle activity, eye movements, electrocardiographic activity, pulse, respiration or sweating. Extra-physiological artefacts may be due to electrode 'pop', alternating current artefact arising from power lines, interference from electrical equipment, movements in the environment i.e. from respirators. EEG artefacts need to be identified and eliminated by the neurophysiologist if possible.

In a normal EEG, the following patterns during wakefulness are seen (Lee-Chiong, 2006);

- Alpha Rhythm: Frequency 8-12 Hz; located posteriorly; with the morphology of rhythmic, regular, waxing and waning; amplitude between 20 and 100 μV; best seen with the eyes closed.
- Beta Activity: Frequency greater than 13 Hz (typically between 18-25 Hz); located mostly in frontocentral; morphology is usually rhythmic, waxing and waning and symmetric; amplitude between 5-20 μV, boosted during sleep stages 1 and 2. Beta activity is usually increased

in amount and voltage in the EEGs of people who use benzodiazepines and barbiturates.

- Mu Rhythm: Frequency 7-11 Hz; located in centroparietal area; arch like shape or like letter “m”; mostly asymmetric but may be unilateral; attenuates with movement of contralateral extremity, thought of a movement or with tactile stimulation.

Two other EEG rhythms that can be identified in EEGs are (Sanei and Chambers, 2007);

- Delta waves: have frequency 0.5–4 Hz. These waves are primarily associated with deep sleep and may be present in the waking state in brain injured patients. They may be confused with artefact signals caused by the large muscles of the neck and jaw.
- Theta waves: have frequency of 4–7.5 Hz and originate from thalamic structures. Theta waves appear as consciousness slips towards drowsiness. A theta wave is often accompanied by other frequencies and seems to be related to the level of arousal. The theta wave plays an important role in infancy and childhood but large amounts of theta wave activity in the waking adult are abnormal and are caused by various pathological problems.

EEG provides a direct measure of the behaviour of large-scale neuronal networks with a millisecond temporal resolution and gives information on functional properties and states of brain functioning and information processing. For example, Fingelkurts et al. found that the EEG microstates (transient, patterned, quasi-stable states or patterns of an electroencephalogram lasting from milliseconds to seconds) and spectral patterns of EEG (i.e. the repertoire, duration and oscillatory type of EEG microstates in resting) were quantitatively related to level of conscious expression in brain-damaged patients and healthy/ conscious subjects (Fingelkurts et al., 2012). In the same study, it was shown that patients with

disorders of consciousness had EEGs characterized by lack of fast-alpha-rhythmic spectral pattern types (normal>=MCS>VS) and predominance of delta- and theta-rhythmic spectral pattern types (VS>MCS). It was suggested that, EEG fast-alpha-rhythmic microstates were necessary for full-fledged consciousness and may be considered a minimally sufficient neural condition for consciousness to be expressed.

2.1.2 Sleep EEG/ Polysomnography

Sleep causes distinctive changes in EEG. Therefore, EEG recordings can be extended to longer periods to capture electrical activity of brain during wakefulness and sleep. Depending on the purpose of the study, the study may be performed overnight, over 24 hours, or even, for few days continuously. In addition to EEG, further data is usually obtained via electromyography (EMG), electro-oculography (EOG) and respiratory muscle (plethysmography) electrodes.

Stages of sleep can be identified by examining EEG recordings during sleep. Based on the EEG features (waveforms, EMG changes and eye movements), sleep can be divided into two categories: non-rapid eye movement (NREM) and rapid eye movement (REM) sleep.

NREM sleep then further divided into three subcategories (stages 1, 2, 3 & 4) which are roughly representative of the depth of the sleep. For example, while arousal threshold is very low in stage 1 (light sleep); in stage 3 & 4 it is the highest (state of deep sleep). Since the publication of the American Academy of Sleep Medicine (AASM) Manual in 2007 (Silber et al., 2007), stages 3 & 4 of the sleep are combined as stage N3. (Please note: from here on, both the terms of stage 3 & 4 and stage N3 will be used to keep in line with the terminology of literature referred.) The EEG features of NREM sleep include sleep spindles, K-complexes (stage N2) and high-voltage slow waves (stage N3).

REM sleep is characterized by typical EEG desynchronization, muscle atonia and episodic bursts of rapid eye movements. This is the state of a sleep where dreaming occurs. In other words, REM sleep is a state where brain is active, and body is almost completely paralyzed.

EEG features of different sleep stages are shown in figure 2.2. Time spent in different stages of sleep in healthy adults and EEG, EOG, EMG characteristics of sleep stages in healthy adults (Attarian, 2012) are summarized in figure 2-3 and table 2-3.

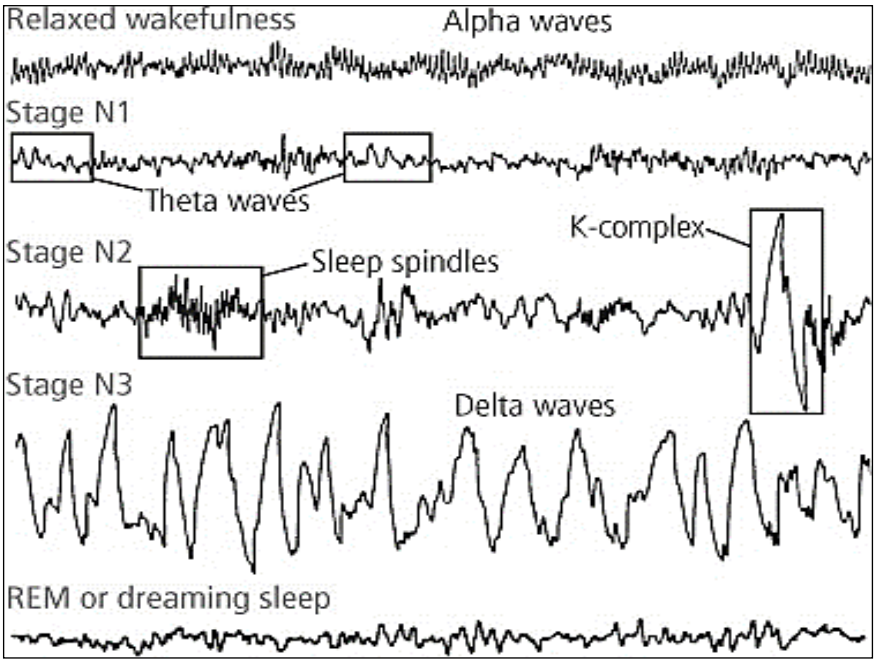


Figure 2-2: EEG features of different sleep stages (Modelling the variability of electrical activity in the brain - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Brain-activity-during-different-sleep-stages-The-detection-of-transient-sleep-spindles_fig7_280647883)

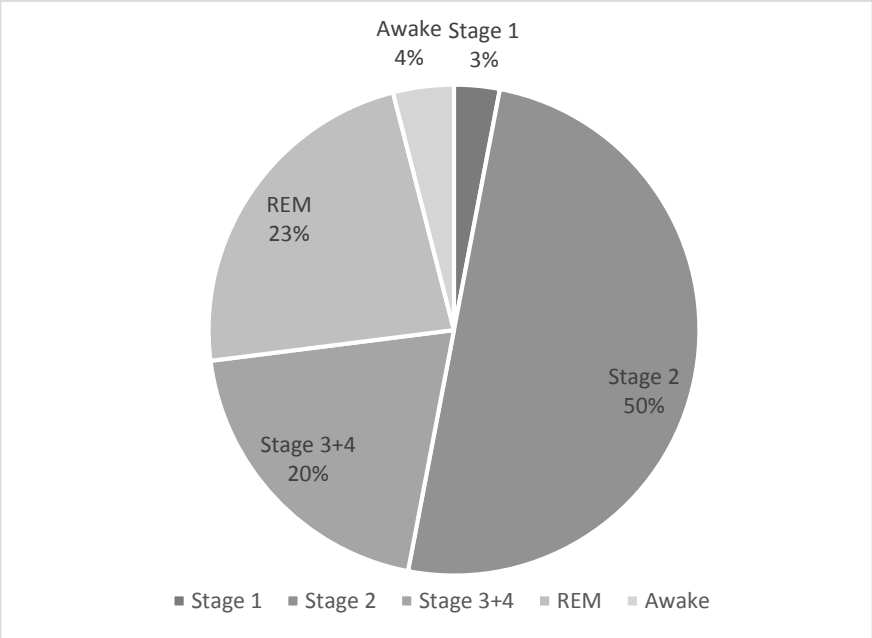


Figure 2-3: Night sleep- percentage of time spent in different sleep states

Table 2-3: Sleep stages observed during sleep EEG and their characteristic features. (TST= Total Sleep Time, EOG=Electrooculography, EMG= Electromyography)

Sleep Stage	% of TST	EEG characteristics	EOG	EMG
Stage N1 "Drowsiness"	3-5 %	Attenuation of posterior dominant alpha activity with overall slowing of background frequencies to theta (4-7 Hz) for more than 50% of the epoch. Slow-rolling eye movements. Sometimes vertex sharp waves.	Slow	Decreased from awake
Stage N2	50-60 %	Sleep Spindles: series of distinct waves with frequency of 11-16 Hz, duration ≥0.5 seconds, usually over central regions. K Complexes: Well-defined negative sharp-wave, immediately followed by a positive component, duration ≥ 0.5 seconds, mostly over frontal regions. Background EEG has less than 20% delta activity.	None	Decreased from awake
Stage N3 /4 "Deep Sleep"	10-20 %	High amplitude (≥75 μV) and slow frequency (0.5-2 Hz) delta activity. Stage 3 has delta activity in 20-50% of the epoch. Stage 4 has delta activity more than 50% of the epoch.	None	Decreased from awake
REM "Dreaming"	10-25%	Low amplitude and mixed frequency activity in EEG channels; presence of rapid eye movements; low muscle to in the chin EMG channel. Saw-tooth theta waves may be present.	Rapid	Nearly absent

Following evaluation of sleep EEG epochs by a neurophysiologist, a polysomnography report is produced, and values are given for several parameters in addition to description of EEG waves and stage data. The most commonly reported sleep parameters are (Pressman, 2000):

- Total Recording Time (TRT): Total amount of time in minutes, during which the patient is in bed with recording equipment activated.
- Total Sleep Time (TST): Total amount of sleep time scored during TRT.
- TST= Stage 1 + Stage 2 + Stage 3 + REM Sleep.
- Sleep Efficiency (SE): Percentage of Total Recording Time which actually spent in sleep.
  SE= (TST/ TRT) x 100

TST and SE increase with use of hypnotic and sedative medications, during rebound sleep and with lack of usual time cues.

TST and SE decrease with recent intake of alerting medications, caffeine or nicotine, anxiety and depression and environmental disturbances.

- Total Wake Time (TWT): Amount of wake time during TRT. This does not distinguish multiple short periods of wakefulness from long and sustained periods of wakefulness.
- Wake After Sleep Onset (WASO): Wakefulness occurring after defined sleep onset. A good measure of how much wakefulness occurred during sleep and hence, a better reflection of sleep disruption.
- Sleep Latency: Time in minutes from light out to the first epoch scored as sleep.
- REM Latency: Time from sleep onset to the first epoch scored as REM sleep.

Although, the wakefulness and presence of sleep–wake cycle is an indicator of emergence from coma and is part of the definition of the disorders of consciousness, recent studies have demonstrated absence of

electrophysiological characteristics of normal sleep in PDOC patients For example, it has been shown that the presence of EEG sleep spindles and REM sleep is highly correlated with better clinical scores and that more structured sleep is associated with more positive clinical outcomes (Arnaldi et al., 2016, de Biase et al., 2014, Aricò et al., 2016). The topic of sleep EEG/ polysomnography in disorders of consciousness will be reviewed in depth in chapter 4.

2.1.3 Event Related Potentials (ERPs)

Event Related Potential experiments investigate brain responses to specific stimuli such as auditory tones, words or pictures. Paradigms may be designed as passive paradigms which do not require any active involvement of the subject; or as active paradigms which the subject is required to perform a task, such as counting a target word in a list of other words presented during the experiment. During active ERP paradigm experiments the subjects are asked to perform some tasks to detect violations of auditory or visual regularities.

Event related potential techniques have the advantage of having very good (1ms or better) temporal resolution which cannot be achieved by hemodynamic investigations like fMRI or PET scanning. Voltages recorded at the scalp by using the ERP technique reflect what is happening in the brain almost at the same moment of time, due to the speed of electrical conduction. On the other hand, spatial resolution is ERP technique is not well-defined. In other words, we cannot definitively localize the neural generators of ERP effects within the head from the data recorded outside of it (Woodman, 2010).

ERPs reflect post-synaptic potentials which last up to hundreds of milliseconds rather than action potentials which last only about a millisecond. Post-synaptic potentials on the other hand, are largely confined to the dendrites and cell body. These factors allow post-synaptic potentials to summate rather than cancel each other- making measurement of voltage from distant scalp

electrodes possible. Another advantage of the ERP technique is its ability to provide continuous measure of neural processing.

Following a stimulus several ERP waveforms are produced. These waveforms are described according to their latency and amplitude. ERP waveforms are named according to their voltage deflections (positive [P] and negative [N]), followed by a number which indicates its latency. For example, N100 is a negative ERP waveform which occur approximately 100ms after the stimulus.

The early waves (waveforms produced within the first 100 milliseconds after stimulus) are termed ‘sensory’ or ‘exogenous’ as they depend largely on the physical parameters of the stimulus (Sur and Sinha, 2009).

For example, following an auditory stimulus, within the first 10ms auditory brainstem responses occur and they are useful for assessing auditory pathology. Brainstem auditory responses are followed by mid-latency components between 10 and 50ms which most likely arise from the medical geniculate nucleus and primary auditory cortex. These are then followed by the auditory P1 wave at 50ms which are the largest at frontal electrode sites and N1 wave which peaks around 100ms.

The N2 waveform is observed in response to a repetitive, non-target auditory stimulus (stimulus detection). If deviant tones are presented, a larger amplitude N2 wave is elicited (Simson et al., 1977).

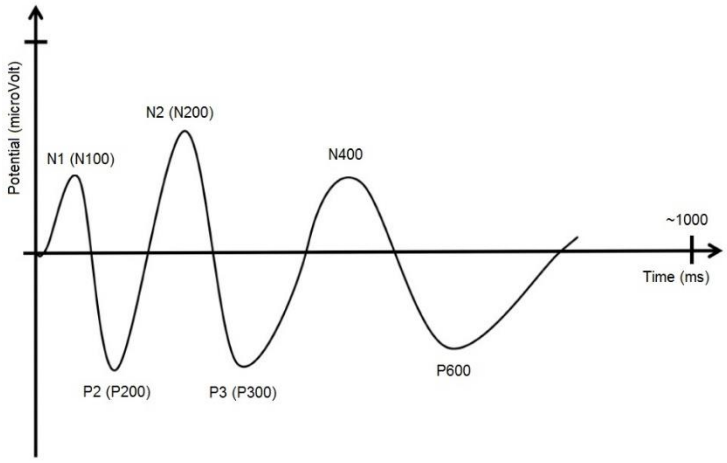


Figure 2-4: ERP waveforms following auditory stimuli. Please note that the ERP is plotted with negative voltages upward, a common, but not universal, practice in ERP research. (redrawn based on available creative commons images)

ERPs generated in later parts reflect the manner in which the subject evaluates the stimulus and are termed 'cognitive' or 'endogenous' ERPs as they examine information processing (Sur and Sinha, 2009). Mismatch Negativity (MMN) and P300 waveforms are examples of cognitive ERPs and can be generated in response to both auditory and visual stimuli.

ERP and MMN studies can be designed to measure brain responses to different types of stimuli. Due to the nature of their clinical presentation patients with disorders of consciousness are unlikely to perform tasks during the experiments using visual stimuli. Therefore, experiments using auditory stimuli are more commonly used in DOC research.

The MMN is observed when subjects are exposed to identical stimuli repetitively with occasional mismatching stimuli. The mismatching stimuli is expected to elicit a negative- going wave at central midline scalp sites reaching to its peak between 160 and 220ms. MMN is observed best if the subject is not using the stimulus stream for the task in other words if there is a competing sequence of stimuli. MMN reflect a reasonably automatic response that compares the incoming stimuli to a sensory memory trace of another stimulus which was previously presented.

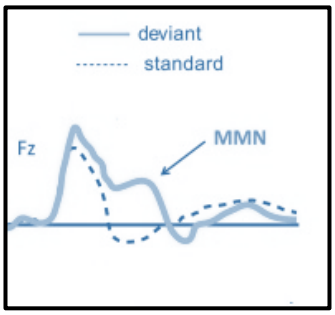


Figure 2-5: Mismatch Negativity (MMN) (Adapted from Sams et al. (Sams et al., 1985)

MMN is regarded as an indicator of pre-attentive sensory memory process. In the recent years several studies examining MMN in disorders of consciousness uniformly have suggested that MMN has potential value in predicting clinical improvement in patients with PDOC, as it may indicate presence of preserved neuronal connections (Qin et al., 2008, Boly et al., 2011, King et al., 2013, Wijnen et al., 2007, Morlet and Fischer, 2013, Fischer et al., 2010).

The P3 (or P300) entirely depends on the task performed by the subject and is not directly influenced by the physical properties of the stimulus presented. P3 is sensitive to target probability. The P3 amplitude gets larger if target probability gets smaller. For example, if the target letter A is presented on 10 percent of the trials and the non-targets are the rest of the letters present in the alphabet, the target will elicit a very large P3 wave. This is most likely due to allocation of more resources to the task. The P3 wave amplitude also increases when subject dedicate more effort to the task and gets smaller if the subject is not sure of whether the stimulus was a target or not. Therefore, the difficulty of task needs special consideration when designing the experiments. For example, a very difficult task may lead to devote more effort to the task and therefore, may increase the amplitude but it may also result in decrease on P3 amplitude by making subjects less certain.

The P300 can often be considered to include two distinct subcomponents: the P3a and the P3b. P3a is frontally centred, usually peaking at 250–300 ms and known to be elicited in a “bottom-up” manner, by novel, unpredictable stimuli,

even if they are irrelevant to the task being performed. P3b is a more posterior waveform, peaking at around 300–350 ms and is thought to be elicited in a "top-down" manner, as a result of response to paying selective attention to stimuli- deemed as task-relevant, and their subsequent entry to conscious awareness and working memory (Chennu and Bekinschtein, 2012).

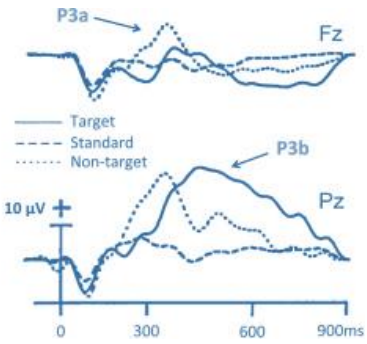


Figure 2-6: P300 waveform. Adapted from Comerchero et al. (Comerchero and Polich, 1999)

N400 waveforms are generated as a result of semantic processing and N600 waveforms are as a result of syntactic processing. These two waveforms are rarely examined in PDOC research studies due to complexity of cognitive processing required to produce these waveforms.

The MMN and P3 ERP components provide objective information on the functional state of the brain without the need to any active participation (Morlet and Fischer, 2014).

Several studies showed that presence of MMN responses is a predictor of awakening and functional outcomes in comatose patients (Kane et al., 1993, Fischer et al., 2006, Fischer et al., 2004, Luaute et al., 2005). The interest then was extended to the patients in vegetative and minimally conscious states. It was found that presence of MMN was associated with better clinical outcomes and also may be used as an additional tool to differentiate VS and MCS (Kotchoubey, 2007, Kotchoubey et al., 2005, Wijnen et al., 2007).

P3 responses obtained during novelty oddball paradigms may help to predict outcomes in comatose patients, and also, to differentiate VS and MCS patients by enabling detection of islands of cognition (Fischer et al., 2008, Fischer et al., 2010). Schnakers et. al. examined P3 responses to subjects' own name stimuli in both active and passive conditions and showed that MCS patents had a larger P3 to own name stimuli, even larger in active conditions. In this study, all healthy subjects, all MCS patients and, 3 out of 5 VS patients had P3 responses in passive conditions with no difference to the responses in active conditions. The authors suggested that active own name ERP paradigms may help to detect voluntary brain function in patients with PDOC (Schnakers et al., 2008).

ERP techniques seem to have a significant prognostic and diagnostic role in coma and disorders of consciousness.

2.2 NEUROIMAGING TECHNIQUES

Structural MRI studies can give valuable information about the extent of the brain damage, especially when combined with additional imaging techniques such as diffusion tensor imaging (DTI), which provides additional information on the state of the brain's white matter tracts, which are not usually visible on conventional neuro-imaging techniques, such as computerized tomography and MRI. DTI technology is based on the molecular diffusion physical principle: any molecule in a fluid is randomly displaced by means of thermal energy (Brownian motion) (Cavaliere et al., 2014). DTI technique has been most commonly used to assess white matter tracts after a traumatic brain injury and brain ischaemia. In the recent years, however, the role of DTI has been extended to developmental disorders and even to psychiatric illnesses. However, DTI scanning is not only susceptible to artefacts from gradient hardware and motion, but also carries significant limitations for the interpretation of the images regardless of the robustness of the tracking algorithms used (Le Bihan et al., 2006). There is a growing interest in using DTI technique to explore the pathophysiological basis of DOC as well as to

monitor change (Bruno et al., 2011a, Fernandez-Espejo et al., 2011, Fernandez-Espejo et al., 2010). Despite the current technical difficulties associated with DTI, it has a potential to be used more frequently in the future for assessment of pathophysiology and progress in DOC.

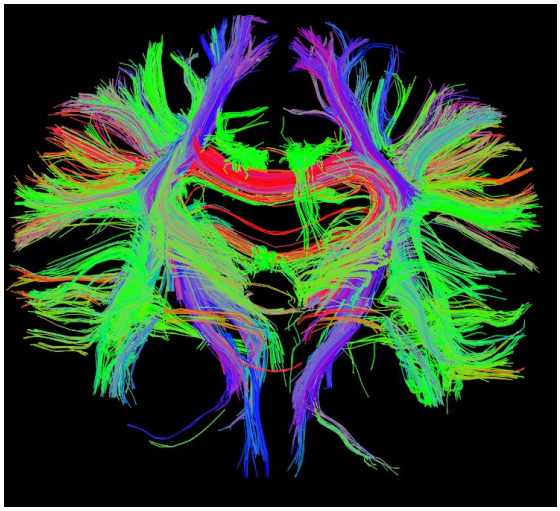


Figure 2-7: A DTI imaging showing white matter tracks. (from http://www.martinos.org/neurorecovery/technology.htm)

Single photon emission tomography (SPECT) and positron emission tomography (PET) are nuclear medicine scintigraphy techniques which enable assessment of regional brain function based on its metabolic activity. Both are non-invasive techniques. Compared to SPECT technology, PET offers a higher spatial resolution and the possibility of true quantitative assessment of physiological processes (Beuthien-Baumann et al., 2005).

Positron emission tomography (PET technique is based on the detection of radioactivity emitted after injection of a small amount of radioactive tracer (usually labelled with oxygen-15, fluorine-18, carbon-11, or nitrogen-13) (Berger, 2003). The brain uses glucose for its energy metabolism. Therefore, 18-fluorodeoxyglucose (18F FDG) is a commonly used radiolabelled glucose analogue which measures of glucose consumption rate in the tissues. PET scans may also be used to measure the blood flow and oxygen consumption

in different parts of the brain (stroke and dementia evaluation) as well as by tracking chemical neurotransmitters (for example tracking dopamine to evaluate Parkinson's disease).

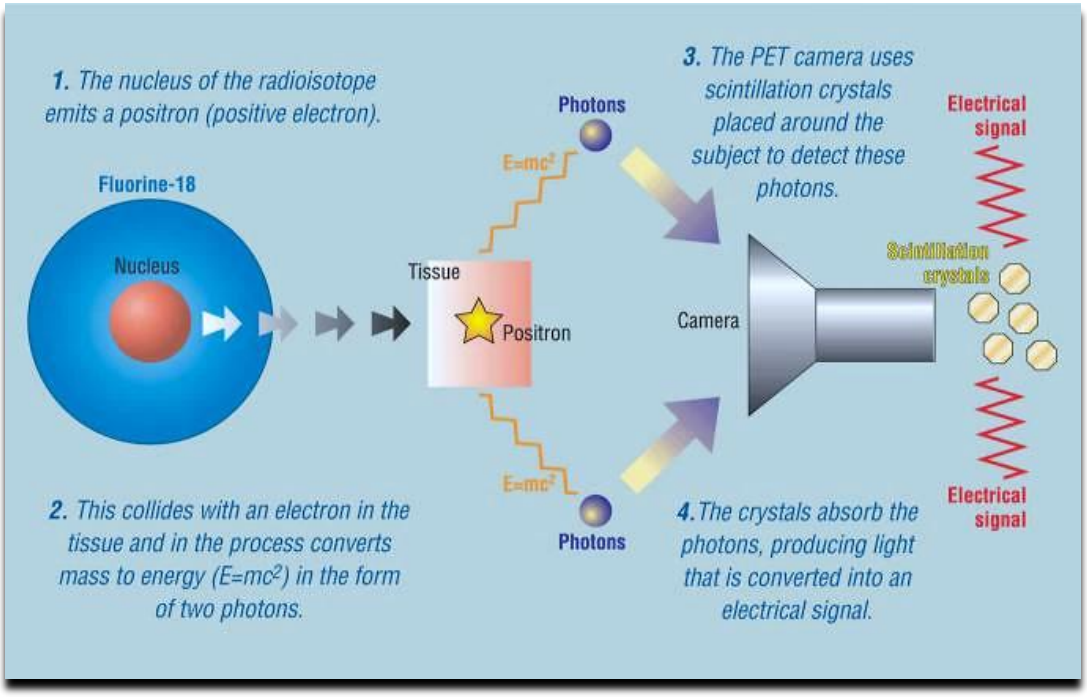


Figure 2-8: PET scanning principles (from Berger,2003)

Using 18F FDG, it has been demonstrated that in VS there is a widespread metabolic dysfunction. On the other hand, in MCS the thalamus and intrinsic network (also known as Default Mode Network (DMN) and based on midline fronto-parietal networks) is impaired (see chapter 1 for further information on intrinsic network). As the consciousness levels increase, the volume of dysfunctional structures decreases (Thibaut et al., 2012).

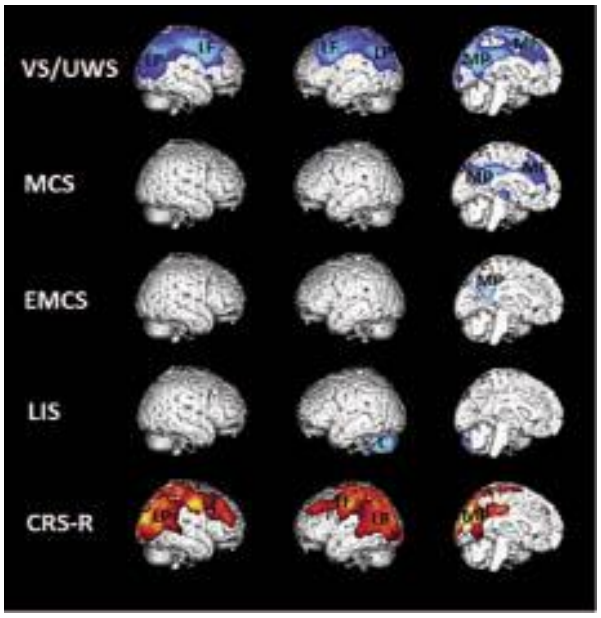


Figure 2-9: Metabolic activity in external and internal awareness networks in severely brain-damaged patients (From Thibaut et al. J Rehabil Med 2012; 44: 487–494)

PET scans may also help to differentiate reflex and non-reflex behaviors in disorders of consciousness. For example, in a study performed by Bruno et.al. it was concluded that visual fixation is not a sign of consciousness as VS patients with and without visual fixation had identical brain metabolism in their PET scans (Bruno et al., 2010).

In another study $H_2{}^{15}O$ PET blood flow studies were used to study pain processing in 18 VS patients. In this study, noxious somatosensory stimuli activated midbrain, contra-lateral thalamus and primary somatosensory cortex in all patients even in the absence of detectable cortical evoked potentials. In healthy controls however, the noxious stimuli activated both primary and secondary somatosensory cortices as well as bilateral insular, posterior parietal and anterior cingulate cortices. Therefore, it was suggested that primary somatosensory cortex was activated VS patients, but this was functionally disconnected from 'higher order' associate cortical areas (Laureys et al., 2002).

Despite of the promising studies PET scanning has significant limitations. First of all, as it is a tomography technique, PET scanning expose patients to radiation (similar to the amount of radiation exposed during computerized tomography scans) and therefore repeated investigations often cannot be instigated. There are also difficulties in relation to lack of statistical criteria for disorders of consciousness patients as this requires group studies which are difficult to perform in heterogeneous and small patient population of disorders of consciousness (Coleman et al., 2009).

Functional MRI studies have attracted the attention of clinicians and neuro-scientists who are interested in the topic of detecting covert signs of consciousness in PDOC patients or in patients who actually suffer from complete locked-in syndrome and cannot show any motor sign of consciousness. fMRI data can be obtained in both resting conditions and also during active paradigms where patients are presented with stimuli and tasks to perform.

Resting fMRI can measure the brain's default activity looking at spontaneous haemodynaemic fluctuations in blood-oxygen-level-dependent (BOLD) signal in specific areas of the brain. For example, internal self-related thoughts result in activation in midline cortical structures and external sensory perceptions result in activation in lateral fronto-parietal areas. Based on this principle, Boly et al. were able to show that VS patients had partially preserved 'default mode network' connectivity but none in brain death patients (Boly et al., 2009). In another study, it was shown that default network connectivity reflected the level of consciousness in non-communicative brain damaged patients (Vanhaudenhuyse et al., 2010).

On the other hand, active paradigm fMRI scans are used as almost like a brain-computer interface technique using motor and spatial imaginary tasks. Functional MRI motor and spatial imaginary tasks were previously validated in healthy control subjects. These tasks are associated with distinct activity in the

supplementary motor area and the parahippocampal gyrus. In a study which used fMRI motor and imagery tasks, 5 out of 54 patients (four VS, one MCS) were wilfully able to modulate their brain activity. One patient was even able to apply the imaginary technique in order to answer yes/no questions (Monti et al., 2010).

These results suggest that different structural and functional imaging modalities (especially when used in combination) may provide a powerful means for reaching diagnosis and prognostic prediction in PDOC patients.

Near infrared spectroscopy (NIRS) is another non-invasive bed side technique which measures changes in brain tissue oxygenation and recently was used to detect residual cognitive functions in patients with prolonged disorders of consciousness (Kempny et al., 2016). This study demonstrated the feasibility of using NIRS for the assessment of brain function in PDOC patients using a motor imagery task however, further studies are needed to form an evidence base.

2.3 EEG TECHNIQUES VERSUS NEURO-IMAGING

Figure 2-10: EEG system (creativecommons.org/licenses /by/2.0/deed.en_GB)

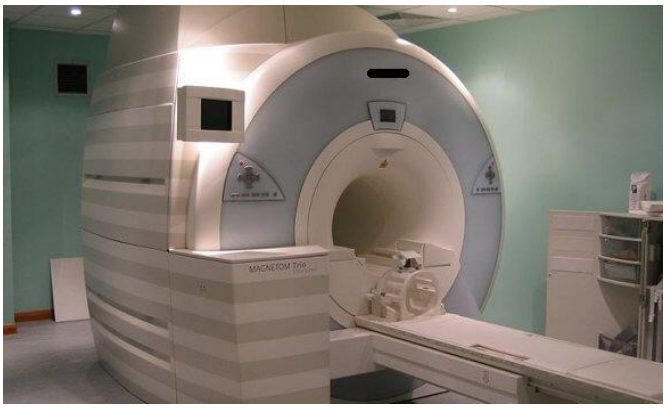


Figure 2-11: MRI scanner (creativecommons.org/licenses /by/2.0/deed.en_GB)

We have seen that, there have been significant amount of research using different fMRI or PET techniques in order to detect covert consciousness in

patients who appear to be in vegetative state on the basis of clinical assessments. As shown in Figure 2-11 the equipment required to perform neuro-imaging tests are large pieces of equipment only which can be housed in neuro-radiology departments. Due to cost and maintenance issues they are not widely available. Patients need to be transported to the units where equipment is available. Most patients with severe brain injuries have contraindications and/or patient related difficulties for MRI scanning such as pieces of metal in the body, connection to clinical machines, severe involuntary movements and sometimes even severe contractures of lower limbs which can prevent going in the MRI scanner. PET and fMRI scanning procedures may result in increased stress in brain injured patients due to noise, long duration and other technological circumstances which in turn may influence the brain functional state. Tomography based techniques carry additional problem of radiation exposure.

Apart from practical difficulties, these techniques present a significant challenge of accurate mapping of responses onto available reference atlases, since complex and severe brain injuries often result in secondary atrophy and loss of both grey and white matter volume loss. They provide indirect measures of brain activity. Whereas the EEG signals recorded at single sensor location are the product of activity arising from part or parts of brain and neutral with respect to the problems of localization of primary source and volume loss.

EEG techniques have several advantages over fMRI. First of all, EEG is much less expensive than fMRI and can be used at the bedside of patients. These make it easily accessible for many centres and also, eliminate the difficulties arising from transferring patients to another centre. Even if the initial EEG recordings are sub-optimal, they can be repeated another time as once the system is set-up, the cost of running is minimal. EEG studies can be performed even in intensive care units, if necessary by using only few electrodes. Recently, with the use of cap systems which contain 32 to 256 electrodes preparation time is greatly reduced.

ERP techniques have the advantage of having very good (1 ms or better) temporal resolution which cannot be achieved by hemodynamic investigations like fMRI or PET scanning. Voltages recorded at the scalp by using the ERP technique reflect what is happening in the brain almost at the same moment of time due extremely high speed of electricity. On the other hand, spatial resolution of ERP technique is undefined as several sources are activated during an ERP experiment. ERP technique, however, is not an invasive technique and is inexpensive in comparison with hemodynamic measures.

Table 2-4: Advantages and disadvantages of neuro-imaging and electrophysiological tests

	Neuro-imaging Techniques (PET, DTI, fMRI)	**Electro-physiological Techniques (EEG, ERP studies)**
Spatial Resolution	Excellent	Poor
Temporal resolution	Poor (up to minutes)	Excellent (milliseconds)
Bedside testing	No	Yes
Equipment Cost	Up to £3 million	Up to £100k
Repeatability in practice	Possible but not easy	Easy
Direct marker of brain activity	No	Yes
Data	Based on statistics	Actual neural activity
Subjectivity to motion artefacts	High	Low
Discomfort to patient (i.e. noise/ claustrophobia)	Yes	No

2.4 CHAPTER SUMMARY AND CONCLUSIONS

Both EEG based techniques and neuro-imaging techniques can aid assessment of consciousness. Both have their own advantages and disadvantages and give information which can complement the whole diagnostic and prognostic work-up in this particular patient group. In an ideal world, the clinicians and researchers would have wanted to perform a battery of tests which can enable them to understand this peculiar condition, minimize misdiagnosis and predict clinical outcomes more precisely. However, due to several constraints which can be, patient, resource or even time related, there is often a need to choose a few amongst them, so the research and clinical questions on the table can be answered most accurately. It should also be kept in mind that most of the techniques summarized above are mainly available for research purposes and not routinely used in most clinical settings even in the most developed countries. The results presented on research papers need to be interpreted cautiously as all of them have significant limitations due to technical and analytic difficulties associated with them. As the research study presented in this thesis is about optimizing circadian rhythm and sleep of patients with disorders of consciousness, EEG techniques which reflect brain's electrical activity directly, will be most informative as they can inform both electrical activity during sleep and, brain re-activity in response to stimuli given. The next chapter will focus on regulation of sleep and circadian rhythms in humans.

Chapter 3 INTRODUCTION: REGULATION OF SLEEP AND CIRCADIAN RHYTHM IN HUMANS

The aim of this thesis is to find out if circadian rhythm and sleep-wake cycle normalization lead to improved brain functions of people with disorders of consciousness. This chapter therefore, will focus on how the sleep and circadian rhythm are regulated in normal subjects, which will then help to understand how the assessments and interventions can be planned on patients with PDOC.

Almost the entire temporal organization of human life is orchestrated by the inputs from main circadian clock which is located in the suprachiasmatic nucleus (SCN), and sleep-wake state of an individual which is regulated by the homeostatic regulatory system (Kryger et al., 2011). Inputs from peripheral circadian clocks, external environment, person's lifestyle and habits contribute to the regulation of these two systems. For example, the timing of food intake, travel between time zones, shift-work all affect the regulation of sleep- wake state and circadian rhythmicity. In this chapter, regulation of the sleep- wake state, circadian rhythm, interactions between different systems and other factors that contribute to their regulation will be summarized.

3.1 REGULATION OF SLEEP- WAKE STATE

The sleep-wake state is regulated by two mechanisms: the homeostatic regulatory system of the hypothalamus and a circadian clock. While the homeostatic regulatory system manages acute changes in sleep duration and quality, for the changes that occur in longer time frames the circadian clock intervenes. For example, if a person is acutely deprived of sleep, the homeostatic system manages the situation by initiating recovery sleep. If the sleep-wake cycle changes, in a repetitive and chronic manner, such as in the event of shift-work, the circadian system adapts to the situation and changes

how it regulates the related temporal events. Alterations to the circadian clock can lead to sleep disorders.

3.1.1 Homeostatic regulation of sleep

The regulation of sleep and arousal is dependent on the interactions between the sleep and arousal promoting systems (Ono and Yamanaka, 2017). It was shown that the ventrolateral preoptic nucleus (VLPO) and the monoaminergic cell groups form a flip-fop switch which is stabilized by orexins. (Saper et al., 2001). It was suggested that these two areas of the hypothalamus inhibit each other, which then results in promotion of stable wakefulness and sleep patterns (hypothalamic switch). The VLPO inhibits the ascending arousal regions and, is in turn inhibited by them, thus forming a mutually inhibitory system resembling what electrical engineers call a “flip-flop switch.”

3.1.2 Circadian regulation of sleep

In Latin “Circa” means about, and “dies” means day. The term of “circadian rhythm” therefore describes behavioural and physiological events that occur and repeat themselves in a period of a day/ 24 hour.

Human circadian rhythm has two control mechanisms: internal and external. Human circadian rhythm is mainly regulated by the internal clock in the SCN and environmental stimuli (time cues or zeitgebers) contribute greatly to the regulation of human circadian rhythm.

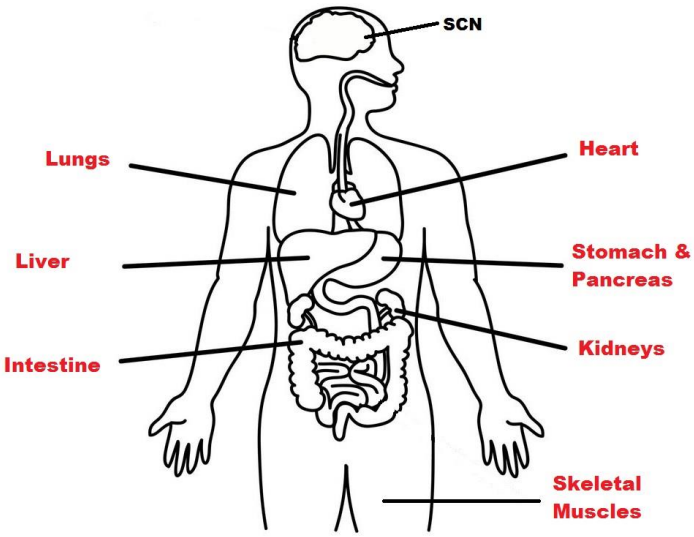


Figure 3-1: Human circadian clocks. SCN= Suprachiasmatic nucleus. The organs labelled in red represent peripheral clocks. SCN is the central/ master circadian clock.

While the internal clock consists of an endogenous component of human circadian rhythm, exogenous component is driven by the individual's lifestyle and environmental factors. Both endogenous and exogenous components interact with each other closely. Entrainment of circadian rhythm can be achieved by use of environmental factors known as zeitgebers (time-givers) such as light, temperature, social cues and rhythmic feeding (Arendt, 1995). For example, secretion of melatonin is suppressed by bright light; the levels of several hormones are modified by food ingestion. The SCN in mammals drives the circadian rhythms that entrain to the daily 24-hour light-dark cycle. It is widely believed that the SCN is the main pacemaker that orchestrates synchrony among the circadian oscillators of peripheral organs such as the liver, lungs, heart, and kidneys, as well as limbic, cerebral, and cerebellum brain (Shibata, 2007).

Most peripheral clocks of human circadian rhythm are mainly entrained by feeding cycles rather than light- dark cycles. It was shown that human

molecular clocks may be regulated by feeding time and timed meals therefore play a role in synchronizing peripheral circadian rhythms in humans. The two-way interaction between the central and peripheral clock of humans appear to be complex as food intake influences activity of enzymes and hormones involved in metabolism as well as circadian gene expression (Froy, 2011, Froy, 2007, Wehrens et al., 2017, Johnston, 2014).

3.1.3 Study of circadian rhythmicity

Chronobiology is a field of science that studies the biology of time and internal biological clocks such as circadian rhythms. Medical chronobiology is a developing field of medicine, and is concerned with two most important issues: (1) chronopathology—the effect of circadian rhythms and the manifestations of disease; and (2) chronopharmacology as many pharmacologic agents influence biological rhythms (Chokroverty, 2009).

If a person is suspected to have a long-term abnormality of sleep-wake cycles (chronic sleep problems) examination of circadian rhythmicity as well as any medications that they are on, is essential. The information gathered then informs the treatment options. The following sections of this chapter will focus on the tools that can be used in assessment of circadian rhythmicity as well as medications that can influence it, and finally what can clinicians do to resolve circadian sleep disorders.

3.1.4 Markers of circadian rhythmicity

Internal clock not only regulates the sleep rhythms but also the body temperature and endocrine cycles. Thus, assessment of human circadian rhythm can be done not only by studying sleep wake cycles but also by studying the temperature cycle and endocrine cycles (Hofstra and de Weerd, 2008).

In practice, the most widely used measures used as markers of circadian rhythm are cortisol levels, melatonin levels, and core body temperature changes.

When doing so, the following parameters are used to describe characteristics of a circadian rhythm and report chronobiological events during a cycle:

- Phase (ϕ) of a circadian rhythm refers to any point within a cycle.
- Acrophase is the phase angle corresponding to the maximal value of the rhythmic parameter studied.
- Rate of cycling (τ) characterizes the time elapsed between each successive occurrence of a phasic event.
- Amplitude refers to range of values throughout the cycle. It is the measure of one half of the extent of the rhythmic change estimated by the mathematical model (e.g., cosine curve) best fitting the data (e.g., the difference between the maximum and the rhythm-adjusted mean of the best fitting curve).
- Peak is the highest point in a series of measurements obtained as a function of time. For example, melatonin peak occurring at 2 am.
- Tau refers to the 'natural' period of a biological rhythm free-running in constant conditions.

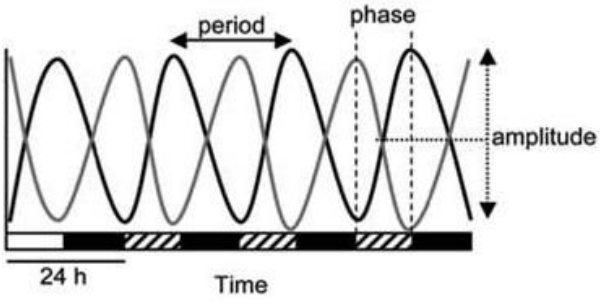


Figure 3-2: Key properties of a circadian rhythm. (Adopted from Biological rhythms workshop-1 by Khulman et. al. Cold Spring Harb Symp Quant Biol. 2007; 72:1-6)

3.1.4.1 Cortisol

Cortisol is a corticosteroid hormone that is produced in the adrenal cortex. Cortisol shows a circadian rhythmicity through multi-synaptic suprachiasmatic nucleus-adrenal pathway (Buijs et al., 1999). Its levels can be measured in

serum and saliva; however, several factors influence the secretion of cortisol such as physical and psychological stress and high-protein diet and therefore it is unlikely to be a first choice tool to assess circadian rhythms in patients with multiple medical problems and on artificial nutrition and hydration (see methods development chapter for further discussion).

3.1.4.2 Melatonin

Melatonin is a hormone, which is secreted by the pineal gland, and it plays a crucial role in regulation of circadian rhythm. Melatonin levels can be influenced by posture, exercise, caffeine and certain drugs such as non-steroid anti-inflammatory drugs (NSAIDs) and beta blockers. However, its rhythmicity remains reasonably preserved. Not only measuring levels of melatonin can be used as a marker of human circadian rhythm, it can also be given to people with circadian rhythm disorders as a treatment. It is secreted in the pineal gland which is located deep in the centre of brain. It was once called “the third eye”.

3.1.4.2.1 Pineal Gland

The existence of the pineal gland has been known for more than 2000 years- with the earliest anatomical description by Galen (130-200AD). Most remarkably, in 1662 the French philosopher Descartes thought that the pineal gland coordinated the psychophysiological functions of humans and the stimulus for pineal function arose from the visual input to the retina, which is effectively true today.

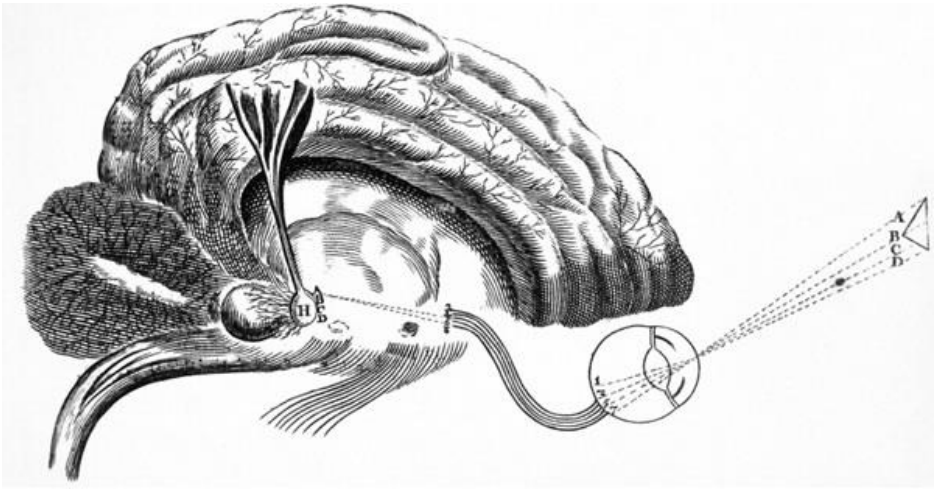


Figure 3-3: Descartes: The Nervous System. Diagram of the brain and the pineal gland,1662 (Wellcome Library, London)

The mammalian pineal gland is a small, singular, central structure with secretory function. The average adult weight of the human gland has been reported as 100mg to 150mg (Arendt, 1995). It originates as an evagination of the diencephalon and it is closely associated with the third ventricle of the brain. It has a very rich vascularization, but it lacks an endothelial blood-brain barrier enabling it to react to peripherally acting medications.

The human pineal gland consists of a central core composed of lobules and a cortex with a more diffuse distribution of neurons. The principal cell type of the pineal parenchyma is the pinealocyte. Human pinealocytes are not capable of direct light sensitivity but they retain many characteristics of pineal photoreceptors found in other species such as lizards and birds (the third eye). Another interesting histological feature of the pineal gland is the presence of calcareous deposits on both parenchymal and intercellular tissue which gives the most distinguishable radiographic characteristic of the pineal gland (Macchi and Bruce, 2004).

Sympathetic and parasympathetic fibres, as well as fibres originating from central nervous system peripherally innervate pineal gland. Sympathetic fibres

arising from the superior cervical ganglion are the most important afferents for the pineal gland. These neurons receive regulatory input from the suprachiasmatic nucleus of the hypothalamus, which receives direct input from retinal ganglion cells (European Pineal Study Group et al., 1979). Described noradrenergic sympathetic innervation of the gland is essential for the appropriate production of melatonin, which is the major hormone of the pineal gland.

Figure 3-4: Melatonin. This image brings together a timepiece, a brain and a melatonin molecule to represent the connection between melatonin, the brain and the body clock. The watch is placed more or less in the brain region of the pineal gland which is where melatonin is produced in mammals. Anna Tanczos, Wellcome Images

3.1.4.2.2 Secretion and metabolism of melatonin

Melatonin is synthesized from tryptophan in the pinealocytes of pineal gland under the control of master circadian clock located in the suprachiasmatic nucleus as illustrated below.

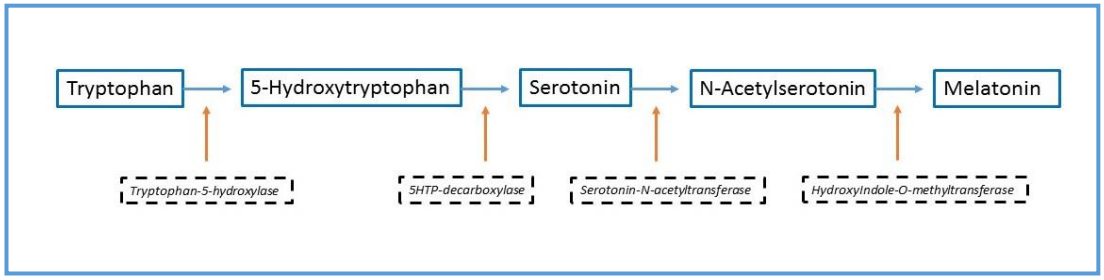


Figure 3-5: Synthesis of melatonin

Melatonin levels start to increase before sleep in the evening, peak in the early hours of the morning and decrease after waking. Hence, dim light melatonin onset (DLMO) and dim light melatonin offset (DLMOff) are commonly used as circadian phase markers. Although, within individual subjects the timing, duration and amount of melatonin secretion are very stable, there is a large variability between subjects. For example, peak saliva melatonin values ranging from 2 to 84 pg/ml in healthy subjects were reported (Burgess and Fogg, 2008).

Following the synthesis, melatonin is released into capillaries and mainly metabolized in the liver but also in the kidney. Therefore, liver and kidney pathologies alter the clearance rate of melatonin. The main metabolite of melatonin in the urine is 6-sulfatoxymelatonin which reflects the qualitative and quantitative aspects of melatonin secretion in humans (Arendt, 1995).

3.1.4.2.3 Light and control of melatonin synthesis

The most prominent regulator of melatonin synthesis is the daily changes of light and darkness. Light has both visual and non-visual effects on human physiology. Its non-visual effects include resetting the timing of circadian pacemaker- SCN, acutely improving subjective and objective measures of alertness and suppressing pineal melatonin production (Lockley and Foster). SCN not only controls the melatonin production but also receives feedback from melatonin via its melatonin sensitive receptors. This contributes to proper synchronization of internal rhythms.

Recently, in addition to the rods and cones, a third class of photoreceptor system called photosensitive retinal ganglion cells (pRGCs) were discovered. pRGCs are involved in irradiance detection and non-image-forming responses to light including pupil constriction, circadian entrainment and regulation of sleep as well as visual information processing and contain a light-sensitive pigment called melanopsin (Hughes et al., 2012). Retinal photic signals then reach the SCN through the monosynaptic retinohypothalamic tract, which originates from retinal ganglion cells (Berson et al., 2002, Hattar et al., 2002).

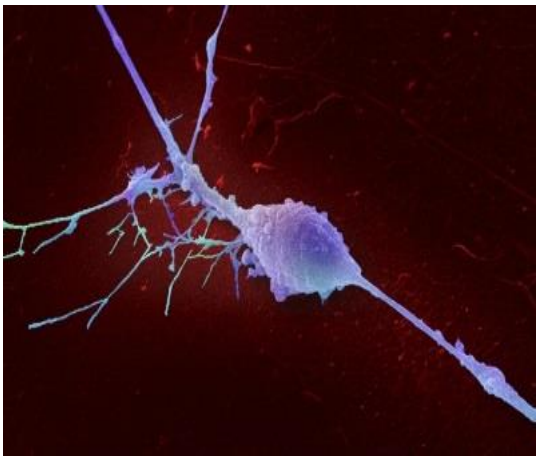


Figure 3-6: An isolated retinal ganglion cell. This is a type of neuron typically located near the inner surface of the retina of the eye that receives visual information from photoreceptors via two intermediate neuron types. Retinal ganglion cells collectively transmit visual information from the retina to several regions in the thalamus, hypothalamus, and midbrain. They vary significantly in terms of their size, connections, and responses to visual stimulation but they all share the defining property of having a long axon that extends into the brain. These axons form the optic nerve, optic chiasm, and optic tract. Annie Cavanagh, Wellcome Images

Melanopsin has a peak sensitivity of approximately 480 nm (blue light). Light intensity, duration of exposure to light, wavelength and timing of exposure are the factors that can have an effect on circadian rhythms. Light not only shifts the timing of the circadian clock but also suppresses melatonin production, reduces sleepiness and improves reaction times. In the absence of light as a synchroniser, the melatonin circadian rhythm free-runs and becomes out of phase with the external light-dark cycle. This phenomenon is clearly seen in blind individuals who completely lack light perception (Lewy et al., 2004).

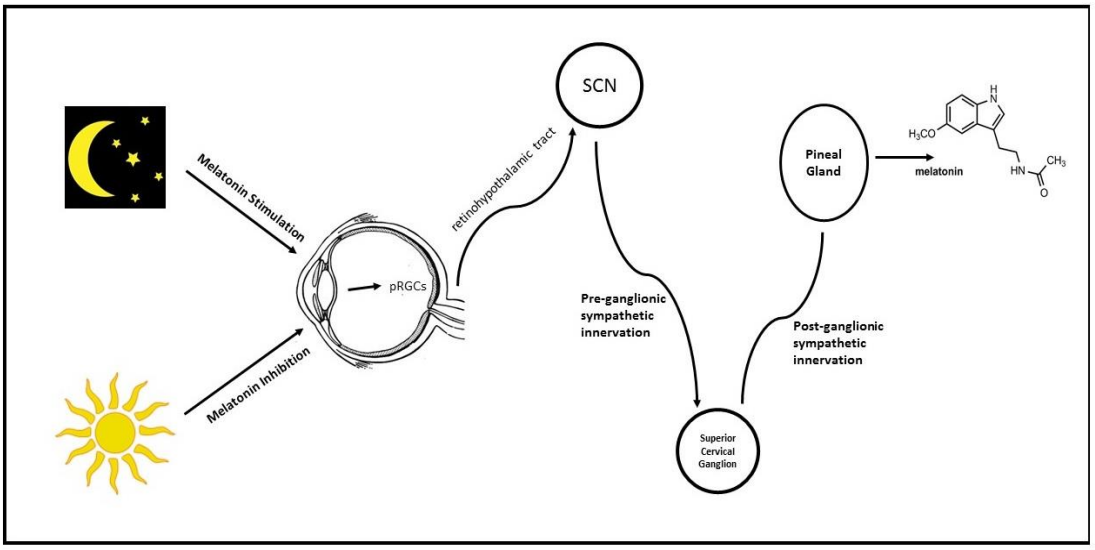


Figure 3-7: Light and the control of melatonin synthesis

3.1.4.3 Body temperature as a marker of circadian rhythm

Thermoregulatory and sleep regulatory system are driven by two independent, but closely interacting physiological principles: homeostasis and circadian system (Kryger et al., 2011).

The human body achieves stable body temperature levels by balancing the production and loss of heat. Heat loss is mainly achieved by the peripheral tissues of the body and affected by environmental temperature. Thermoregulatory distal skin blood flow is the main regulator of the heat loss. For example, to achieve a more efficient heat exchange from core to the distal skin (to increase heat loss) dilatation of arteriovenous anastomoses happen in the distal body parts which then promotes the rapid onset of sleep (Kräuchi et al., 2000).

Core body temperature (CBT) includes the temperature of the brain and other internal organs and usually regulated around 37º C. CBT displays a circadian oscillation as circadian signals are transmitted from suprachiasmatic nucleus to the pre-optic anterior hypothalamus which is responsible for

thermoregulation. The interaction between the hypothalamic thermoregulatory and suprachiasmatic circadian systems was described by Moore et. al. in 2002. It was shown that suprachiasmatic nucleus makes three projections to the different parts of hypothalamus: dorsal projections to the paraventricular hypothalamus which control pineal melatonin production; rostral projections to the anterior hypothalamic/preoptic areas which mediate the CBT rhythm and finally caudal projections to the subparaventricular zone and hypothalamic arousal systems located in the posterior and lateral hypothalamic areas which control the rhythm in rest-activity (Moore and Danchenko, 2002).

CBT exhibits a daily decline in the evening and rise in the morning. Distal skin temperatures on the other hand, show an inverse circadian rhythm activity in comparison to the of core body temperatures. In addition to this inverse relation between the circadian rhythms of core and peripheral body temperature, it was shown that peripheral circadian activity is ahead about 100 minutes (Krauchi and Wirz-Justice, 1994). Distal-proximal skin temperature gradient provides a selective measure of distal skin blood flow and again shows an inverse circadian activity of the CBT under constant routine conditions (constant bed rest, no sleep, regular food and drink consumption). Proximal skin temperatures (thigh, infraclavicular area, abdomen, and forehead) follow a similar change to CBT (Krauchi and Wirz-Justice, 1994) (see figure 3.8). A change from standing position to supine position induces redistribution of heat from core to the peripheral tissues and therefore, increase of skin temperatures, decrease of CBT and increase of sleepiness in healthy subjects (Krauchi et al., 1997).

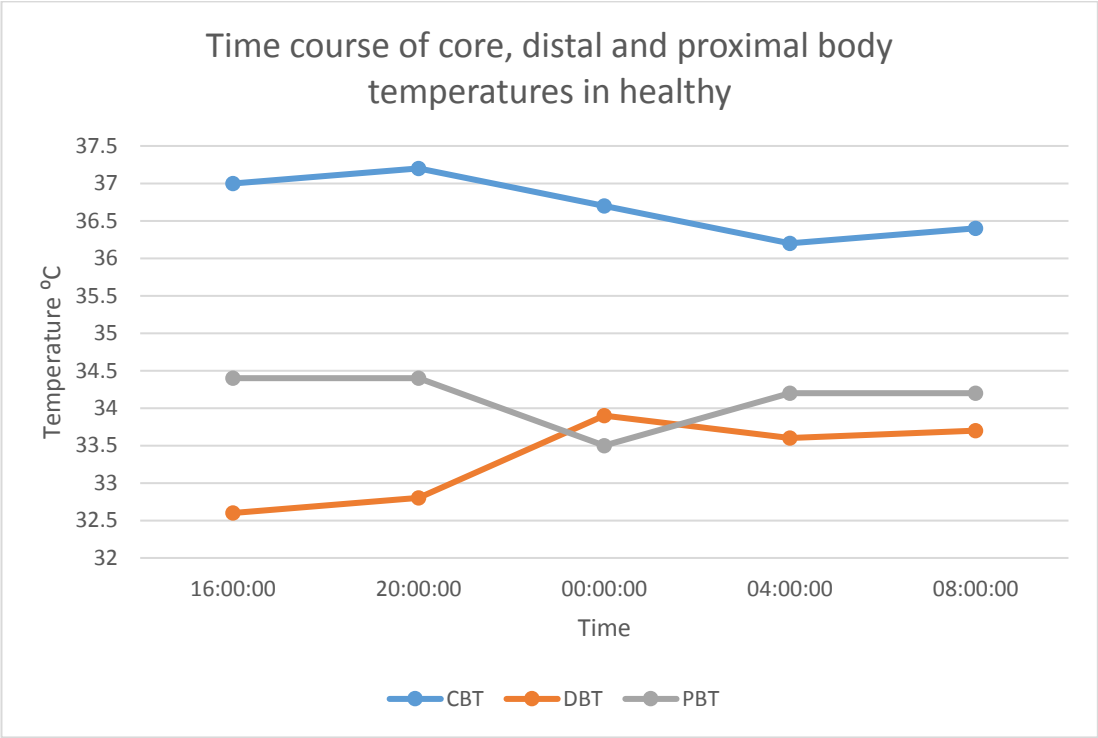


Figure 3-8: Simple representation of time course of core, distal and proximal body temperatures (Adapted from Krauchi K et al. Functional link between distal vasodilatation and sleep-onset latency? Am J Physiol Regul Integr Comp Physiol. 2000; 278 (3)) CBT= Core Body temperature, DBT= Distal Body Temperature, PBT= Proximal Body Temperature

3.2 REGULATION OF SLEEP, PHYSIOLOGICAL AND PATHOLOGICAL CHANGES OF SLEEP

3.2.1 Regulation of sleep

Regulation of sleep is complex. Interactions between the homeostatic and endogenous circadian processes, habits and behavioural factors as well as environmental factors influence sleep regulation. Abnormality of one or more of these factors may lead to temporary dysregulation of sleep and circadian rhythm sleep disorders if they are not normalized effectively and promptly.

This homeostatic process is influenced by prior wakefulness and plays an important role in the sleep quality. For example, the longer the person is

awake during the day, the deeper the following sleep period will be, which will be evidenced by increased slow wave activity on sleep EEG (Dijk et al., 1990).

On the other hand, the endogenous circadian process which is regulated by the internal clock in the suprachiasmatic nuclei affects sleep quantity and duration which is mostly influenced by when a person goes to sleep.

Habits and behaviours have a great impact on sleep. Examples of such habit and behaviours include going to bed at regular hours thinking that we need to get up early to go to work or resisting the urge of an afternoon nap while working at our desks knowing that it is not appropriate to do so. Daily activity and stimulation levels and consumption of stimulants such as coffee are also part of habits and behaviours. Habits and behaviours play a strong role in sleep regulation and may even take priority over homeostatic and circadian processes.

Similarly, environmental factors –zeitgebers whether they are part of habits or behaviours or occurring because of events, which are not in the control of individuals, form external circadian factors and affect sleep regulation in great extent.

All of the factors listed above impact on sleep regulation. In order to have a well-timed and high quality and quantity sleep a person needs to meet the following requirements:

- To have intact neural structures and pathways to regulate circadian rhythm and sleep regulation,
- To be able to keep awake for a substantial amount of time during the day in order to reach high quality sleep at night evidenced by slow wave sleep/ deep sleep activity,
- Improved habits and behaviours to facilitate function of circadian and homeostatic factors,

- Optimized environmental factors/ external zeitgebers in order to enhance external control of circadian rhythm.

3.2.2 Normal sleep

The Oxford dictionary defines sleep as a condition of body and mind, which typically occurs several hours every night, in which the nervous system is inactive, the eyes closed, the postural muscles relaxed, and consciousness is practically suspended. In simple terms, it is a reversible state of perceptual disengagement with the environment.

Normal human sleep includes two states: rapid eye movement (REM) sleep and non-REM sleep. These two states alternate during sleep and both can be identified on EEG recordings due to their distinctive EEG features (see 2.1.2 for details). Normal sleep starts with NREM state, followed by the first REM sleep state, usually occurring 80 to 100 minutes after sleep onset. Thereafter, NREM & REM sleep cycles repeat themselves roughly every 90 minutes. During a night's sleep, healthy adults go through 4 to 6 cycles. Slow-wave sleep predominates in the first third of the night and REM sleep predominates in the last half of the night.

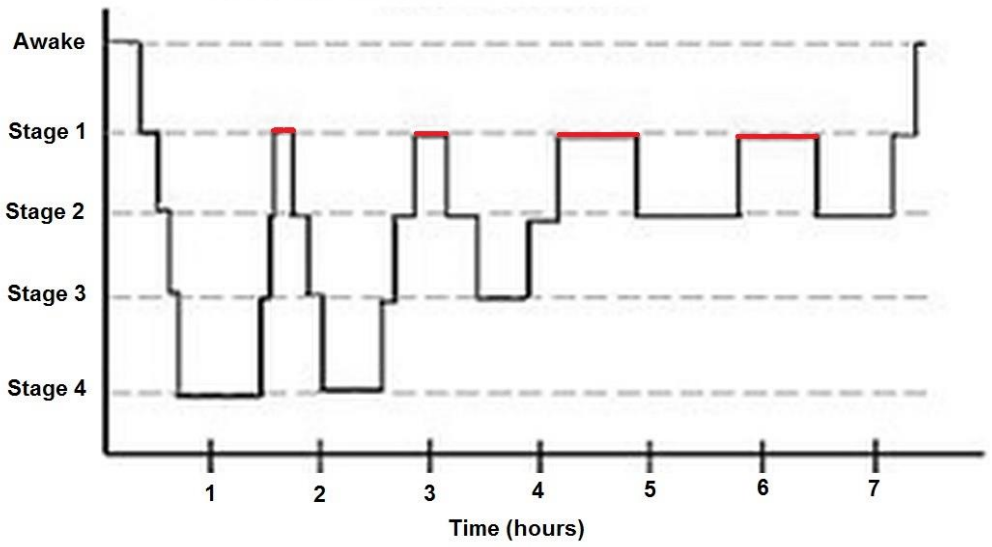


Figure 3-9: Alternating sleep stages during night sleep. REM sleep is shown with red lines.

Identification of sleep onset is not straight forward as very often EEG patterns are not associated with a healthy subjects' own reports. For example, while polysomnography and behavioural changes suggest onset of sleep, subjects report that they were awake (Kryger et al., 2011). At the sleep onset, the electromyography (EMG) shows gradual loss of muscle tone, electrooculogram (EOG) shows slow, asynchronous eye movements; and the EEG shows clear rhythmic alpha activity mainly located on the occipital region or EEG features of stage 1 sleep which is characterized by low-voltage, mixed frequency EEG pattern. Even the observation of stage 2 sleep on PSG may not be perceived as sleep by subjects (Agnew and Webb, 1972). This mismatch between the self-reports and PSG feature may be explained by the fact that humans most often drift in and out of arousal at the beginning of sleep.

Sleep stage distribution may be influenced by age, quality of sleep during previous night, circadian rhythm disorders, environmental temperature, ingestion of medications, alcohol and drugs and sleep disorders such as narcolepsy, sleep apnoea and other medical conditions, which lead to increase of sleep fragmentation.

3.2.3 REM sleep and dreaming

Dreaming has been described as (Kryger et al., 2011);

"a universal human experience occurring during sleep in which fictive events follow one another in an organized, story-like manner and into which are woven hallucinatory, primarily visual, images that are largely congruent with an ongoing confabulated plot."

Although some dreaming occurs in NREM sleep, the majority of dreaming takes place during REM sleep when intensive gamma frequency (30 to 80 Hz) and fast brain oscillations are recorded (Gross and Gotman, 1999). During REM sleep a significant increase of neural activity was shown in subcortical regions of the brain as well as visual processing areas of the cortex accompanied by relative deactivation of lateral prefrontal cortex (Maquet and

Phillips, 1998, Maquet et al., 2005). It was also shown that, REM sleep dreaming exhibits default mode network processing but with some disconnection between network subsystems (deactivation of dorsomedial/posterior structures and activation of ventromedial/ prefrontal structures) and therefore, a good access to self-referential, emotional, cognitive memories but with a limited access to autobiographical memories occur during dreaming (Buckner et al., 2008).

While many studies using positron emission tomography and functional MRI imaging, consider the associations with dreaming and neural activity in various parts of the brain, the question of “why we dream?” continues to attract attention in the area of sleep research. REM sleep serves to help offline consolidation of memories and learning in certain domains during certain timelines of sleep (Stickgold et al., 2001, Wamsley et al., 2010, Stickgold et al., 2000, Gais et al., 2000). In humans, the first half of the sleep is dominated by NREM, and the second half by REM sleep. This enables design of research studies, where timing of different cognitive processes can be examined. For example, it was shown that while improvement of a visual discrimination skills and declarative memory correlates with early night sleep, procedural memory consolidation mainly happens during late sleep (Gais et al., 2000, Plihal and Born, 1997). Furthermore, it was suggested that dreaming is a state, where highly complex analysis and recasting of emotional events occur and there might be a positive association between REM sleep and the selective consolidation of central, negative aspects of complex scenes which helps sleeping brain to preserve emotionally significant memories (Payne et al., 2012).

3.3 HOW MUCH SLEEP DO WE NEED?

Total sleep time (TST) varies between five and ten hours in healthy young adults (300 to 600 minutes). According to a report of an expert panel seven to nine hours of sleep is recommended for adults aged 25 to 64 years and seven to eight hours of sleep recommended for older adults (≥ 65 years) (Hirshkowitz et al., 2015).

The 2007 AASM Manual for the Scoring of Sleep and Associated Events defines arousals as sudden change of EEG frequency that lasts between 3 and 15 seconds following at least 10 seconds of stable sleep. This is accompanied by increase in muscle tone. Wakefulness should not occupy more than 5% of sleep (Hirshkowitz et al., 1992). Hence, a healthy adult with good sleep is expected to have sleep efficiency of 95%, with swift sleep onset (less than 15 minutes) and have few and brief nocturnal awakenings only (Hirshkowitz, 2004).

3.3.1 Sleep deprivation

3.3.1.1 Acute sleep deprivation

Sleep deprivation may be due to total sleep loss or increased sleep fragmentation. Several studies showed that acute sleep deprivation result in apparent changes in mood, alertness, and performance (Goel et al., 2013, Lo et al., 2012, Babson et al., 2010, Pires et al., 2016). Acute sleep deprivation result in production of a recovery sleep, which shows increased slow wave sleep state followed by increased REM activity (Borbely et al., 1981, Nakazawa et al., 1978).

3.3.1.2 Chronic sleep deprivation

Chronic sleep deprivation can be due to sleep disorders as well as work and life-style conditions. Regardless of the reason for chronic sleep deprivation, it results in reduction of daytime functioning, cognitive performance, safe operating of machinery/ driving (Reynolds and Banks, 2010). It also negatively effects mood and physiological functions. In the recent years, there have been

great interests in the disturbance of immune, cardio-vascular, endocrine and metabolic functioning due to chronic sleep deprivation. For example, chronic sleep deprivation lead to decreased glucose tolerance, elevation of evening cortisol levels which then lead to development of diabetes and obesity (Spiegel et al., 2000, Spiegel et al., 2003, Spiegel et al., 1999, Van Cauter et al., 2007, Knutson and Van Cauter, 2008); reduction of immune function due to changes in natural killer cell activity, cellular adhesion molecule expression, lymphokine- activated killer cell activity, interleukin-6 and soluble tumour necrosis factor activity (Redwine et al., 2004, Redwine et al., 2000, Shearer et al., 2001, Irwin et al., 1996); and increase of cardiovascular events and morbidity (Kohansieh and Makaryus, 2015, Mullington et al., 2009, Tobaldini et al., 2017, Tobaldini et al., 2014).

3.4 EFFECT OF SLEEP ON LEARNING, MEMORY AND BRAIN PLASTICITY

In the recent years there have been several studies published suggesting that sleep plays an important role in learning, memory consolidation and brain plasticity. This is particularly important in patients with prolonged disorders of consciousness. If their circadian rhythm and sleep- wake patterns are severely deranged, would it be ever possible to make a recovery of their brain functions?

Memory consolidation is defined as a time-dependent process, which converts labile memory traces into more permanent and enhanced forms over hours, days, or even years (McGaugh, 1966). This process is facilitated by brain plasticity which can be simply defined as ability of the brain to modify its own structure and function following changes within the body or in the external environment (2012) and sleep takes part in this process. Furthermore, different stages of sleep have distinct memory related functions. Therefore, ability to maintain normal sleep structure, where different sleep stages are adequately achieved, is imperative. For example, REM sleep is necessary for synaptic re-modelling, which is essential for learning and cognition; NREM Stage 2 sleep

takes part in synaptic plasticity and targeted bidirectional plasticity in the neocortex; NREM Stage 3 &4 (slow wave-dependent activity) would serve to strengthen memory circuits and therefore consolidation of memories. In addition, while motor skills learning happens in NREM, visual learning happens mostly in slow wave sleep and REM (Stickgold and Walker, 2007, Walker and Stickgold, 2006). (see Table 3-1: Summary of different memory and learning events in distinctive sleep stages.)

Table 3-1: Summary of different memory and learning events in distinctive sleep stages.

DECLARATIVE MEMORY CONSOLIDATION	
Semantic memory	REM and Stage 2 sleep (Brualla et al., 1998, Bastuji et al., 1995, Bastuji et al., 2002, Daltrozzo et al., 2012, Perrin et al., 1999)
Episodic memory	Slow wave sleep, spindle activity (Plihal and Born, 1999, Plihal and Born, 1997, Fogel and Smith, 2006, Clemens et al., 2005)
NONDECLERATIVE MEMORY CONSOLIDATION	
Perceptual learning	Both REM and slow wave sleep (Karni and Sagi, 1991, Atienza et al., 2004, Gottselig et al., 2004)
Motor learning	Stage 2 sleep (Walker et al., 2002, Walker et al., 2003, Backhaus and Junghanns, 2006)
Priming	REM sleep (Wagner et al., 2003)

3.5 SLEEP AND AGING

Although only minor differences were found between healthy young adult men and women, sleep parameters differ significantly with aging. In a review paper published in 2017 (Mander et al., 2017), changes in sleep with aging summarized as below:

Macro sleep changes:

- Advanced sleep timing (earlier bed times and rise times),
- Longer sleep-onset latency,
- Shorter overall sleep duration,
- Increased sleep fragmentation,
- Higher likelihood of being woken by external stimuli,
- Reduced amount of deep/ slow wave sleep,
- Increased time spent in Stages 1 and 2,
- Shorter and fewer NREM-REM sleep cycles,
- Increased time spent awake during the night,
- Increased daytime naps.

Micro sleep changes:

- Reduced slow wave activity
- Reduction in amplitude and density of slow wave activity especially over the frontal lobe topography,
- Reduction in spectral power in the frequency of sleep spindles due to decline of sleep spindles density especially over frontal EEG derivations.

Sleep Quality Recommendations published by National Sleep Foundation (USA) give values for sleep continuity measures, sleep architecture measures and naps as indicators of good sleep quality among healthy individuals across the life span (Ohayon et al., 2017). Table 3-2 gives the summary of the recommendations defined as appropriate and uncertain (where there was a

disagreement between the experts) for the sleep measures of adults and older adults:

Table 3-2: Summary of National Sleep Foundation's recommendations for good sleep quality. The values in brackets represent the ranges where there was a disagreement amongst the experts.

Sleep Continuity Measures		
	ADULTS (26-64 years)	OLDER ADULTS (≥65 years)
Sleep Latency	0-30 mins (31-45 mins)	0-30 mins (31-60 mins)
Awakenings > 5 mins	1 (1-3)	2 (2-3)
Wake after sleep onset	0-20 mins (21-40 mins)	0-30 mins (31- 60 mins)
Sleep efficiency	>85% (75-84%)	>85% (75-84%)
Sleep Architecture Measures		
	ADULTS (26-64 years)	OLDER ADULTS (≥65 years)
REM Activity	21-30% (<10- 20% and 31-40%)	(<10- 40%)
N1 Sleep	<5% (6-20%)	(0-25%)
N2 Sleep	(up to 80%)	(up to 80%)
N3 Sleep	16-20% (6-15% and >26%)	(≥26%)
Naps		
Number of naps	(0-3)	(0-3)
Nap duration (mins)	(0-100)	(0-100)

Circadian rhythm changes with aging play an important role in the age-related sleep changes. In a recently published review paper, it was suggested that progressive yellowing and thickening of the lens may reduce sensitivity to light. In addition, reduction of arginine vasopressin and vasoactive intestinal polypeptide expression and fewer GABAergic synapses may decrease signalling within the SCN, leading to a decrease in the overall amplitude of its firing rhythm. As a result, a weaker SCN output signal may in turn reduce the strength of downstream oscillators in central and peripheral tissues, including the cortex, pineal gland, liver, kidney, thyroid, and spleen. Therefore, providing other zeitgebers such as scheduled meals, which act on the circadian system

via extra-SCN pathways, may help entrain an aging circadian system (Hood and Amir, 2017).

3.6 ASSESSMENT OF SLEEP

Three main techniques to assess sleep will be discussed here with their advantages and disadvantages.

3.6.1 Actigraphy

Figure 3-10: Examples of actigraphy devices (source: http://actigraphcorp.com/solutions/sleep-assessment/)

An actigraph is a small watch-like device that is worn by the individual to measure movement (gross motor activity) throughout the day using an accelerometer. It aims to assess sleep habits objectively. Interpretation of actigraphy results requires assumption of sleep period where there is lack of motion and there is overestimating sleep duration on patients who do not / cannot move when they are awake. Moreover, comparisons made between actigraphy and polysomnography have shown that actigraphy was highly correlated with PSG for differentiating sleep from wake in healthy people but not necessarily in assessment of sleep onset latencies and wake time after sleep onset. This leads to reduced reliability of actigraphy method for detecting

sleep as sleep become more disturbed (Jean-Louis et al., 2001, Jean-Louis et al., 1997b, Jean-Louis et al., 1997a, Ancoli-Israel et al., 2003, Pollak et al., 2001). The advantage of actigraphy over polysomnography is that, actigraphy can record for long periods; it is a relatively cheap equipment in comparison to polysomnography and can provide accurate information of sleep/ wake status in healthy people.

Actigraphy has also been compared to direct observations of sleep and to sleep logs. It was found that both self-completed sleep logs and actigraphy yielded similar data for sleep timing, sleep duration, sleep onset and sleep offset but not for sleep latency, number and duration of awakenings or number of naps (Lockley et al., 1999). Nursing observations of sleep in psychiatric patients as well as the research staffs' observations of nursing home residents showed that direct observations and actigraphy results were similar when assessing sleep wake status (Ancoli-Israel et al., 1997, Krahn et al., 1997).

Although the role and validity of actigraphy is well documented in sleep/ wake scoring in healthy population; using actigraphy has its own challenges in specific populations such as people with tetraplegia. A study published by Spivak et al. (Spivak et al., 2007) showed that comparable results to controls were obtained with placing actigraph on the forehead of patients with C4 tetraplegia. However, patients with disorders of consciousness are usually tetraplegic and have very poor head control. When in bed or in their wheelchair they require several positioning and postural management equipment such as head straps, pillows, splints and other support apparatus to keep them in comfortable and in a safe posture. These equipment at the same time may limit spontaneous movements. Actigraphy recording would likely to contain some levels of exogenous activity due to re-positioning in bed, hoisting, moving limbs during personal care and therapies. Moreover, patients with disorders of consciousness often have spontaneous movements, which may not necessarily be indicative of synchronized cerebral waking.

3.6.2 Sleep logs

Individuals who have sufficient cognitive, communicative and physical abilities can complete sleep logs. In healthcare institutions, it can also be completed by the nursing/ care staff by direct observation of patient and identifying sleep/ awake status using external behavioural signs such as eyes open/ close, change in respiration and body positioning, snoring etc. However, like actigraphy this method carries risk of overestimating sleep (i.e. when eyes are closed despite being awake) and is less specific when considering sleep parameters such as sleep latency, sleep onset, sleep offset data.

3.6.3 Polysomnography

Polysomnography is the most reliable measure of sleep as it provides detailed information on sleep measures including sleep stages. However, it is relatively expensive, as it requires an EEG system as well as input from trained professionals on setting up the recording and for evaluating the data obtained. It may be uncomfortable for the patient and may restrict physical management (positioning, hoisting) and care of the individual. Additional information can be gained with video recording during PSG as well as through monitoring physiological parameters such as respiration, blood oxygenation, electromyography and electrocardiographic data, alongside of EEG.

3.7 SLEEP AND MEDICATIONS

People with PDOC often suffer from several other premorbid medical conditions such as heart disease, asthma, diabetes and high blood pressure. Following the brain injury, they also are prescribed medications to treat neurological complications of brain injury such as spasticity, dysautonomia, seizures and involuntary movements. Most of these medications act on the central nervous system and have influence on important neurotransmitter systems that take part in regulation of arousal and sleep. Some medications also influence melatonin levels, hence have an effect on circadian rhythm regulation. Therefore, it is important to be aware of these neurotransmitter systems as well as the medications which influence sleep and wakefulness

when interpreting sleep studies and circadian rhythms (Bourne and Mills, 2004, Papagiannidou et al., 2014, Landolt, 2008, Snel and Lorist, 2011, Drake et al., 2006).

Several medications can affect sleep parameters and when interpreting polysomnography reports this should be considered. Based on the changes observed in different sleep stages they can be simply grouped as;

- Medications that can suppress REM Sleep: tricyclic antidepressants, MAO inhibitors, amphetamines, barbiturates, alcohol.
- Medications that can affect stage 2 spindle activity: benzodiazepines
- Medications that can suppress stage 3 & 4 Sleep: benzodiazepines, tricyclic antidepressants or barbiturates.

The neurotransmitter systems that take part in regulation of arousal and sleep are;

- for maintaining wakefulness: include norepinephrine, serotonin, acetylcholine, dopamine, excitatory amino acids, hypocretins and histamine;
- play a role in NREM sleep: include adenosine, Gamma amino butyric acid (GABA) and prostaglandins;
- play a role in REM sleep: include acetylcholine, serotonin, norepinephrine and hypocretin (latter three by inhibiting cholinergic and cholinoceptive neurons which are implicated in initiation of REM sleep) (Monti et al., 2008).

This section focuses on medications which may have been prescribed to people with PDOC and therefore, indirectly may have an effect on measures and intervention outcomes used in this research project.

3.7.1 Medications with hypnotic effects

3.7.1.1 Medications that modulate GABA-A receptor and sleep

The GABA-A receptor is the most wide-spread receptor mechanism of inhibitory synapses in the central nervous system and they are densely located in the hypothalamus (Kryger et al., 2011). Benzodiazepines bind to benzodiazepine receptors which are a modulatory site on the GABA-A receptor and increase the GABA inhibitory effect by increasing the time that chloride channels are open (Johnston, 2005). The “Z” drugs such as Zopiclone, Zolpidem and Zaleplon are non-benzodiazepine medications that bind to various subtypes of GABA-A receptors. The most often used non-benzodiazepine medication which is prescribed to treat insomnia in the UK is Zopiclone. Zopiclone is preferred due to its short half-life and less sedative effects during the day. Zolpidem may be prescribed in cases with PDOC as several case reports in literature suggesting it may help with improvement of consciousness in the short and long-term.

GABA-A receptor agonists lead to promotion of NREM sleep (particularly increased stage 2 sleep with boosted spindle activity but suppressed stage 3 and 4 sleep) and inhibition of REM sleep (Mendelson, 2001) which translates as decreased sleep latency, decreased awakenings, increased stage 2 sleep, decreased stage 3 and 4 sleep, increased REM latency and reduction and fragmentation of REM sleep.

3.7.1.2 Antidepressants and sleep

Almost all antidepressants affect functioning of noradrenergic or serotoninergic systems, or both. Tricyclic antidepressant medications (doxepin, amitriptyline, trimipramine) interact with serotonin, norepinephrine, acetylcholine and histamine receptors and probably due to the combined effects of these neurotransmitter systems, they influence sleep. This translates to sleep studies as a reduction of sleep latency, increased sleep continuity, reduced REM amount and no significant changes in stages 3/ 4. Other antidepressants such as trazadone and mirtazapine also influence sleep by reducing the sleep

latency and increasing sleep continuity. While trazadone increases stage 3 and stage 4; mirtazapine has no significant effect on slow wave sleep or REM sleep (Montgomery et al., 1983, Ruigt et al., 1990, Winokur et al., 2000). Serotonin receptor antagonist antidepressants are non-sedating drugs but have been shown to effect sleep. For example, citalopram led to decrease of REM and a significant lengthening of REM latency and stage 2 sleep, with no changes in sleep continuity (van Bemmel et al., 1993). Similarly, it was shown that Fluoxetine reduced the total sleep time and the duration of REM sleep and increased awake activity and stage 1 sleep during the night (Nicholson and Pascoe, 1988).

3.7.1.3 First generation antihistaminic medications

Although these medications are rarely used for their sedative effects in brain injured patients, they may be prescribed for treatment of other conditions such as allergic conditions. Their H1 antagonist effects cause sedation and changes in polysomnography studies such as reduction of sleep latency and REM sleep, no change or increase in sleep continuity and slow wave sleep amount (Kryger et al., 2011).

3.7.1.4 Gabapentin and Pregabalin

These two medications are often prescribed for brain injured patients for treatment of neuropathic pain, allodynia, spasticity and as an adjunct anticonvulsant medication. Although they are structural analogues of GABA, their main effect occurs due to their selective binding to the $alpha_2$ delta subunit of N-type voltage-gated calcium channels and possibly due to their effects on N-methyl-D-aspartate (NMDA) receptors (Gajraj, 2007, Rose and Kam, 2002). Studies indicate that both drugs lead to reduced sleep latency, increased sleep efficiency, and significant increase in slow wave sleep amount (Foldvary-Schaefer et al., 2002, Hindmarch et al., 2005, Kubota et al., 2001, Roth et al., 2014).

3.7.1.5 Antipsychotic medications

Although rarely, these drugs can be used in brain injured patients for variety of indications such as insomnia, psychotic symptoms following brain injury or on the background of pre-existing psychiatric illness. Olanzapine and quetiapine are most commonly used medications for the patients with psychotic illness. Both medications have antagonist effects on serotonin, alpha-1 adrenergic, dopamine D2 receptors. They were shown to decrease sleep latency, increase sleep time, and efficiency, and increase stage 2, 3 and 4 amounts (Kryger et al., 2011, Salin-Pascual et al., 1999).

3.7.1.6 Medications with wake-promoting effects

Central nervous system stimulants may be prescribed for brain injured patients to combat daytime sleepiness which often is a problematic situation as it negatively effects the rehabilitation and social interaction. For the disorders of consciousness patients' daytime sleepiness may further complicate the clinical assessment of awareness and may contribute to well-recognized misdiagnosis rates as high as 43% (Andrews et al., 1996, Majerus et al., 2005).

3.7.1.6.1 Amphetamines and Amphetamine-like compounds

Amphetamine has a simple chemical structure which is very similar to endogenous catecholamines and contains three structural components: an aromatic nucleus, a terminal amine and an isopropyl side chain. By modifying these components several therapeutic (methylphenidate for treatment of attention-deficit hyperactivity disorder, modafinil for narcolepsy) and recreational drugs (Ecstasy and Love) have been produced over the years.

Methylphenidate is an amphetamine-like compound and is thought to block the reuptake of norepinephrine and dopamine into the presynaptic neuron and increase the release of these monoamines into the extra-neuronal space. It has attracted some attention recently. Published studies indicate that it may have a role in increasing motor and cognitive function after stroke and traumatic brain injury (Johansson et al., 2015, Johansson et al., 2017, Lokk et al., 2011, Tardy et al., 2006). However, side effects (increase in blood

pressure, gastro-intestinal disturbance, restlessness, agitation, sweating) and contraindications of this medication limits its use in clinical practice (Dymowski et al., 2017).

3.7.1.6.2 Dopaminergic agonists and L-dopa (dopamine precursor)

These medications are used in treatment of Parkinson's disease and Parkinsonism which may be a clinical feature in brain injured patients. Dopamine is a stimulatory transmitter and has an important role in the wakefulness, behaviour regulation, mood, language, cognition and motor control. It works on the basal ganglia-thalamus network which is connected to the supplementary and primary motor areas. It also plays role in modulation of dorsolateral prefrontal cortex and limbic structures which support cognition and emotion. L-dopa may have been prescribed to PDOC patients as it was shown that some patients with brain injury may respond well to treatment with L-dopa especially if they have concomitant symptoms of Parkinsonism and neuro-radiological findings of high intensity lesions in the dopaminergic pathway on T2-weighted MRI scans (Matsuda et al., 2005).

3.7.1.6.3 Modafinil

Modafinil is used for treatment of narcolepsy in the UK. It has been approved for treatment of shift-work circadian disorder and treatment of residual sleepiness in obstructive sleep apnoea syndrome. Clinicians may use (off-licence) Modafinil to treat daytime sleepiness secondary to brain injury. Its mechanism of action is still debated but thought to be due to DAT (Dopamine Transporter) inhibition. Animal studies showed that Modafinil decreases REM and NREM sleep without rebound hyper-somnolence (Edgar and Seidel, 1997).

3.7.1.6.4 Caffeine

Caffeine probably is the most popular central nervous system stimulant in the world as it is present in coffee, tea, cola drinks, chocolate and cocoa. It increases mental alertness, speed of thought processing and wakefulness. It is a Xanthine derivative and its mechanism of action involves nonspecific

adenosine antagonism. Adenosine is an endogenous sleep promoting agent with neuronal inhibitory effects (Basheer et al., 2000, Porkka-Heiskanen, 1999, Porkka-Heiskanen et al., 2002).

The list of medications that are commonly used in brain injured patients and their effects on sleep and/or melatonin levels are shown in table 3.3.

Table 3-3: Medications that effect sleep and circadian rhythmicity (TST=Total Sleep Time, SE=Sleep Efficiency, REM= Rapid Eye Movement, SWS= Slow Wave Sleep, SSRI= Selective serotonin re-uptake inhibitor)

Medication	**Effect on Sleep or Melatonin**
Benzodiazepines	↓REM, ↓SWS
Opioids	↓REM, ↓SWS
Clonidine	↓REM
NSAIDs	↓TST, ↓SE, ↓Melatonin levels
Beta-blockers	↓REM, ↓Melatonin production
Calcium Channel Blockers	↓Melatonin levels
Amiodarone	Nightmares
Corticosteroids	↓REM, ↓SWS, reduced melatonin secretion
Tricyclic antidepressants	↓REM, ↑TST
SSRIs	↓REM, ↓TST, ↓SE
Typical antipsychotics	↑TST, ↓REM, ↑SWS
Atypical antipsychotics	↑TST, ↓REM, ↑SWS
Phenytoin	↑Sleep fragmentation
Phenobarbital, Carbamazepine	↓REM, ↑TST
Gabapentin	↑TST, ↑REM, ↑SWS
Pregabalin	↑SWS, ↓REM
Levetiracetam	↑SWS, ↓REM, ↑TST
Cimetidine	↑melatonin concentration
Fluvoxamine	↑melatonin concentration
Oestrogens	↑melatonin concentration
Caffeine	↓SWS, ↓Melatonin levels

3.8 CIRCADIAN RHYTHM SLEEP DISORDERS

Circadian rhythm sleep disorders are caused by misalignment between the endogenous circadian timing system and the external 24-h environment (Bjorvatn and Pallesen, 2009). There are six types of circadian sleep disorders. The delayed sleep phase type, the advanced sleep phase type, the non-24-hour sleep/wake syndrome and the irregular sleep/wake rhythm are due to primary abnormality of individual's circadian system. Shift work sleep disorder and jet lag disorder on the other hand are due to conflict of surrounding environment with the individual's intrinsic circadian timing (Smith et al., 2008). The key characteristics, diagnostic evaluation and therapeutic options for circadian sleep disorders are summarized in table 3.4.

Table 3-4: Clinical features, diagnostic workup and practical therapeutic options of circadian rhythm sleep disorders (adopted from Sleep Medicine, Smith, 2008)

Circadian sleep disorder	**Clinical features**	**Diagnostic evaluation**	**Therapeutic options**
Delayed sleep phase disorder	Delayed in bed and wake times; bedtime between 2 and 6am, wake time between 10am and 1pm. Symptoms: Morning sleepiness and evening insomnia	Sleep log or actigraphy DLMO and core body temperature minimum to verify delayed endogenous phase	Bright light (2000- 10000 lux) in the morning Melatonin 0.3-3 mg 5 hours before habitual bedtime Chronotherapy: progressively delay bedtimes by 3 hours every 2 days Sleep hygiene
Advanced sleep phase disorder	Advanced bed and wake times; bedtime between 6pm and 9pm, wake time between 2am and 5am. Symptoms: early morning awakenings, early evening sleepiness	Sleep log or actigraphy DLMO and core body temperature minimum to verify advanced endogenous phase	Bright light (2000- 10000 lux) before habitual bedtime Chronotherapy: progressively advance bedtimes by 3 hours every 2 days Sleep hygiene
Non-24-hour sleep/wake syndrome	A progressive delay in bed and wake times Symptoms: sleepiness or insomnia which may wax and wane with time Generally seen in blind individuals	Sleep log or actigraphy for at least 1 week to verify a progressive delay in bed and wake times	Melatonin 10 mg 1 hour before desired bed time, 0.5mg maintenance dose Structured physical and social activity Sleep hygiene
Irregular sleep/ wake rhythm	Presence of at least 3 irregular sleep bouts throughout the 24-hour day Total sleep time over 24 hours is age-appropriate Generally seen in individuals with underlying neurological disease	Sleep log or actigraphy	Bright light: 2 hours of 3000-5000 lux in the morning; increased bright light exposure during the day Create a favourable night-time sleep environment: reduce noise, reduce light, improve continence care Structured physical and social activity Sleep hygiene

Shift work sleep disorder	Work schedule is at odds with the individual's endogenous sleep/ wake cycle Symptoms: sleepiness during the work shift, insomnia during newly desired sleep time	Sleep log or actigraphy PSG to rule out comorbid sleep disorders	Continuous or intermittent exposure to bright light during 3-6 hours of the work shift, avoidance of light in the 2 hours prior to end of the shift Melatonin 3 mg before desired bed time Behavioural strategies: favourable sleep environment, sleep hygiene, therapeutic napping Stimulants (caffeine or modafinil) at the beginning of the shift Treat comorbid sleep disorders if present
Jet lag disorder	Self-limited disorder associated with jet travel across two time zones Symptoms vary based on direction of travel and number of zones crossed	Further diagnostic evaluation generally not indicated	Strategic exposure to bright light Melatonin 2-5 mg for the initial 4 days after arrival Behavioural strategies -/+ Short acting hypnotic

3.9 ENTRAINMENT OF CIRCADIAN RHYTHM

3.9.1 Light

Light is the most important Zeitgeber and is known to synchronize the SCN to a 24-hour rhythm (entrainment). The effect of light on circadian rhythms follows phase-response curve and according the phase response curve light can have two opposite effects of the circadian rhythm depending on the time of exposure (Khalsa et al., 2003).

While bright light exposure in the early evening results in sleep phase delay, exposure after 3.00-5.00 am advances the next sleep phase. The sleep phases can be shifted by up to 1-2 hour per day by light exposure, according to its timing (Shneerson). The extent of the phase shift also depends on the dose and duration of the light exposure.

Even low intensity light as little as 100 lux, can alter the circadian rhythm but exposure to bright light for 10-20 min is required in order to have a significant impact. Portable light units which produce about 10 000 lux bright light are usually used for 30 to 45 minutes per day to improve the circadian rhythm and treat sleep disorders.

Although it is not necessary to stare at the light continuously, during the treatment patients are required to gaze towards the light source. Therefore, any change in the head position may cause reduction of the therapeutic effect. This can be especially problematic in patients with disorders of consciousness as they often have poor head control and positioning of the head and/ or light unit is dependent on the staff's engagement in the process. Moreover, they are likely to be lying in bed while light treatment is given due to short sitting tolerances and may not be in a close proximity of the light source due to size of the bed and equipment around them. Nevertheless, even light of less intensity and duration will likely be beneficial.

Treatment with the light boxes is safe and probably damage to the retina is the most serious potential side effect. However, there has been no scientific

publication reporting damage to the eyes once light treatment is administered using approved light boxes according to the standard procedures.

3.9.2 Melatonin

Melatonin is synthesized from tryptophan in the pineal gland upon request from the biological clock under the direct control of SCN. Melatonin is produced only at night time and its production is inhibited by light exposure.

Due to the close temporal relationship between the SCN and melatonin production, melatonin often used as phase-marker of human circadian rhythm (Lockley and Foster). Moreover, when mild sedative effect of synthetic melatonin coupled with its ability to shift the timing of the clock, its usefulness become apparent in treatment of sleep disorders associated with circadian rhythm disruption.

In some countries melatonin is sold as an over-the-counter medication as a sleep and circadian rhythm enhancing medication. It has a time to maximum concentration of approximately 30 minutes and elimination half-life of approximately 60 minutes (DeMuro et al., 2000). With this information in mind, it would have been reasonable to expect melatonin to act as a short-acting hypnotic, mainly effective to treat sleep onset difficulties. However, a strict dose-response relationship does not appear to be present for melatonin and its maximum effect may not coincide with its maximum serum concentration levels. Therefore, dose (1mg to 75mg) and administration timing (30 minutes to 3 hours before bedtime) of melatonin vary greatly in published studies. This makes the recommendation for dosage and timing of administration difficult. In the UK, modified-release melatonin is licenced to use to treat insomnia of adults 55 years and over with dose of 2-3 mg to be given 1-2 hours before bedtime (*British national formulary*). On the other hand, the same formulation is licenced to treat insomnia and delayed sleep phase disorder in children with dose of up to 10 mg to be given before bed time (*BNF for children*). No

significant risks or adverse effects have been reported and its abuse potential is minimal.

3.9.3 Caffeine

Caffeine is a weak psycho-stimulant and in addition to its stimulatory action it has circadian effects especially on melatonin synthesis in human. Caffeine can be used effectively to modulate our mental state and is found to be beneficial in restoring low levels of wakefulness and to counteract deteriorations in task performance related to sleep deprivation (Snel and Lorist, 2011).

It was shown that the subjects who suffer from jet lag achieved resynchronization of their circadian rhythm earlier than placebo group subjects when given caffeine alongside of melatonin (Beaumont et al., 2004, Pierard et al., 2001).

After oral ingestion of caffeine, 90% of it is absorbed from the gastro-intestinal tract into the blood stream, peaking 30-60 minutes after ingestion and diffuses throughout the entire body by passing all biological membranes including blood-brain barrier. It has a half-life of five to seven hours. It can inhibit melatonin production due to its adenosine receptor antagonist (mainly A1 and A2A receptors) properties (Ribeiro and Sebastião, 2010).

Adenosine receptors are found in the brain and involved in the regulation of sleep, arousal and cognition. Chronic caffeine use may up-regulate adenosine receptors and increase adenosine levels and some adenosine actions in the brain. As with other regulators of circadian rhythm timing of caffeine consumption is important. For example, if caffeine is consumed late in the day it may negatively affect sleep and may cause daytime sleepiness the next day.

3.10 CHAPTER SUMMARY

In chapter 3, I tried to summarize key information about regulation of sleep and circadian rhythms in humans, which then evolved into brief review of techniques that are used to assess sleep and circadian rhythms, physiological changes with aging, effect of medications and finally, disorders of circadian rhythms. Although further research is required to further understand the refined relationships between learning, memory and plasticity functions of the brain and sleep, which plays an important role in these events. It appears that all sleep stages are almost equally important and most likely they complement each other. Therefore, achievement of good sleep with adequate length, depth and quality is important for people with brain injury and during their rehabilitation process.

People with brain injury often suffer from sleep difficulties due to the physiological and pathological changes in the brain as well as associated processes involved in recovery from brain injury such as medications, reduced ability to be physically active and environmental settings such as busy hospital wards and rehabilitation units. Although this information is considered when discussing a patient's arousal levels and impact on the low arousal levels on assessment process; there are usually no formal processes and procedures in place to optimize patient's sleep-wake cycle as part of their neurological rehabilitation and diagnosis. Furthermore, there is limited research on the impact this optimization could have on patients' recovery.

Now the foundation information about sleep and circadian rhythms in hand; the next chapter will focus on what is known on sleep and circadian rhythms in PDOC population by performing review of literature on the subject.

Chapter 4 CIRCADIAN RHYTHM AND SLEEP IN PDOC - REVIEW OF THE LITERATURE

The previous chapter showed that the regulation of sleep is complex. Interactions between the homeostatic and endogenous circadian processes, habits and behavioural factors as well as the environmental factors influence sleep regulation. Abnormality of one or more of these factors - if not normalized effectively and promptly, may lead to dysregulation of sleep and/ or circadian rhythm and eventually to sleep disorders.

In order to have a well-timed and of high quality/ quantity sleep a person needs;

1. To have intact neural structures and pathways to regulate circadian rhythm and sleep regulation,
2. To be able to keep awake for a substantial amount of time during the day in order to reach high quality sleep at night which is evidenced by slow wave sleep/ deep sleep activity and REM,
3. Improved habits and behaviours to facilitate function of circadian and homeostatic factors,
4. Optimized environmental factors/ external zeitgebers to enhance external control of circadian rhythm.

People with PDOC lack all four requirements listed above. They have severely damaged neural structures and pathways, have poor arousal levels at day time to facilitate high quality sleep at night, have lost all appropriate habits and behaviours and are looked after in a hospital based institutional setting where environmental factors are far from ideal. For example; they are usually prescribed several centrally acting medications to prevent seizures and spasticity which may then reduce daytime arousal levels, often fed continuously including at night, checked at regular intervals during the night by

nursing staff which necessitates increasing illumination levels to do so, being turned and repositioned regularly in order to prevent pressure sores, and constantly being in an environment which is noisier and brighter than an ideal sleep environment.

The aim of this chapter is to review the literature related to sleep and circadian rhythms in PDOC, so that the appropriate assessment and treatment methods can be identified and used in this PhD research study.

4.1 LITERATURE SEARCH PROCEDURE

PubMed, MetaLib and Science Direct search engines were used to find published literature. The search was performed using the Medical Subject Heading (MeSH) terms “Vegetative state and sleep”, “Minimally conscious state and sleep”, “apallic syndrome and sleep”, “unresponsive wakefulness and sleep” and finally “disorders of consciousness and sleep” (Figure 4-1: *Literature review process*). Due to wide range of terminology used to describe the condition it may not have been possible to capture all the literature published. However, by using the most commonly used terminology, it can be confidently said that majority of the literature on the subject have been identified. Papers that are not in English language, on non-human subjects and exclusively for paediatric patient group were excluded. Abstracts of the all articles were read and irrelevant papers were excluded. References of the papers were also studied to capture any additional papers that did not come up during initial search.

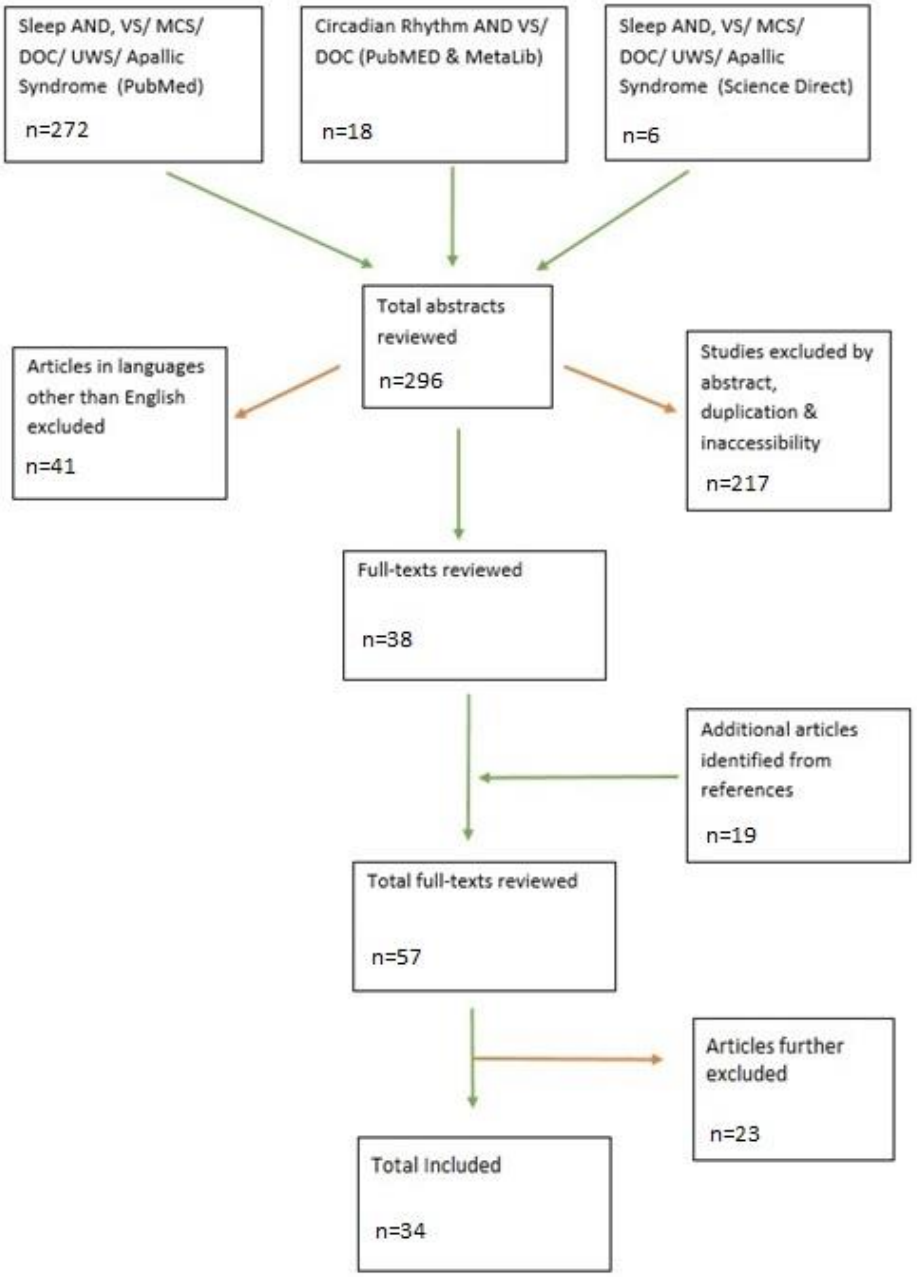


Figure 4-1: **Literature review process**

The literature was then reviewed in chronological order to understand the development of body of knowledge on this subject in line with the developments in overall understanding of disorders of consciousness itself. For example, minimally conscious state was only defined in 2002 which probably then steered the research towards diagnostic differentiation between vegetative and minimally conscious states. Similarly, the research and developments in neuro-imaging and neuro-physiology for disorders of consciousness might have added an additional dimension to assessment of sleep and circadian rhythms in DOC. An additional subgrouping was performed, to review the papers with a focus on review of literature, treatment, circadian rhythm and neuro-physiological assessment of sleep. (Table 4-1: *Papers published prior to 2002 and after 2002, sub-grouped according to topic*)

Table 4-1: **Papers published prior to 2002 and after 2002, sub-grouped according to topic**

	Papers published prior to 2002	Papers published after 2002	Total
EEG/ PSG	10	9	19
Circadian parameters	3	5	8
Review Paper	0	4	4
Focus on Treatment	0	3	3
Total	13	21	34

4.2 SLEEP AND CIRCADIAN RHYTHMS IN DOC- PRIOR TO DEFINITION OF MCS IN 2002

4.2.1 EEG/ PSG studies

Examination of polysomnography recordings may yield more reliable results in assessing sleep-wake patterns of the patients with disorders of consciousness. Moreover, detailed analysis of EEG patterns may give information on the sleep stage as well as the electrical activity/ spectral changes over long periods of time. However, obtaining good quality polysomnography recordings is very challenging and recordings are often of poor quality due to artefacts. The very early studies concentrated on exploring the association between broad EEG/ PSG features and the severity of brain injury in DOC population.

For example, in an early study which investigated the sleep EEG patterns as a marker of prognosis in post-traumatic coma, it was found that organized sleep EEG patterns were associated with a better prognosis in 16 patients (Bergamasco et al., 1968) whereas, patients with biphasic (consisting slow and fast phases only) and monophasic (slowing or flattening of background EEG activity) night sleep EEG recordings all died.

Later, it was shown that the presence of EEG patterns similar to those of sleep was associated with a favourable outcome (Bricolo 1979 & 1983). Moreover, REM sleep percentage increases were seen with recovery of cognitive functions on brain injured patients (Harada et al. 1976, Ron et al. 1980).

Matsuo et al. (Matsuo, 1985) described three cases of apallic syndrome due to anoxic brain injury. In all cases diffuse EEG attenuation of severe degree was demonstrated. Differences between aroused and quiescence were observed on PSGs without characteristic differential features of NREM sleep stage I-IV.

From the 1990s onwards, however, more detailed examination of sleep EEGs were performed which paid attention to sleep staging, spindling activity and REM sleep.

Gordon and Oksenberg investigated the presence of spontaneous nystagmus in 6 VS (secondary to traumatic brain injury (TBI), aged 20-53, time since brain injury onset 3-96 months) patients across the sleep wake cycle using 24 hours PSGs (Gordon and Oksenberg, 1993). Although in this study, authors focused on the nystagmus which was present in awake, Stage 1 and REM stages (but not in Stage 2 and slow wave sleep); it was reported that all patients had REM sleep between 11.7 and 44.1 mins during the 24-hour PSG recording.

In 1994, D'Aleo et al. investigated sleep in the initial stages and in the last remission stages of vegetative state (D'Aleo et al., 1994b, D'Aleo et al., 1994a). In a study that involved 30 patients with vegetative state (20 TBI, 10 hypoxic brain injury, aged 16-41). Sleep EEGs of 25 patients were examined with an emphasis on the spindling activity. It was found that only 11 (44%) of the patients had spindling activity: 8/15 (53.3%) traumatic versus 3/10 (30%) of the hypoxic patients. While sleep staging was not achieved in this study, the following observations made with comparison to the control group: The patients had higher percentage of non-REM sleep, greater fragmentation of sleep, higher percentage of wakefulness after re-awakenings, slower EEG frequencies and greater oscillations of cardiac and respiratory frequencies during REM. According to the old staging of the vegetative state none of the patients in stage 0 or 1 showed any evidence of spindling. (Stage 1 of this staging score corresponds to vegetative state whereas stages higher than 1 may fulfil the current criteria for MCS (von Wild et al., 2012)).

In another study the relationships between spindles and clinical outcomes of patients in post-anoxic stupor or coma were examined based on the 20 minutes EEG recordings. It was found that the absence of spindles or EEG reactivity was associated with poor outcome (Hulihan and Syna, 1994).

Sleep abnormalities in traumatic apallic syndrome were investigated by Giubilei et al. (Giubilei et al., 1995). 10 patients with traumatic apallic syndrome (awake without being aware), aged 15 to 55, range 47 to 180 days from head injury were investigated with computerized tomography (CT) scans, 12-hour PSG recordings and Glasgow Outcome Scale (GOS) on admission and at six months follow up. In this study 9 out of 10 patients had sleep patterns. There was no significant difference in sleep architecture between patients and controls, except increase in time spent awake after sleep onset and higher number of awakenings. One patient who did not show any evidence of sleep pattern had the lowest Barthel Index total score, remained in VS and died before the 6-month follow up. It is worth noting that in this study four of the patients were classified as with severe disability on GOS; three of them with Barthel Index total score of 35 and one of them with Barthel Index total score of 60. Therefore, it is very likely that at least these four patients were in MCS or higher rather than in VS.

In another study nine male patients with vegetative state (aged 17-40, 8 TBI, 1 non-TBI) were investigated using 24-hour PSG and penile circumferential change measurements to examine relations between sleep related erections and REM sleep (Oksenberg et al. Sleep Vol 23, No. 7, 2000). In this study, it was found that while 95% of the sleep related erections were associated with REM sleep, 64.6% of the REMs were associated with sleep related erections. No statistically significant difference was found between REM or sleep related erections between the recovered and non-recovered patients. Although, this study was unable to suggest that sleep related erections could be used as an indirect measurement of REM sleep; it provided some important clues with respect to disruption of sleep-wake cycles in vegetative states as REM sleep with or without sleep related erections were found both nocturnal and diurnal periods.

Again, Oksenberg and his colleagues further investigated the phasic activities of REM sleep in 11 vegetative state patients (aged 17-53, 10 males, 1 female,

TBI). It was found that nocturnal REM sleep time was significantly lower in patients than in control group. Mean duration of REM sleep periods was also lower in the patient group than in controls over 24-hour period. The patients also had significantly lower density of rapid eye movements, chin twitches and leg muscle twitches. These were unrelated to recovery of patients. The authors suggested that the decrease in the densities of REM and muscle twitches during REM sleep may be related to possible damage to the cholinergic mechanisms of the pedunculopontine tegmentum.

Isono et al.(Isono et al., 2002) investigated 12 patients with EEG, evoked potentials, MRI and 24-hour PSG studies. The patients included in the study aged between 10 and 69, 3 to 18 months post-brain injury (anoxic brain injury in three, diffuse axonal injury (DAI) in four and cerebro-vascular disease in five). Four of the patients were found to have no sleep-wake cycles (eyes usually closed and opened briefly after stimulation). The presence of brain stem lesions was the common finding of these four patients with no sleep-wake cycles. The remaining eight patients showed obvious sleep-wake cycles indicated by sleeping several hours at night and brief sleep episodes during the day. REM sleep and spindles were not seen any of the patients with sleep-wake cycles. Although this study was performed prior to definition of diagnostic criteria for MCS, it was noted by the authors that there were no differences between the clinical conditions of the patients with and with-out sleep wake cycles. It was also noted that none of the 12 patients included in the study show any evidence of awareness (therefore, may all have been in vegetative state).

With an aim to find an association between sleep patterns and prognosis, Valente et al. investigated 24 patients (post-traumatic, aged 16 to 74 years, 7-15 days after brain injury) with 24-hour PSG and according to the type of sleep pattern found they were classified into 5 groups from simplest monophasic EEG to more complex alternating REM/ NREM sleep patterns (Valente et al., 2002). It was found that the clinical outcome was significantly associated with

the PSG pattern. Better outcomes were related to better PSG patterns. Moreover, outcome showed a significant inverse correlation with age. Interestingly, no association was found between GCS on admission or with severity of lesions shown by CT brain scans. It was concluded that PSG pattern was a reliable prognostic marker of functional recovery in sub-acute stages of coma secondary to head injury. Presence of rudimentary sleep patterns were a good prognostic marker for survival but not for functional recovery. Presence of alternating REM/ NREM sleep patterns were associated with favourable outcomes (good recovery or moderate disability).

4.2.2 Circadian rhythm studies

Emergence from coma to vegetative and minimally conscious states are characterized by return of the simultaneous eye opening and exhibition of sleep wake cycles but this does not necessarily indicate preservation of circadian rhythmicity. Moreover, observation of naturally occurring circadian rhythms can be used in investigating the integrity of brain functioning in severely brain injured patients.

Early studies concentrated on assessment of circadian rhythms by using endocrinological markers such as cortisol, prolactin and growth hormone as well as physiological markers such as heart rate and blood pressure.

Normal circadian variation of mostly studied hormones are summarized in Table 4-2: *Circadian variation of endocrine hormones* (Minors and Waterhouse, 1981);

Table 4-2: **Circadian variation of endocrine hormones**

Cortisol:	Highest in the morning, lowest before retiring
Growth Hormone:	Marked peak in the first part of sleep (just after midnight)
Prolactin:	Highest levels at night, peak at or just after waking
Melatonin:	Higher at night (30-70 times higher than daytime concentrations), usually peak between 2 and 4 am.

In healthy subjects, blood pressure and heart rate show a circadian rhythmicity with higher values during daytime and lower values during the night time (RICHARDSON et al., 1964, Millar-Craig et al., 1978). While this is regulated by the autonomic nervous system, both physiological parameters are under the influence of many intrinsic and extrinsic factors such as posture, pain, spasms, medications and coexistent illnesses.

In a 1981 study (Ratge et al., 1981) 6 patients with apallic syndrome and four healthy volunteers were investigated over a period of 48 hours with 2-hourly plasma and urine catecholamine (norepinephrine, epinephrine and dopamine) levels. Existence of circadian rhythmicity was determined upon analysis of variance for paired data between the sampling times. There was no circadian rhythmicity of dopamine in both groups. Five out of six apallic patients had no recognizable rhythmicity of epinephrine and norepinephrine levels neither in plasma nor urine. The authors also measured plasma prolactin and cortisol levels and urinary cortisol excretions and published another paper of the same study. In this paper, they reported that all apallic patients the circadian rhythm of prolactin was abolished. In 2 patients a rhythm of cortisol was still detectable (Ratge et al., 1982).

24-hour profiles of growth hormone, prolactin and cortisol were studied on 11 patients with vegetative state (aged 16-57 years, 40-219 days after brain

injury, 9 TBI, 2 hypoxic)(Vogel et al., 1990). In comparison to age and sex-matched controls patients in VS fewer growth hormone peaks, higher baseline levels of prolactin and cortisol, diminished nocturnal increase of growth hormone and prolactin levels. Cortisol on the other hand, showed preserved circadian rhythm. Six of these patients were also examined with sleep EEGs. None of the patients had REM sleep and only one had atypical sleep spindles.

In an earlier study by Fukudome et al. (Fukudome et al., 1996) studied the circadian variations of body temperature, blood pressure, heart rate as well as urinary excretions of hormones (epinephrine, norepinephrine, 17-hydroxycorticosteroids), water and sodium on 16 patients with VS (aged 26-91 years, mixed aetiology, 9 of whom with cerebrovascular accident (CVA), 33.7 months post brain injury). It was found that circadian variations of blood pressure and heart rate were diminished. On the other hand, circadian rhythms for body temperature and urinary excretion of catecholamines, corticosteroids, water and sodium were preserved.

Table 4-3: Summary of Literature before 2002 (prior to definition of MCS)

EEG/ PSG Studies before 2002									
Authors/ Year	**Main Outcome Measure**	**Diagnosis**	**Time since BI**	**Cause of BI**	**Age**	**N=**	**Sex**	**Methods used**	**Main Findings**
Bergamasco et al. / 1968	EEG patterns as a prognostic criterion	Post-traumatic coma	N/S	Trauma	3-66	18	N/S	Serial Polygraphic recordings (1h daytime, 7h night-time)	Presence of sleep patterns were associated with good prognosis.
Matsuo et al./ 1985	Sleep EEG features	Apallic Syndrome	9m,17m & 6y	Anoxia	24,37 & 41	3	2F,1M	Overnight sleep EEG	Differences between aroused and quiescence were observed on prolonged EEG.
Gordon & Oksenberg/ 1993	Presence of spontaneous nystagmus across sleep-wake cycle	VS	3-96 months	TBI	20-53	6	(5M, 1F)	24h EEG recordings	Spontaneous nystagmus was present in awake, stage 1 and REM stages only. All patients had REM sleep (11.7 min- 44.1 min).
D'aleo et al./ 1994	Sleep EEG features with an emphasis on	VS	48 days to 823 days	TBI (20) Hypoxia (10)	16-48	30	19M, 11F	Nocturnal polygraphic recordings	44% of the patients had spindling activity. This was more common in patients with TBI than in patients with hypoxic brain injury. No

	spindles and REM								subjects in full stage of VS (apallic syndrome had spindles). In comparison to controls patients had less REM, more fragmented sleep and longer wakefulness after awakenings.
Hulihan et al./ 1994	Background reactivity and Presence of spindle activity	Stupor	0 to 43 days	Anoxia	Mean age: 59	14	4F, 10M	20 min EEG	The absence of spindle activity was associated with poor outcome.
Giubilei et al./ 1995	Presence of sleep patterns, sleep structure	Apallic Syndrome (at least 4 in MCS as Barthell index=>35)	47-180 days	TBI	15-55	10	3F,7M	CT brain, 12-hour PSG, Glasgow outcome scale on admission and at 6 months	Only one patient did not have a sleep/wake patterns who remained in VS and died before the 6-month follow up. Two patients had good recovery, and all others reached level of severe or moderate disability at 6 months.
Oksenberg et al. /2000	Sleep related erections and REM sleep	VS	1-8 months	TBI(8), non TBI(1)	17-40	9	9M	24-hour PSG, Penile circumference measurements	95% of sleep related erections were associated with REM sleep. There was no significant difference

									between REM or sleep related erections in relation to prognosis.
Oksenberg et al./2001	Assessment of the phasic components of REM	VS	2-5 months	TBI	17-73	11	(10M, 1F)	24-hour PSG	Nocturnal REM sleep time was significantly lower in patients than in control group. Patients also had lower density of REM, chin twitches and leg muscle twitches. REM activity and characteristics were not related to recovery of patients.
Isono et al. /2002	Presence of Sleep/ wake cycles, sleep structure	VS	3-18m	Anoxia(3), DAI(4), CVA(5)	10-69	12	4F, 8M	EEG, EPs, MRI, 24-hour PSG	8 of the patients (3 anoxia, 2 DAI, had sleep/wake cycles but no REM sleep or spindles. There were no differences between the clinical conditions of the patients with or without sleep cycles.
Valente et al./ 2002	Association between the sleep patterns and prognosis	Coma	7-17 days	TBI	16074	24	5F, 19M	24-hour PSG, Glasgow Outcome Scale	The presence of organized sleep was highly predictive of better clinical outcomes.

CIRCADIAN RHYTHM STUDIES BEFORE 2002									
Authors/ Year	**Main Outcome Measure**	**Diagnosis**	**Time since BI**	**Cause of BI**	**Age**	**N=**	**Sex**	**Methods used**	**Main Findings**
Ratge et al. /1980	Circadian rhythms of catecholamines	Apallic Syndrome	N/S	N/S	14-83	6	N/S	Dopamine, adrenalin and noradrenalin levels (2 hourly blood and urine samples for 48h)	No dopamine rhythmicity 1/6 patient had catecholamine rhythmicity
Vogel et al./ 1990	24h profiles of GH, Prolactin and Cortisol	VS	40-219 days	TBI (9) Anoxia (2)	16-57	11	2F, 9M	PSG on 6 patients Blood samples for hormone levels every 20 mins	All patients had abnormal organization of sleep stages, none of the patients had REM, only 1 patient had spindles Hormonal circadian rhythm was preserved.
Fukodeme et al./ 1996	Presence of circadian rhythmicity for endocrine and physiological measures	VS	2-92 months	Stroke (10) SAH (2) TBI (1) CO poisoning (2)	29-91	16	9F, 7M	24-hour blood pressure and heart rate (every 30 mins) (n=16) 24-hour body temperature using intravesical thermistor	Circadian rhythms of blood pressure and heart rate were diminished. Body temperature and endocrine circadian rhythms were preserved.

				Anoxia (1)				catheters (every one minute) (n=12) 6 hourly urine samples for sodium, potassium, norepinephrine, epinephrine, 17-hydroxycorticosteroid levels (n=14)	

4.2.3 Key messages from literature prior to 2002

1. Sleep/ wake cycles and sleep EEG patterns are abnormal in patients with DOC.

2. REM and Spindles are more common in TBI than in brain injury secondary to non-traumatic aetiology.

3. The more organized the sleep EEG patterns the better the diagnosis.

4. Absence of spindles and EEG reactivity are associated with poor prognosis.

5. Presence of alternating REM/ NREM sleep patterns are associated with good recovery.

6. Blood pressure and heart rate circadian rhythms are diminished and / or unreliable in DOC.

7. Catecholamines, dopamine, prolactin and growth hormone rhythmicity are diminished.

8. Cortisol and body temperature rhythmicity is usually present.

4.3 SLEEP AND CIRCADIAN RHYTHMS IN DOC- AFTER DEFINITION OF MCS IN 2002

4.3.1 EEG/PSG studies

Electrophysiological correlates of behavioural changes in vigilance in VS and MCS were investigated by Landsness et al.(Landsness et al., 2011). Sleep studies were performed on 11 patients (6 MCS and 5 VS) using 256-electrode high density EEG for 12 hours between 8pm and 8am. Behavioural sleep patterns, stages 2 & 3 and periods of REM sleep were observed in the 5/6 MCS patients. Spindles were observed in all MCS patients. On the other hand, in all VS patients behavioural transition to sleep was not accompanied by EEG changes. No stage 2 &3 or REM sleep were observed in VS patients. No

spindles were observed in VS patients in this study. It was suggested that electrophysiological sleep features could be a reliable indicator of level of consciousness in PDOC and can help to differentiate VS from MCS. With respect to failing to show EEG correlates of behavioural changes in vigilance in VS the authors suggested two possibilities: insufficient modulation of cortico-thalamic activity by a deficient ascending reticular activating system such as in pontine damage and failure in cortico-thalamic function to transmit signals from the ascending reticular activating system. The latter can also explain the severe impairment of consciousness in VS. It was further argued that as REM sleep generating mechanisms are located in the dorsal pontine tegmentum, brain-stem generated features of REM sleep may be present in some VS patients. A comparison of slow-wave activity between wake and sleep was performed which provided an additional quantitative assessment of EEG changes in relation to changes in vigilance.

Cologan et al. (Cologan et al., 2013) studied 24-hour PSG on 10 vegetative and 10 minimally conscious state patients in the subacute stages of their illness (1 to 12 months after brain injury). EEG relative spectrum, EMG, EOG and heart rate parameters were used to define rest periods which then aided to classify patients in three groups: cycled, uncycled and ultradian where at least two periodic rest-wake patterns were found. In addition to assessment of cycles sleep staging was performed using the following criteria:

Table 4-4: **Sleep staging criteria used by Cologan et al.**

Waking stage: High frequency (>10 Hz) desynchronized EEG, high muscular tone on EMG, eye blinks in EOG
Stage 1: Slower and synchronized EEG, low muscle tone, the absence of eye blinks, and occurrence of slow eye movements
Stage 2: Presence of sleep spindles
SWS: high amplitude (75-140 μV) synchronized EEG in the δ band (20% in stage 3 or 50% in stage 4), low muscular tone, absence of eye blinks and movements.
REM: synchronized EEG on θ band, muscular atonia, presence of REM phasic events and muscular twitches.

PSG sleep cycle was found in 3/10 VS patients and in 5/10 MCS patients. An ultradian pattern was present in one VS and two MCS patients. The authors noted that the standard temporal progression of sleep stages (wake→ stage 1→Stage 2 → SWS →REM Sleep →awakening) was not seen in only two patients. In this study sleep staging was performed by 3 different examiners and most discrepancies were observed in scoring SWS and REM Sleep especially in MCS patients. It was argued that this was due to having unclear boundaries in SWS in some patients, presence of episodes resembling REM Sleep in brain injured patients and of course due to differences in experiences of the scorers. The scoring conflicts were resolved by reaching a common agreement. Spindles, SWS and REM Sleep were more prevalent in MCS patients than in VS patients but none of these parameters could be statistically compared due to small number of patients. The persistence of REM sleep was correlated with MCS patients (10/10 MCS patients had REMS in comparison to 3/10 VS patients). Sleep spindles were present more in patients who clinically improved within 6 months indicating the restoration of thalamo-cortical connectivity during recovery of consciousness in these patients. The authors suggested that VS patients with REM sleep should be further

investigated with active paradigms to test the persistence of minimally conscious state.

PSG studies, stimulus related evoked potentials and clinical features of PDOC patients were examined by de Biase et al. with the aim of increasing diagnostic accuracy in PDOC (de Biase et al., 2014). In this study 32 patients with prolonged disorders of consciousness (27 VS and 5 MCS) were investigated using clinical evaluation tools (CRS-Revised, Glasgow Coma Scale (GCS) and Disability Rating Scale (DRS)) and neuro-physiological evaluation tools which included 24-hour polysomnography, somatosensory evoked potentials (SEPs), brainstem auditory evoked potentials (BAEPs) and visual evoked potentials (VEPs) with the aim of identifying possible correlations between clinical evaluation and neurophysiological variables. The patients included in the study were aged between 26 and 71 years. The causes of the PDOC were traumatic brain injury (n=10), hypoxic/anoxic brain injury (n=15) and haemorrhagic stroke (n=7). The time since brain injury ranged between 3 months and 12 years). REM sleep and simultaneous presence of all sleep elements (sleep-wake cycle, sleep spindles, K-complexes) were more frequent in MCS than in VS. REM sleep was present in all MCS patients and only on 4 VS patients (two of them had REM only once and only for few epochs). Only 7.4% of VS patients showed simultaneous presence of all the examined sleep elements. While the simultaneous presence of REM sleep and sleep spindles together were more adequately correlated with CRS-R scores, there were no significant associations between evoked potentials and CRS-R scores. The authors argued that PSG recordings may help clinicians to differentiate between VS and MCS patients. As REM sleep is associated with dreaming and information reprocessing during sleep; it was suggested that presence of REM sleep in VS patients could be used as an indicator to investigate these patients further with functional neuro-imaging techniques to detect covert signs of consciousness.

Again in 2014, Kang et al. suggested a simple score to predict outcome for Unresponsive Wakefulness Syndrome (UWS)/ VS (Kang et al., 2014). A clinical prospective study was conducted to identify prognostic markers for recovery of awareness and to develop a simple bedside score to achieve more reliable outcome prediction for unresponsive wakefulness syndrome. 56 patients aged 10 to 73 year old, 3 to 12 weeks post brain injury, were included in the study where they were investigated with CRS-R, serum neuron specific enolase levels and bedside neurophysiological tests which included 24-hour video-EEG and somatosensory evoked potentials. Pupillary light reflexes, corneal reflexes, motor responses to painful stimulation and main patient characteristics (age, sex, time from brain injury and type of brain injury) were examined. Presence of EEG reactivity, sleep spindles and cortical N20 responses were considered. The neurological outcomes were no recovery of awareness (GOS 1 and 2) and recovery of awareness (GOS 3,4,5 and additional category for patients in MCS). Type of brain injury, motor response to pain, EEG reactivity, sleep spindles and N20 differed significantly between the recovered and not recovered patients. Interestingly although all these variables had high specificity for recovery awareness; only sleep spindles showed a high sensitivity (70.8%). A scoring tool called TMSEN (type of BI- Motor response- sleep spindles- EEG reactivity- N20) was developed to be used in outcome prediction for UWS. While a score of 3 or more translates into 80.77% probability of recovery within one year (positive predictive value), score of 0 to 2 translates into 90% negative predictive value. The TMSEN score awaits further validation.

Table 4-5: TMSEN Score as developed by Kang et al.

Relative factor	**Categories**	**Points**
Type of Brain Injury	Trauma	1
	Non-trauma	0
Motor response to pain	Flexor	1
	Extensor or absent	0
EEG Reactivity	Present	1
	Absent	0
Sleep Spindles	Present	1
	Absent	0
N20	Present	1
	Bilaterally absent	0
Total		**5**

Rossi Sebastiano et al. investigated the value of multiple neurophysiological tests which included EEG, 18-hour polysomnography, auditory event related potentials and multi-modal evoked potentials in classifying DOC in patients with chronic VS and MCS (Rossi Sebastiano et al., 2015). In this study 142 patients (aged 50.9±14.1 and after 38.8±34.6 months after acute brain injury) were included. 85 of the patients in VS and 57 were in MCS as diagnosed with CRS-R. EEGs were evaluated using the Synek scale. See Table 4-6: Synek Scale- EEG patterns for coma prognostication in adults (Synek, 1988). Sleep was evaluated on 18-hour long EEG recordings and was scored using an arbitrary scale developed by the authors rather than standardized sleep staging criteria published by AASM. The multimodal evoked potential responses were scored arbitrarily (absent, abnormal, normal), as the MMN/ P300 responses (absent, present). It was found that combined multimodal EPs and sleep EEG scores provided the most valuable information as sleep patterns were robust indicator of DOC severity regardless of the aetiology and EPs informed the clinicians on patients' susceptibility to specific stimuli to tailor

customised rehabilitation programmes. Although, standard spectral EEG analysis outcomes were significantly related to DOC classes and CRS-R scores they did not make a significant additional contribution to DOC classification.

Table 4-6: Synek Scale- EEG patterns for coma prognostication in adults

Grade	EEG Characteristics	Prognostication
Grade 1	Predominantly regular alpha activity, some scattered theta activity	Favourable
Grade 2	Dominant theta activity with some alpha and delta (EEG can be reactive or non-reactive to external stimuli)	Favourable if reactive EEG
Grade 3	Dominant diffuse delta activity, mostly seen in coma. Several subgrades defined based on rhythmicity of delta activity, presence of sleep patterns and amplitude of delta.	Depends to subgrade.
Grade 4	Burst-suppression pattern. Four subgrades: alpha pattern coma, epileptiform, theta pattern coma, low-output EEG.	Poor
Grade 5	Absence of EEG activity	Brain death

Generation of slow wave activity (SWA) requires activity of cortico-thalamo-cortical loops as well as preservation of cortical plasticity which then enables sleep-related restorative, learning and memory functions. Pisani et al. hypothesized that modulation of SWA using repetitive transcranial magnetic stimulation (rTMS) may help to differentiate MCS and VS (Pisani et al., 2015).Ten patients (4 MCS, aged 26 to 72, five TBI and five anoxic brain injury, 4 male, 4-23 months post-ictus) were included in the study. The patients underwent 24h PSG recordings which was followed by a real or sham 5Hz-

rTMS and a second PSG recording. Presence of sleep-wake cycles and standard temporal progression of sleep stages (wakefulness/ NREM/ REM/ awakening) were only seen on the MCS patients. Furthermore, only MCS patients had showed significant after-effect following real rTMS but not following sham rTMS. None of the VS patients had any significant after-effect following either the real or sham rTMS. This provided further evidence that assessment of sleep- wake cycles and sleep stages may help to diagnose disorders of consciousness and that being able to elicit responses to rTMS may provide additional supporting evidence for inconclusive cases.

Arico et al. explored combining sleep studies and pain evaluation to increase diagnostic accuracy in DOC (Aricò et al., 2016). 14 patients with DOC (6 MCS, 8 VS, aged 31 to 72, 7 TBI and 7 non-TBI) were studied with CRS-R assessments, 24-hour PSG and laser evoked potentials. Clinical and neurophysiological data were compared in the two groups by means of un-paired t-tests (p<0.05). A Fisher's test was carried out for correlation analysis. It was shown that patients in MCS showed a more structured sleep than those in VS. This was independent of DOC aetiology. Patients in VS showed more delayed N2P2 LEP (laser evoked potentials) component latency and reduced amplitudes than MCS. It was suggested that presence of clear LEP components and more structured PSG may indicate a more organized thalamo-cortical system.

Bedini et al. investigated the contribution of Per3 genotype on sleep quantity and consciousness recovery level in patients with disorders of consciousness (Bedini et al., 2015). The Per3 gene has been recognized to have a role in circadian rhythmicity generation (Dijk and Archer, 2010) and has different genotypes (5/5, 4/5 and 4/4 genotypes) which are related to different sleep phenotypes and sleep-wake disorders. For example, EEG alpha activity in REM sleep, theta/alpha activity during wakefulness and slow wave activity in NREM sleep are elevated in Per3 5/5 which is present in 10% of the population. In this study 48 patients with DOC (44 VS and 27 MCS, 26 TBI/45

vascular, aged 17-81 years, 2-22 years post brain injury) were investigated with PSG (2pm to 9am the following day) and Per3 genotyping. The median total CRS-revised score in Per3 5/5 group was significantly higher than 4/4 genotype suggesting Per3 5/5 genotype may be associated with a better outcome. Analysis of the 48 PSGs revealed that all patients had stage 2 sleep, 41% had Slow Wave Sleep and 22 % had REM sleep. The only significant difference between the VS and MCS patients was in NREM 2 + SWS duration (p=.047). Although statistical significance was not achieved, SWS and total sleep time in Per3 5/5 patients was higher than Per3 4/4 and 4/5 patients. The authors speculated that the higher total sleep time in Per3 5/5 patients may be associated with a protective role of this genotype in preserving a better sleep homeostasis. Furthermore, SWS could be related to better functional outcomes by leading to preservation of main cerebral connections, memory consolidation and learning.

In a recently published work (Arnaldi et al., 2016) it was shown that better clinical outcomes were associated with the presence of sleep integrity, a better baseline clinical status and younger age. In this study 31 patients with DOC (18 males, age 18 to 81 years, 1-6 months post-anoxic/ haemorrhagic brain injury and 1-12 months post-TBI) were investigated with 24-hour PSG studies. Sleep integrity was defined using the scoring structure index (SSI) established by Valente et al. (Valente et al., 2002). Three patients had no sleep pattern (SSI=1), 12 had sporadic NREM markers only (SSI=2), three had sporadic REMs (SSI=3) and nine patients had identifiable sleep stages (SSI=4). The patients were then monitored with CRS-R assessments for 30 months. Association with better baseline clinical status and younger age was well-known. However, the predictor value of presence of sleep integrity was stronger than the other two factors tested. It was suggested that sleep investigations should be included as part of routine evaluation of patients in the sub-acute stages of DOC.

4.3.2 Circadian rhythm studies

People with brain injuries often suffer from dysautonomia (Baguley et al., 1999, Baguley et al., 2009) which can render assessments of rhythmicity for blood pressure and heart rate challenging. Nevertheless, circadian blood pressure and heart rate changes in patients in a persistent VS after TBI was investigated (Pattoneri et al., 2005). In this study 10 patients with VS (TBI, aged 19-39, 27.3 ±5.6 days after trauma) and 10 healthy controls underwent ambulatory heart rate and blood pressure monitoring for 24 hours. The patients presented with symptoms of dysautonomia. It was found that patients had higher 24-hour mean values of blood pressure and heart rate than the controls. There was no physiological nocturnal dip in the patients. Day-night differences of blood pressure and heart rate were significantly lower in patients. These results suggested that dysautonomia after TBI is associated with alteration of circadian blood pressure (BP) and heart rate (HR) pattern.

Bekinschtein (Bekinschtein et al., 2009b) investigated the circadian temperature rhythms on five patients with vegetative state (two traumatic brain injury, two anoxic brain injury, one stroke) using iButton sensors which measured skin temperatures every 15 minutes over 13-16 days continuously. In addition, the degree of cortical and subcortical atrophy was assessed using a 5 point visual scale where atrophy levels are graded between no atrophy to highly severe atrophy (Galton et al., 2001). Two patients with traumatic brain injury had well-formed circadian temperature rhythms and had more reflexive behaviours and relatively low cortical and sub-cortical atrophy, whereas the three patients from anoxic-hypoxic origin demonstrated no cycles or rhythmic behaviour. In discussing the reason for the difference between traumatic and non-traumatic patients, authors pointed that anoxic and ischaemic brain injuries lead to diffuse, cortical, basal ganglia and hippocampal damage particularly affecting the medical and inner parts of the brain where the biological rhythms are regulated. Finally, it was suggested that circadian rhythms may be used as a measure of physiological state and/or prognosis as

hypothalamic and midbrain functions can be deduced from circadian parameters.

Guan et al.(Guan et al., 2011) investigated the infradian (circaseptan) and circadian rhythms of blood pressure and heart rate on 26 patients (15 males and 11 females, age 43.7-/+ 6.3) in persistent vegetative state secondary to traumatic brain injury. Heart rate and blood pressure were recorded hourly for 10 days. Time since the brain injury or specifics of the monitoring equipment used were not reported. Cosinor analysis of the data suggested that both infradian and circadian rhythms were present in VS patients. However, infradian rhythm had greater amplitude than the circadian rhythm.

Cruse et al. investigated the circadian sleep-wake cycles by using actigraphy in 55 patients with disorders of consciousness (Cruse et al., 2013). The actigraphs were applied to the limb with the highest range of movement over four days and cosinor analyses were performed to assess circadian rhythmicity. This study has shown that significant proportion of patients with disorders of consciousness did not exhibit statistically reliable sleep-wake rhythms. In addition, findings of this study suggested that the circadian sleep-wake cycles of patients with MCS were significantly statistically more reliable than those of VS patients. Interestingly this study also indicated that patients with disorders of consciousness were most active in the late afternoon which may have been related to being institutionalized. (While activity peaks of healthy individuals occur earlier in the day between approximately 13.30 pm and 16.00 pm this is delayed in institutionalized patients due to abnormal fluctuations of light as well as low light levels) (Shochat et al., 2000, Ancoli-Israel et al., 2002). However, due to practical limitations of actigraphy in this particular patient population, the findings of the above study need to be interpreted with caution as the results were not supported with use of another measure such as sleep/ wake logs and/or polysomnography.

Nocturnal melatonin regulation in vegetative state (6 patients, 2 female, 6 to 18 months after TBI) was assessed by Guaraldi (Guaraldi et al., 2014). He demonstrated that VS patients did not have physiological elevation of nocturnal melatonin levels. When exposed to light the control subjects had significant suppression of melatonin levels. On the other hand, all VS patients lacked such physiological response. The importance of circadian rhythm assessment was re-iterated, and it was suggested that melatonin supplementation may provide benefit to VS patients.

4.3.3 Review Papers

Bekinschtein et al. reviewed the factors underlying wakefulness and their interactions with each other in 2009 (Bekinschtein et al., 2009a). It was recommended that; at least four days of recording of continuous temperature and motor activity with actigraphy, three days (and nights) blood sampling every 6 hours to assess cortisol and melatonin day-night differences and 24-hour EEG should be performed in order to gather adequate information on circadian rhythmicity which can aid to plan behavioural assessments accordingly. Importance of performing behavioural assessments when patient is systemically healthy and homeostatically stable.

Cologan et al. (Cologan et al., 2010) published a review paper in 2010 where they focused on the importance of evaluation of the biological rhythms in DOC, methodological issues around sleep staging in DOC and finally on the available data on sleep according to different states of consciousness namely in brain death, coma, vegetative state, in recovery from coma and in locked-in syndrome. Sleep in minimally conscious states was not discussed on this paper, as at the time of the preparing this paper the authors were not able to find any published data on sleep of patients with MCS.

De Salvo et al. reviewed the role of neurophysiological testing for clinical differentiation and outcome evaluation in VS and MCS patients (De Salvo et al., 2012). The challenges of interpreting sleep studies in DOC as well as role

of sleep studies in differentiating MCS from VS and its role on prognostication have been highlighted.

Blume et al. reviewed the literature neuroimaging methods and sleep on a paper published in 2015 (Blume et al., 2015). It was highlighted that in DOC some rhythms may recover while some do not. For example, previously it was shown that circadian rhythm of body temperature and urinary excretion of hormones may be observed in VS patients. On the other hand, studies failed to observe preservation of blood pressure and heart rate circadian rhythms. Authors suggested that interventions targeting restoration of circadian rhythmicity may be a promising tool to support assessment and rehabilitation process in DOC. Furthermore, upon review of the PSG literature the authors recommended to use of PSG for reliable and valid assessment of sleep in DOC patients. Bright light therapy was suggested as in intervention to restore the circadian rhythmicity and normalize sleep as well as to stimulate patients right before assessments of consciousness.

4.3.4 Publications with a Focus on Treatment

In a paper published by Sará et al. two different mechanisms were identified that may explain why, in some patients, an improvement of consciousness following intra-thecal baclofen pump implantation occur. The authors hypothesized that intrathecal baclofen might have helped the clinical improvement by increasing stabilization and coherence of the circadian rhythms, through the activation of postsynaptic GABA-B receptors, by enhancing metabotropic glutamate receptors 1 and mediated excitatory transmission. It was further suggested that effects of baclofen on serotonin release may be linked to its action on alertness (Sara et al., 2009). The authors concluded that the strategic use of drugs aimed at modulating the arousal-circadian dynamics might orient neural plasticity and therefore, may result in clinical improvement.

De Weer et al. examined four patients with disorders of consciousness (2 TBI, 2 anoxic brain injury, aged 19-61, 3 MCS and 1 coma). Only two of the patients with MCS following TBI were able to exhibit increased motor activity on their actimetric measurements and their arousal levels when they were expose to light irradiation (De Weer et al., 2011). Hence, bright light may be used to re-establish circadian rhythms in disorders of consciousness by increasing day time arousal and promoting sleep at night.

Blume et al. investigated the presence of circadian temperature rhythms across 6 to 7 days using external skin temperature sensors as well as the relationship between CRS-R scores and circadian rhythmicity in 18 patients with DOC (Blume et al., 2017). In this study all patients had preserved circadian rhythmicity of skin temperature and the integrity of circadian variations were correlated with CRS-S scores, especially the arousal subscale scores. Eight of the patients were then given bright light treatment for one three times a day. It was found that the bright light treatment led to not only improvement of circadian rhythmicity but also improvement of CRS-R scored in three patients.

Table 4-7: Summary of Literature after 2002 (after definition of MCS)

CIRCADIAN RHYTHM STUDIES AFTER 2002									
Authors/ Year	**Main Outcome Measure**	**Diagnosis**	**Time since BI**	**Cause of BI**	**Age**	**N=**	**Sex**	**Methods used**	**Main Findings**
Pattoneri et al./ 2005	Circadian blood pressure and heart rate changes	VS patients with dysautonomia	27.3-/+5.6 days	TBI	19-39	10 patients, 10 controls		Blood pressure and heart rate measurements every 15 minutes for 24 hours Differences on day and night time values	Patients had higher values of blood pressure and heart rate than controls at night time. Night time blood pressure dip was not observed in patients (non-dipper pattern). Day-night difference was also lower in patients.
Bekinschtein et al./ 2009	Circadian temperature rhythms/ Ibutton	VS	5-24 m	TBI (2), Anoxia (2), Stroke (1)	22-67	5	3F, 2M	iButton measurements	Two patients with TBI had preserved circadian

								every 15 mins over 13-16 days Severity of cortical atrophy	rhythmicity of temperature. These patients had less cortical atrophy. Circadian rhythms can be used as a measure of physiological state/ prognosis as hypothalamic and midbrain functions can be deduced from circadian parameters.
Guan et al./2011	Circadian and infradian rhythms of blood pressure and heart rate	VS	N/A	TBI	43.7-/+ 6.3	26	11F, 15M	Hourly blood pressure and heart rate measurement for 10 days	Cosinor analysis of the data suggested that both rhythms were present but with a greater amplitude with infradian rhythm.

Cruse et al./ 2013	Circadian sleep-wake rhythms	MCS (37) VS (18)	1 to 290 months	TBI (31) Non-TBI (24)	11-67	55	17F, 38M	CRS-R, actigraphy for 4 days	Significant proportion of patients did not have sleep-wake cycles. Sleep-wake cycles were more impaired in VS than in MCS and also in non-traumatic group than in traumatic.
Guaraldi et al./ 2014	Assessment of nocturnal melatonin regulation	VS	6-18 months	TBI	28-47	6	2F, 3M	Night-time plasma melatonin levels at baseline (00:30 am to 3:30 am), Melatonin suppression in response to light	At baseline while controls had significant increase of plasma melatonin levels, VS patients had no significant increase. VS patients also had no significant

									suppression of melatonin levels when exposed to blue light. It was suggested that DOC patients may benefit from Melatonin supplementation.
EEG/ PSG STUDIES AFTER 2002									
Authors/ Year	**Main Outcome Measure**	**Diagnosis**	**Time since BI**	**Cause of BI**	**Age**	**N=**	**Sex**	**Methods used**	**Main Findings**
Landness et al. / 2011	Presence of sleep stages	MCS (6) VS (5)	25 days to 25 years	TBI (4) Anoxia (3) Other (4)	19-75	11	6F, 5M	12-hour high density EEG between 8pm and 8am.	Stages 2 &3 and periods of REM were observed on 5 MCS patients. These were not observed on any of the VS patients.

Cologan et al./ 2013	Sleep-wake cycles and sleep stages	MCS (10) VS (10)	1-12 months	TBI (9) Anoxia (6) Stroke (5)	16-74	20	N/A	24-hour PSG	Sleep wake cycles were present in five MCS and three VS patients. All patients with MCS had REM but only three of the VS patients. spindles were more common on patients with clinical improvement at 6 months.
De Biase et al. / 2014	Sleep structure, evoked potential (EP) responses	MCS (5) VS (27)	3months-12 years	TBI (10) Anoxia (15) Haem. Stroke (7)	26-71	32	9F, 23M	CRS-R, 24-hour PSG, SEPs, VEPs, BAEPs	Sleep was more structured in MCS patients than in VS patients. Presence of REM sleep and sleep spindles together were more

									correlated with CRS-R scores. There were no significant associations between EP responses and CRS-R scores.
Kang et al./ 2014	Development of a simple bedside score to predict clinical outcome using EEG reactivity, sleep spindles, and cortical N20 responses	MCS/awareness (24) VS/ unawareness (32)	3-12 weeks	TBI (23) Non-traumatic (33)	10-73	56	15F, 41M	CRS-R, serum neuron specific enolase, 24-hour video EEG, SSEP	All variables showed high specificity for recovery of awareness but only sleep spindles showed high sensitivity. A scoring tool called TMSEN (type of BI, Motor response, sleep spindles, EEG reactivity,

									N20) was developed.
Sebastiano et al./ 2015	Synek scores of EEG, arbitrary scoring of PSG, presence/ absence of EP responses and MMN/ P300 responses, spectral EEG analysis	MCS (57) VS (85)	38.8 -/+34.6 months	Anoxia (53) TBI (40) Vascular (49)	50.9 -/+14.1	142	52F, 90M	EEG, 18-hour PSG, auditory event related potentials, multi-modal evoked potentials	Combined multi-modal EPs and sleep EEG scores provided the most valuable information on DOC severity and establishment of customised rehabilitation programmes.
Pisani et al./ 2015	Presence of sleep/ wake cycles and temporal progression of sleep stages Modulation of SWA	MCS (4) VS (6)	4-23 months	TBI (5) Anoxia (5)	26-72	10	6F, 4M	Repetitive transcranial magnetic stimulation (real & sham) 24- hour PSG	Sleep-wake cycles, temporal progression of sleep stages and SWA modulation with real TMS were only seen on MCS patients. This may

									help to differentiate VS and MCS patients.
Arico et al./ 2016	Sleep structure, laser evoked potential responses	MCS (6) VS (8)	12-60	TBI (7) Non-TBI (7)	31-72	14	6F, 8M	24-hour PSG, Laser evoked potentials, CRS-R	Sleep was more structured in MCS patients than in VS patients. VS patients also had more delayed N2P2 LEP component latency and reduced amplitudes than MCS patients.
Bedini et al./ 2016	Contribution of Period3 genotype on sleep quantity and consciousness	VS (44) MCS (27)	2 to 22 years	TBI (26) Vascular (45)	17-81	71	30F 41M	Period3 genotyping, 2pm to 9am PSG on 48 patients,	Period 3 5/5 genotype was found in 12.7% of the patients. These patients has significantly higher total sleep time and

	recovery in DOC.								total CRS-R scores than 4/4 genotype.
Arnaldi et al./ 2016	Prognostic value of sleep patterns/ structure	MCS minus (2) MCS plus (4) VS (20)	1 to 6 months post anoxia 1 to 12 months post TBI	Anoxia (8) Trauma (13) Haemorrhage (6)	18-81	27	9F, 18M	24-hour PSG, CRS-R at baseline and at follow up (6 to 38 months after PSG)	The more structured sleep is a valuable predictor of a positive clinical outcome. The predictive value of sleep integrity is higher than of age and baseline clinical condition.
STUDIES WITH A FOCUS ON TREATMENT									
Authors/ Year	**Main Outcome Measure**	**Diagnosis**	**Time since BI**	**Cause of BI**	**Age**	**N=**	**Sex**	**Methods used**	**Main Findings**
Sara et al./ 2009	Influence of Intrathecal	VS	6-12 months	TBI (2), Anoxia (1),	23-54	5	1F, 4M	CRS-R sores before and 6	Two of the patients showed marked

	baclofen treatment on recovery			Haemorrhage (2)				months after intrathecal baclofen pump insertion for treatment of spasticity.	improvement in CRS-R score, One patient had a marked improvement transiently but declined, two patients had improvement alertness only.
De Weer et al. /2011	Changes on motor activity and arousal levels following exposure to light irradiation	MCS (3) Coma (1)	24 to 40 days	TBI (2) Anoxia (2)	19-61	4	N/A	Actimetry recordings for 4 to 9 days. Video recording of environmental changes.	Increase of motor activity was observed in two patients with MCS due to TBI when they were exposed to light irradiation. Bight light may be used to re-establish circadian rhythms in DOC.

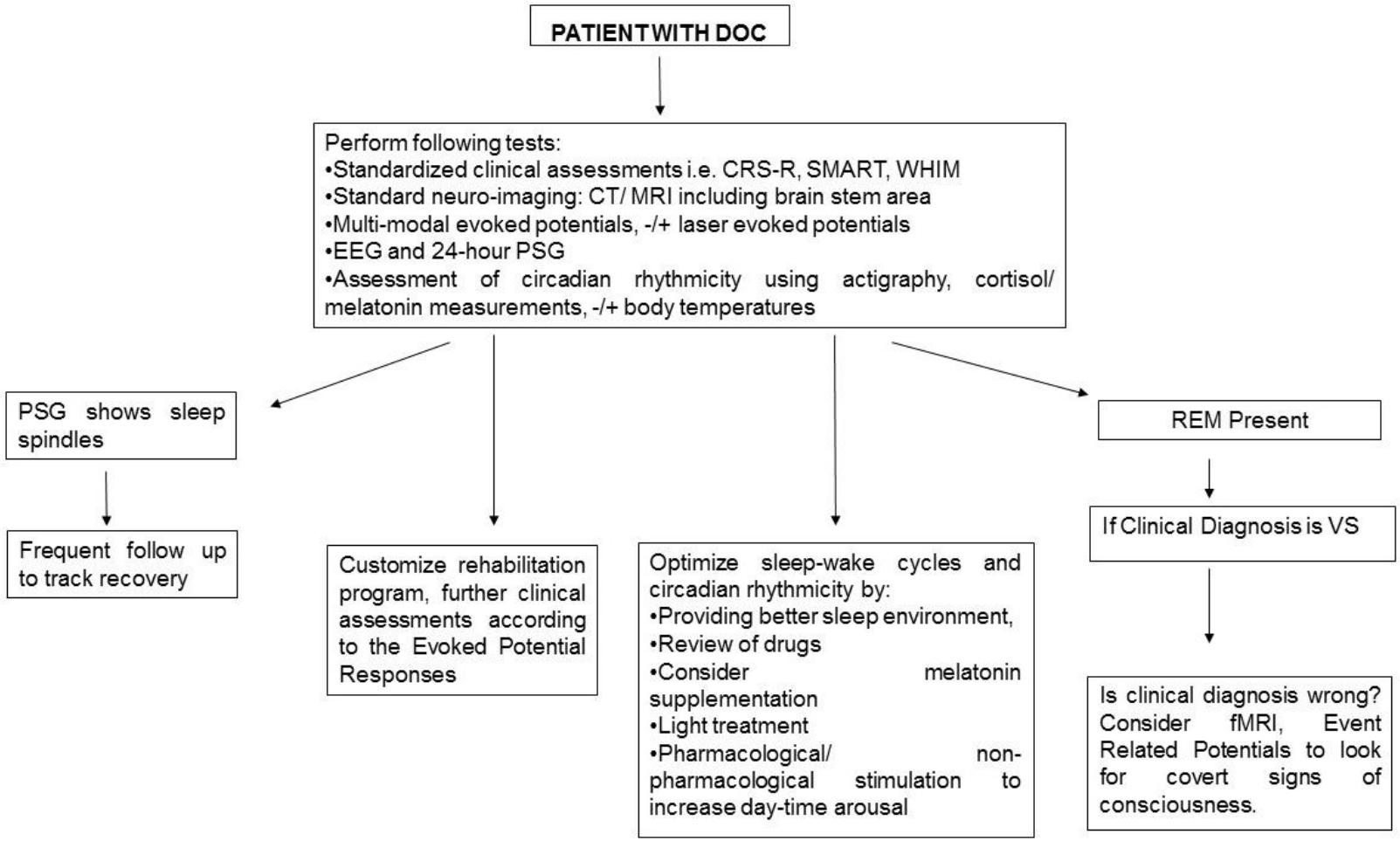
Blume et al. 2017	Changes in circadian rhythm measures and CRS-R following bright light treatment	VS (6) MCS (0) MCS_{exit} (2)	5-98 months	Non-traumatic	25-70	8	3F, 5M	Bright light treatment three times a day. CRS-R at baseline and post-intervention	Three of the eight patients had increase in CRS-R score and diagnosis change (VS to MCS)
REVIEW PAPERS									
Bekinschtein et.al/ 2009	Review Paper	Review of the factors underlying wakefulness							
Cologan et al. /2010	Review Paper	Sleep in disorders of consciousness							
De Salvo et al./ 2012	Review Paper	Neurophysiological studies in DOC							
Blume et al. /2015	Review Paper	Neuroimaging and sleep in DOC							

4.3.5 Key messages from literature after 2002

1. Body temperature measurements and actigraphic recordings may aid to assess circadian rhythmicity in DOC.
2. Melatonin circadian regulation is impaired in VS.
3. Light therapy and melatonin supplementation may be used to restore circadian rhythmicity.
4. Strategic use of drugs may be required to modulate arousal- circadian dynamics.
5. Presence of spindles indicate favourable prognosis.
6. Spindles and REM are much more common in MCS therefore may help to differentiate VS and MCS.
7. VS patients with REM may need to be investigated further to detect covert signs of consciousness.
8. Battery of neurophysiological tools which include PSG can be used for diagnostic accuracy, predicting recovery as well as identifying patients who may need customised rehabilitation programmes.
9. Novel investigations such as genotyping and rTMS may play role in further assessment of sleep and circadian rhythms in DOC patients.

4.4 CONCLUSION

This literature review illustrates the temporal progress of research on the area of sleep and circadian rhythms in DOC. In addition to the clinical assessment tools and neuro-physiological/ neuro-imaging techniques, it is reasonable to conclude that sleep and circadian rhythm studies will become part of battery of tools that will be used to differentiate VS and MCS but also to predict prognosis. Most importantly, optimizing the circadian rhythmicity may help to generate electrophysiological features of normal sleep such as REM and sleep spindles which in turn help to aid increasing brain functions and recovery. Based on evidence extracted from the literature reviewed the following flow chart may be used to help design clinical assessment and treatment protocols.


PATIENT WITH DOC
Perform following tests:
•Standardized clinical assessments i.e. CRS-R, SMART, WHIM
•Standard neuro-imaging: CT/ MRI including brain stem area
•Multi-modal evoked potentials, -/+ laser evoked potentials
•EEG and 24-hour PSG
•Assessment of circadian rhythmicity using actigraphy, cortisol/ melatonin measurements, -/+ body temperatures
PSG shows sleep spindles
Frequent follow up to track recovery
Customize rehabilitation program, further clinical assessments according to the Evoked Potential Responses
Optimize sleep-wake cycles and circadian rhythmicity by:
•Providing better sleep environment,
•Review of drugs
•Consider melatonin supplementation
•Light treatment
•Pharmacological/ non-pharmacological stimulation to increase day-time arousal
REM Present
If Clinical Diagnosis is VS
Is clinical diagnosis wrong? Consider fMRI, Event Related Potentials to look for covert signs of consciousness.

Chapter 5 : BACKGROUND WORK

The previous chapters of this thesis showed that PDOC is a complex pathological and, sleep is a complex physiological condition. Research studies indicated that sleep in PDOC is often deranged, but there has been no systematic attempt to treat it. Furthermore, most research studies were performed in a variety of clinical settings where clinical practices and environmental factors differ. For example, a patient who is in an intensive care setting soon after brain injury will have different contributory factors to his condition than another patient who is in chronic stage of his illness and resident in a nursing home setting.

It is clear from the previous chapter that a study which looks at the impact of normalizing circadian rhythm in patients with PDOC. However, before that we needed to know the answers to the following questions:

1. Is late recovery of awareness possible in PDOC patients?
2. What are their sleep wake patterns like?
3. What are the characteristics of environmental factors in our study setting?

The aim of this chapter is to summarize the three pieces of foundation work, which were performed to answer above questions.

5.1 EXPLORING LATE RECOVERY OF AWARENESS IN PDOC- A CROSS-SECTIONAL COHORT STUDY (Yelden et al., 2017a)

According to the Royal College of Physicians Guidelines on Prolonged Disorders of Consciousness, the likelihood of significant functional improvement diminishes over time and cause of brain injury is a strong determinant of outcome for both VS and MCS (Royal College of Physicians of London).

The objective of this work was to detect any improvement of awareness in prolonged disorders of consciousness in chronic stages. If present, such improvement may indicate a potential for naturally occurring neuro-plasticity, which can be potentially accelerated by treatment and/or therapy.

Thirty-four patients with prolonged disorders of consciousness (16 male and 18 female; aged 21 to 73) who were residents in the specialist nursing home section of the Royal Hospital for Neuro-disability (RHN) were included in the study.

The causes of brain injury were: anoxic brain injury in 15; severe subarachnoid haemorrhage (SAH) (The World Federation of Neurosurgeons, classification Grade IV and V) in 11; traumatic brain injury (TBI) in six; and massive ischaemic stroke in two participants.

All patients were initially diagnosed with SMART assessment while they were inpatients in the specialist neurological rehabilitation unit of the hospital and eventually were transferred to specialist nursing home for long term care. 27 (79%) of the patients had a diagnosis of vegetative state on initial SMART assessment, and the remaining seven (21%) had diagnosis of minimally conscious state. Time from brain injury to initial diagnosis varied between five and 38 months.

Time from brain injury to re-assessment was between 2 and 16 years. The main outcome measure for re-assessment was recovery of awareness, which was measured using CRS-R.

The results showed that although all patients remained severely disabled, 32% of the patients showed improvement of awareness of themselves or their environment (See Table 5-1, Table 5-2). Most of the late recovery of awareness occurred in patients with SAH (5/11, 45.5%). The age of patients within the late recovery group (Mean=45, SD=11.4) and non-recovery group (Mean+43.5, SD=15.5) was not statistically different (p=0.76).

Only one patient, who suffered from severe SAH and was previously in minimally conscious state, had progressed to the level of functional verbal communication and object use. The remaining six patients with MCS failed to improve.

Table 5-1: Demographic and clinical features of patients according to cause of brain injury (BI=Brain Injury, CRS-R=Coma Recovery Scale-Revised)

		TBI (n=6)	Anoxic (n=15)	Subarachnoid haemorrhage (n=11)	Ischaemic Stroke (n=2)	Total Sample (n=34)
Mean Age (SD)		38.5(10.9)	49.2(12.5)	53.6(9.2)	58(21.2)	49.2(12.4)
Mean Months BI to SMART (SD)		10.2(2.8)	12.9(10.1)	9.19(3.3)	8.5(2.1)	10.9(7.2)
Baseline SMART diagnosis	VS	4	15	8	0	27
	MCS	2	0	3	2	7
	MCS+	0	0	0	0	0
Years BI to CRS-R (mean±SD)		4.9±2.1	6.9±3.9	4.9±3.0	7.0±1.4	5.9±3.4
CRS-R Outcome (MCS+/MCS/ VS)		0/4/2	0/3/12	1/8/2	0/1/1	1/16/17
Improvement		33% (2/6)	20% (3/15)	46% (6/13)		32% (11/34)

Table 5-2: CRS-R Scores of patients with improvement of consciousness

Patient ID Sex/Diagnosis/ aetiology	Time BI to SMART (months)	Time SMART to CRS-R (years)	Auditory function	Visual function	Motor function	Oromotor/ Verbal function	Communication	Arousal	CRS-R Total Score
Patient 1 M/ VS/ SAH	7	9	Reproducible movement to command	Visual startle	Object manipulation	Vocalization/ Oral movement	Non-functional: intentional	Eye opening without stimulation	13
Patient 2 F/ VS/ SAH	8	2	Consistent movement to command	Visual pursuit	Flexion withdrawal	Oral reflexive movement	None	Eye opening without stimulation	12
Patient 3 F/ VS/ TBI	15	3	Reproducible movement to command	Visual pursuit	Flexion withdrawal	Oral reflexive movement	None	Attention	12
Patient 4 F/ VS/ TBI	12	2	Localization to sound	Visual pursuit	Abnormal posturing	Vocalization/oral movement	None	Attention	11
Patient 5 M/ VS/ Anoxia	12	2	Reproducible movement to command	Object localization/ reaching	Object manipulation	Oral reflexive movement	None	Attention	15
Patient 6 F/ VS/ SAH	9	10	Localization to sound	Visual pursuit	Localization to noxious stimulation	Vocalization/oral movement	None	Attention	13
Patient 7 F/ VS/ Anoxia	38	4	Reproducible movement to command	None	Flexion withdrawal	Vocalization/ oral movement	None	Attention	10
Patient 8 M/ MCS/ Infarct	7	5	Consistent movement to command	Object recognition	Functional object use	Intelligible verbalization	Functional: accurate	Attention	23
Patient 9 M/ VS/ Anoxia	10	2	Auditory startle	Fixation	Flexion withdrawal	Oral reflexive movement	None	Eye opening w/o stimulation	8
Patient 10 F/ VS/ SAH	12	2	Reproducible movement to command	Fixation	Object manipulation	Intelligible verbalization (lip read)	Non-functional: intentional	Attention	16
Patient 11 F/ VS/ SAH	10	2	Reproducible movement to command	Object recognition	Object manipulation	Vocalization/ oral movement	None	Attention	17

5.1.1 Contribution to thesis

First, this study showed that late improvements in awareness were not exceptional even in non-traumatic PDOC cases. Second, while it highlighted the importance of long term follow up of patients with disorders of consciousness, regardless of the aetiology, age and time passed since the brain injury; it also indicated that despite having profound brain injury and being in vegetative or minimally conscious state for such a long time, neuro-plasticity and axonal regrowth can still occur.

Currently most interventional research studies are performed in the more acute/ subacute stages of PDOC. Finally, these patients also have an additional advantage of being medically stable and being on established medication and feeding regimes. Hence, any changes in the clinical status during an interventional study are least likely to be due to other clinical factors such as resolution of an infection, seizures, dysautonomia, hydrocephalus or, changes made to the medication regimes. Therefore, patients who are in chronic stages of their illness may also be included in studies that are designed to explore effect of intervention on consciousness and/ or awareness.

5.2 OBSERVATION OF SLEEP-WAKE PATTERNS OF PATIENTS WITH PDOC

The aim of this work was to investigate the sleep patterns and arousal levels of patients with disorders of consciousness. Five patients with DOC were observed for 40 days during their inpatient stay in a specialist rehabilitation unit while undergoing assessment and rehabilitation. Clinical assessments (SMART, WHIM, Music Therapy Assessment Tool for awareness in DOC (MATADOC)) indicated that all 5 patients were in vegetative state.

Presence of the sleep state was identified using the physical signs of this condition, which include lack of movement, reduced postural muscle tone, closed eyes, and more regular and relaxed breathing, usually accompanied

with an increase in upper airway noise. Presence of awakening was simply identified by opening eyes.

Based on these criteria, sleep/ wake status was recorded every 4 hours; during therapy/ assessment sessions; and during physical nursing care. No changes were made to delivery of care or therapy/ assessment sessions.

On average patients had 223 four-hourly observations. Between 8am and 8pm, the patients were awake during 60% of the observations. Midnight and 4 am observations revealed that the patients were awake 30% of the time. Arousal levels were higher in the afternoon and evening hours.

Arousal levels were recorded during 74 therapy/ assessment sessions in total. While in 35% of the sessions patients were awake throughout the session, in 25% of sessions they were asleep most of the session or throughout the session. In the remaining 40% of sessions patients were awake more than half of the therapy session duration.

Arousal levels were recorded during 137 nursing care episodes. In 55% of nursing sessions patients were awake. In 8% of the nursing sessions they were asleep most of the session or throughout the session despite being exposed to high levels of stimuli provides during nursing care which involved re-positioning in bed, hoist transfers and personal care.

5.2.1 Contribution to the thesis

As a result of this simple observational study, it was concluded that sleep/ wake patterns of patients with prolonged disorders of consciousness were very abnormal possibly indicating presence of circadian sleep disorder.

Disturbed night-time sleep is likely to have a negative impact on rehabilitation and recovery of PDOC patients. Moreover, low arousal levels observed during assessment sessions, may contribute to misdiagnosis and missing subtle but conscious behaviours which can be used for rehabilitation. Improvement of

sleep-wake cycle is essential in PDOC, so the patients can be assessed, diagnosed and rehabilitated appropriately.

5.3 ENVIRONMENTAL CHARACTERISTICS OF PATIENT ROOMS AND SLEEP ENVIRONMENT (Yelden et al., 2015)

Fatigue, lethargy, day time reduced arousal level and low mood are common problems that are encountered in hospital and residential care settings. Most of the time, these problems are attributed to physical or if present, cognitive impairment of patients. However, very little attention is paid to sleep patterns. It has been previously demonstrated that sleep fragmentation caused by relatively brief interruptions, leads to measurable changes in daytime alertness, cognitive functioning, neuroplasticity, memory, psychological functioning and learning (McCoy and Strecker, 2011, Martin et al., 1997, Roehrs et al., 1994, Stepanski et al., 1987, Djonlagic et al., 2012). Daytime sleepiness and fatigue have been shown to be related to difficulties in sleeping (Goodchild et al., 2010). The commonest preventable causes of sleep disturbance in healthcare settings are background noise and light. Sudden increase in their levels cause interrupted sleep. Environments that are too hot or cold may also be disturbing.

The World Health Organization (WHO) and The Society of Light and Lighting published standards for ward-based hospital environments to minimise sleep disturbance.

According to the WHO Guidelines for Community Noise (Berglund et al., 1999), the noise levels in hospital rooms should be 35 dB or less. The recommended maximum intensity for individual events at the patient's bedside is 45 dB. Temperature and humidity should be maintained between 20-22 C° and 30-60 % respectively (World Health Organization. Regional Office for South-East Asia., 2002). The Society of Light and Lighting states that night

and observation illuminance in patient rooms should be below 5 lux and should not exceed 10 lux (2002).

Night time environmental characteristics of patient rooms, namely light, noise, temperature and humidity levels need to be optimised not only to provide comfortable accommodation but to improve sleep quality and consequently to minimize the problems that may be faced due to poor sleep.

Having established that the patients with prolonged disorders of consciousness have abnormal sleep wake patterns; there was a need to understand influence of environmental factors on their sleep-wake status. How much of this disturbance is due to extrinsic factors rather than intrinsic circadian sleep disorders?

This study aimed to obtain background information on light, noise, temperature and humidity levels of patient rooms which can affect the circadian rhythms and sleep-wake pattern of patients with DOC at the Royal Hospital for neuro-disability. As sleep hygiene is one of the most important treatment methods for circadian sleep disorders; understanding the environmental characteristics of sleep environment and, if not ideal, trying to optimize the sleep environment as part of planned interventional study was essential.

To examine the sleep environment of patients with DOC, measurements of environmental characteristics were recorded on three non-consecutive nights by data loggers in a 12-bedded ward. The obtained measurements were compared with the recommendations of the WHO and the Society of Light and Lighting.

Recordings were within recommended levels for light and humidity overnight. Light intensity levels were reasonably maintained within the recommended levels between 11pm and 7am. Mean temperature levels were higher than recommended. Average noise levels were above the recommended levels.

There were abrupt increases of light and noise levels which were high enough to cause sleep fragmentation.

Mean sound levels in all occupied rooms were consistently in excess of the 35 dB and therefore, not within the recommendations made by the WHO for healthy sleep environment in healthcare settings. Moreover, there were abrupt increases of sound levels as high as 84 dB which is equivalent to sound of an alarm clock at 2 ft distance. Hence, the 45-dB threshold of recommended maximum intensity for individual events at the patient's bedside was exceeded.

5.3.1 Contribution to the thesis

This study indicated that activities of the night staff significantly contributed to the increased noise levels in patient rooms as it is unlikely that the patients with disorders of consciousness would be the source of noise. Following this work, an internal review of the ward equipment and fixtures & fittings were performed by the estates maintenance department. Series of educational events were delivered for nursing and care staff on importance of avoiding sudden increases in light and noise levels at night, as well as on importance of sleep for our patients.

5.4 BACKGROUND WORK: CONCLUSIONS

1. Patients with PDOC who are in chronic stages of their illness may also be included in our interventional study which will be designed to explore effect of intervention on their consciousness and/ or awareness levels as their brains continue to have level of neuro-plasticity which was indicated presence of natural improvement of their consciousness over years/ decades. Moreover, these patients are usually medically stable and on established medication regimes.

2. Our simple observational study on sleep-wake states of patients with disorders of consciousness showed that their sleep/ wake patterns were very abnormal possibly indicating presence of circadian sleep disorder. However,

visual assessment of the subject is not enough for the characterisation of sleep stages. Physiologically, the electroencephalogram (EEG), the electromyogram (EMG) and the electrooculogram (EOG) provide a higher level of quantification in the description of the different sleep stages. Incorporation of detailed assessment methods examining the sleep stages and circadian rhythms are vital in design of our interventional study.

3. Our work on environmental factors in patients' rooms indicated that there was a need to provide education and training of night staff to raise awareness on importance of sleep and environmental factors and development of strategies to reduce noise levels at night with the objective of providing better sleep environment and reduce possible complications of interrupted sleep as part of interventional study.

Chapter 6 METHODS DEVELOPMENT

The main aim of this research project is to optimize sleep of people with PDOC using simple interventions and to assess effect of these on their consciousness levels. The introductory chapters showed that many techniques are available to assess sleep, circadian rhythm and levels of consciousness but in this particular patient population not all techniques are sensitive and practical. When designing an interventional study, which includes patients who cannot consent to research, are vulnerable in many ways and who require care for all their needs, the researchers need to choose techniques which are easy to perform, informative, repeatable, and least harmful to the patients.

Due to the clinical characteristics of patients in vegetative and minimally conscious states it was clear from the beginning that adjustment of standard techniques that are normally used for assessing circadian rhythm and obtaining neurophysiological data was needed. The presence of involuntary movements, an inability follow commands, an inability to express discomfort and pain were the biggest concerns. Therefore, several techniques were trialled in order to find out the most practical, comfortable and efficient ways of collecting data. This chapter will the describe the development of the methods used in this study, including the ethical issues, study design and development of data collections methods.

6.1 ETHICAL CONSIDERATIONS

This study involved individuals who do not have the capacity to consent for themselves. We followed the criteria for involving people who lack capacity to consent to participate in a research study, as laid down in the Mental Capacity Act (Health, 2005). We liaised with the ward managers/ nursing staff to identify a suitable consultee (someone who has known the patient when s/he had capacity and is willing to be consulted on what the patient's wishes and feelings would be towards participating in the project). The consultees were provided

with written information and any questions raised by them were answered. Signed assent was obtained.

This study conforms to the World Medical Association Declaration of Helsinki and ethical approval was obtained from National Research Ethics Service. (Research Ethics Committee ref: 11/LO/1233, Site Specific Assessment ref: 11/LO/2052)

6.2 STUDY DESIGN

This research study aims to find answers to following questions:

1. Do the patients in vegetative state and minimally conscious state maintain any circadian rhythm? If so, is it normal?
2. If their circadian rhythm is abnormal, can we optimize it by using simple and inexpensive clinical interventions namely blue light, melatonin and caffeine?
3. Could these interventions lead to improvement of consciousness/ brain functions measured using either clinical assessment tools and/ or neurophysiological investigations?

To be able to answer the questions above, randomized controlled trial (RCT) and cross-over trial study designs were considered.

RCTs are considered the gold standard for a clinical trial but they require two groups of patients who are assigned to treatment and control subgroups. Blinding is recommended when performing RCTs. RCTs are most suitable for the diseases with high number of patients and treatments which can easily be concealed for blinding. Number of patients who can be enrolled in a RCT trial and ways of blinding the interventions were considered. The number of people with DOC due acquired brain injury and medically stable in our hospital was too small to enable a robust RCT design. In addition to this limitation, some difficulties with blinding were identified. These were the use of placebo drugs

for people who lack capacity to consent, and difficulty of administering sham blue light treatment.

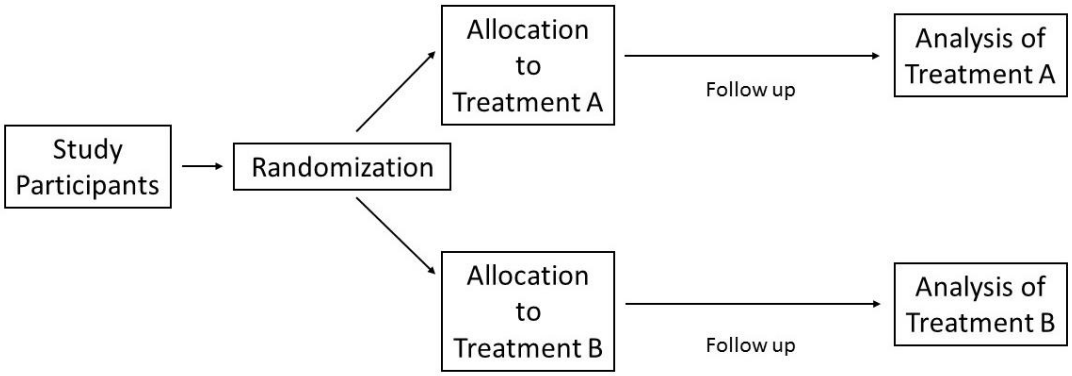


Figure 6-1: Randomized controlled trial design illustration

In cross-over trials there are usually two groups in parallel. The subjects who are first assigned to the treatment group and, after a brief interval of wash-out, are moved into the placebo /alternative treatment group and vice versa. The alternative treatment can be the usual practice. The subjects act as their own control at the end of the study.

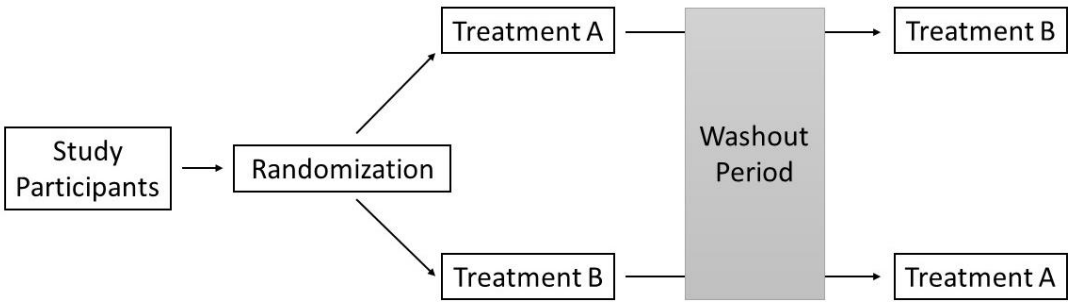


Figure 6-2: Cross-over study design

Due to the difficulties given for a RCT trial, cross-over design was explored in length. As normalization of circadian rhythmicity was not previously studied in

this patient group, treatment- wash out- alternative treatment design was rejected. The main reason for this was, uncertainty over the duration of effect once circadian rhythm is optimized. Therefore, the study was designed in a way that the participants still act as their own controls by utilizing a design which allowed us to look at individual participants and the group: Investigations performed twice to examine any fluctuations at baseline- treatment- repeat investigations.

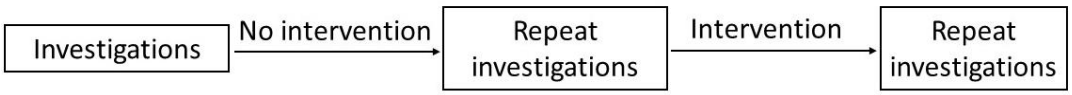


Figure 6-3: PhD study design

6.3 ASSESSMENT OF CIRCADIAN RHYTHM

Circadian rhythmicity can be demonstrated by assessing endocrine markers and physiological changes. In practice, the most widely used endocrine markers are cortisol and melatonin; and most commonly used physiological marker is core body temperature. Therefore, these measurements were considered predominantly to assess circadian rhythmicity and explored in detail to choose best method of data collection for our research participants.

6.3.1 Endocrine markers of circadian rhythm

Cortisol is a corticosteroid hormone that is produced in the adrenal cortex. Cortisol shows a circadian rhythmicity through multi-synaptic suprachiasmatic nucleus-adrenal pathway (Buijs et al., 1999). Its levels can be measured in serum and saliva; however, several factors influence the secretion of cortisol such as physical and psychological stress and diet. Although cortisol measurement was considered as a method to assess circadian rhythm, due to the influencing factors it was identified as a less sensitive indicator of circadian rhythm in our patient population as people with PDOC are immobile, artificially

fed and suffer from many other physio-pathological processes such as dysautonomia, pain and discomfort due to immobility and increased muscle tone.

Melatonin levels can be measured in plasma or saliva, and levels of its metabolite, 6-sulfatoxymelatonin, in the urine. Although melatonin levels can be influenced by posture, exercise, caffeine and certain drugs such as non-steroid anti-inflammatory drugs (NSAIDs) and beta blockers, its rhythmicity remains reasonable preserved. Therefore, it was identified as a choice of endocrine marker for this study.

Patients with DOC may have had a urinary catheter inserted in the acute phases of their illness, however, they are usually without the urinary catheter in the chronic phase and urinary and faecal incontinence is managed with using incontinence pads. Therefore, collection of urine for melatonin measurement was not practical. Insertion of a urinary catheter carries risk of urinary tract infection as well as discomfort. Similarly, plasma collection for melatonin measurement was impractical, as it required the invasive procedure of venous cannula insertion. Hence, it was concluded that saliva collection procedure was the most sensitive, practical and risk-free way of measuring melatonin levels as a marker of circadian rhythm in the patients with DOC.

Salivary flow rate is influenced by many factors such as hydration state and body positioning. Saliva samples can be efficiently collected when person is sitting upright with the head slightly tilted forward and the eyes open. Time of the day (peak values in the afternoon), subject's medical status and medications can also influence saliva production. In addition to these factors, patients with DOC often sleep mouth open, often have a sucking reflex and clench their teeth tightly if any object is placed in the oral cavity.

Saliva can be collected by using four main methods: draining, spitting, suctioning and swab (Navazesh, 1993). Occasionally draining may be possible

in the patients with DOC. This requires the patients to have hyper-salivation which may lead to spontaneous drooling in a sitting position. However, this is not possible in all patients and highly dependent on the position of the patient. Spitting entails active effort and involvement of the patient, hence was not possible to use in this study. Suction necessitates insertion of a test tube into the mouth floor, which may be hazardous in DOC patients due to not being able to co-operate, sucking reflexes and clenching teeth. The swabbing method requires use of pre-weighted gauze, cotton wool or sponge.

Owing to the difficulties listed above it was imperative to choose a collection swab which was safe to use, absorbent enough and also free from pre-treatment with chemicals which could interfere with melatonin assays in the laboratory. Therefore, advice from the scientists who perform the assays, dental practitioners, nursing staff and speech and language therapists was sought. Several types of collection swabs were initially trialled on healthy subjects and then on patients for absorbency and suitability with laboratory processing.

Figure 6-4: Oral foam swabs

Oral swabs with foam head are commonly used for the oral care of patients in the hospitals. These swabs were not suitable in our patient group due to the possibility of detachment of the sponge head from the stick during use.

Figure 6-5: Cotton bud swabs

Large cotton bud swabs were safe and easy to use in our patients. However, when sent to laboratory for melatonin testing, the results were unsatisfactory due to the interference of an unknown chemical with the melatonin essay.

Figure 6-6: Salimetrics paediatric oral swabs

Salimetrics paediatric oral swabs (Salimetrics, USA) are long enough to allow one end of the swab to be held while it is in the mouth and it is made of a durable polymer, which withstands chewing. The volume of the sample recovered is typically in the range of 200-1000 µL which is sufficient for

melatonin essays. When tested for melatonin in the laboratory there was no chemical interference. Hence, these swabs were chosen to be used for saliva melatonin measurements in our study.

Having decided on what to measure and how to collect the samples, we needed to decide when and how often to collect samples.

In practice, measurement of dim-light melatonin onset (DLMO) is commonly used, as 24-hour melatonin sample collection is time-consuming and also inconvenient for the subject. DLMO reflects the phase of the circadian rhythm only if the individual's phase is approximately normal and measured over more than one cycle (Hofstra and de Weerd, 2008). However, due to the severity of brain injury sustained people with DOC are likely to have free running circadian rhythms and therefore, abnormal phase and peak times. Hence, frequent measurements of melatonin levels throughout a 24-hour period were a necessity in order to assess peak times and even longer periods of sample collection to assess rhythmicity. Hence, it was decided that in our study, samples should be collected every 4 hours, for two days.

6.3.2 Physiological markers of circadian rhythm

Circadian pacemaker in the suprachiasmatic nucleus not only generates and maintains circadian rhythms of sleep/wake cycle and secretion of hormones; but also, many physiological features such as blood pressure and body temperature. Measurement of the temperature circadian rhythm is commonly used, usually by means of measuring core body temperature (CBT). CBT rhythm is characterized by nocturnal decline reaching its minimum at approximately 5am and reaching its maximum during the afternoon around 5pm. This rhythm reflects the combined effects of the body clock, sleep, and physical and mental activity and can be described by a cosine curve. The regulation of body temperature rhythms is provided by the circadian pacemakers and thermoregulatory system located within the hypothalamus and influenced by several external factors such as activity levels, external

temperatures and perspiration levels (Refinetti and Menaker, 1992, Weinert and Waterhouse, 2007). It was also shown that melatonin has suppressing effects on core body temperature (Deacon et al., 1994, Deacon and Arendt, 1995).

CBT can be measured by a variety of methods (e.g. intravascular, tympanic, bladder, rectal, oesophageal). These methods are invasive, some may cause discomfort or even wake the patient up during data collection procedure, and consequently negatively influence the circadian rhythms. Most importantly, as patients with DOC are unable to express pain and discomfort it was necessary to find a method which measured changes in body temperature reliably, did not discomfort, did not interfere with personal care or the feeding regime of patients.

Proximal skin temperatures measured at the thorax, breast, abdomen, and thigh have been found to follow the same circadian rhythm as rectal temperature (Krauchi and Wirz-Justice, 1994). Bogh et al. studied the circadian rhythms of temperature measurements from axilla, chest wall and rectum and showed that chest wall and axillary sites gave similar results to rectal measurements but these were both more variable and up to 8 hour later than those for rectal temperature especially in physically active subjects (Bogh et al., 1994). It was also shown that conditions with and without sleep had different effects on the core, proximal and distal body temperatures (Kräuchi, 2002, Kräuchi et al., 2000). Interestingly it was shown that distal and proximal skin temperature increased in parallel after lights out while the core body temperatures fell.

Collecting continuous body temperature measurements require either wired or wireless connection to a recording device or use of battery powered loggers that store data in a memory unit for later download. Use of a wired recording device was not practical for our patients as they were often repositioned in bed,

hoisted to/ from wheelchair and taken out of the ward during the day for participation social activities.

The iButton (Maxim Integrated, USA) has been found to be a reliable and valid measure of body temperature and its application to human skin was shown to be comfortable and tolerable with no significant adverse reactions. Moreover, the iButton is an inexpensive, wireless data logger that can be used to obtain a valid measurement of human skin temperature. It was suggested as a practical alternative to traditional measures of circadian rhythms in sleep/wake research (Hasselberg et al., 2013). It has been used previously to examine circadian rhythms in PDOC patients (Bekinschtein et al., 2009b).

DS1921H high resolution thermochrone ibuttons (Maxim Integrated, USA) measure temperature in 1/8°C increments between +15 and +46 Celsius, at user-programmable intervals from 1 to 255 minutes. Due to its ease of use and reliability ibuttons were chosen as method of recording body temperature as physiological marker of circadian in our study.

Figure 6-7: DS1921H high resolution thermochrone ibuttons

6.4 ASSESSMENT OF SLEEP

Polysomnography is the only tool that can reliably be used for assessment of sleep structure in patients with DOC, but both acquisition and analysis of EEG data is challenging in this patient group. First of all, recording of good quality EEG signals is difficult due to spontaneous movements, excessive sweating, drooling, interference from other electrical devices such as feeding pumps and complex care which require frequent re-positioning of patients to prevent

pressure sores and hoisting for transfers. These may contribute to displacement of electrodes, skin irritation as well as occurrence of artefacts. It is ideal to have long polysomnography recordings for example over 48 to 72 hours, but when this was trialled on two patients, we were able to identify some complications related to this technique, as there was evidence of skin irritation and interference with the patient's personal care which could potentially lead to distress of the patient, carers and relatives. Therefore, 24-hour recording of polysomnography was judged to be an appropriate and safe method of performing sleep studies in our patient group.

Analysis of polysomnography recordings are based on sleep stage scoring which is based on an epoch by epoch approach. Identification of wakefulness, sleep stages 1 & 2, slow wave sleep (stage3/4) and REM is well defined in healthy individuals. Although there are established criteria to report polysomnography in healthy subjects, this is not straightforward in DOC. The EEGs recorded in patients with DOC are heterogeneous in appearance, often because of the varied neuroanatomical abnormalities seen in this patient group due to injuries suffered. The visual inspection of the baseline EEG recordings in these patients tends to show differing proportions of theta and delta activity, impoverished-low amplitude and/or devoid of distinguishing features which is especially a feature of recordings taken from patients in vegetative state. Therefore, it was necessary to document the subjects' baseline EEG whilst awake, before the start of the polysomnography recording. In this way, we would be able to compare the initial awake background activity with any overall change in EEG background, which was taken as in indicator for change in state (sleep onset/sleep). Following this, it was necessary to adapt sleep staging criteria as sleep does not always match the standard scoring criteria for this patient group (Cologan et al., 2010, Cologan et al., 2013).

6.5 BEDSIDE ASSESSMENT OF CONSCIOUSNESS

In the introduction chapter of this thesis, three different bedside assessment tools were described: SMART, WHIM and CRS-R.

WHIM was identified as a useful tool in tracking recovery of patients and it was shown to have good inter-rater and test-retest reliability after a training was given to the assessors. However, the stimuli given to assess responses of patients are not well-prescribed.

The SMART and CRS-R both apply similar stimuli and specify the method of application. CRS-R is an easily performed and reliable assessment tool (Seel et al., 2010). CRS-R includes all the modalities of the SMART assessment with the exclusion of the gustatory and olfactory sensory stimulation techniques. Both, assessments tools use well-described detailed instructions to give stimuli as well as to score responses observed. While a single SMART assessment takes approximately one hour to complete, a single CRS-R assessment takes about 20 mins. SMART also requires training of assessors through a training and certification program, while CRS-R requires training through video.

For this research project, CRS-R was chosen as the bedside tool to assess consciousness levels. In addition to the advantages given above, the researchers who performed the data collection were already trained and experienced in using it.

6.6 NEUROPHYSIOLOGICAL ASSESSMENT OF CONSCIOUSNESS

As shown in the introduction chapter of this thesis, event related potentials can give information on assessing brain responses to specific stimuli such as auditory tones, words or pictures. They can be used in their familiar environment and at the bedside of patients, eliminating transportation to a different facility. For this research study, all ERP methods were considered. Active paradigms were considered to be too complex for our patient group, as

they require following instructions and active involvement of the patient- such as counting deviant stimuli amongst many standards. The paradigms which use visual stimuli were discarded as observation of participants indicated that most patients would not be able to keep their eyes open for the duration of testing or able to focus their eyes on the screen. There was also possibility of some patients suffering from cortical blindness especially following hypoxic brain injury.

Therefore, auditory passive ERP paradigms were chosen as a method of assessing consciousness neuro-physiologically. Although, deafness after a brain injury is rare, it is possible after traumatic brain injury or due to other medical conditions such as ear infections, ear wax and degeneration. Therefore, auditory evoked potential testing was planned to exclude any hearing impairment and false-negative ERP results. A simple oddball paradigm to measure mismatch negativity and a more complex paradigm to measure brain responses to a meaningful and significant stimulus- subjects own name, were developed. Using EEG caps during ERP experiments was identified as the easiest and quickest way of recording EEG responses.

6.7 DEVELOPMENT OF INTERVENTION TECHNIQUE

We aimed to optimize circadian rhythm of patients with disorders of consciousness by improving sleep of patients; by achieving light- dark cycle in their usual environments; by using pharmacological agents to improve circadian rhythmicity and finally by using pharmacological agents to increase daytime arousal.

6.7.1 Improving sleep environment of patients

As identified in the background work on environmental factors in patients' rooms, each participant's room was assessed prior to the interventional study in order to improve sleep environment of patients. Nine of the patients who participated in the study were residents in single rooms. One patient was a resident in a room where two other patients with disorders of consciousness

were also residents. The rooms were not in a close proximity to the nurses' work station. Moreover, nursing staff were informed that the patients were participating in a research study aiming to examine the sleep-wake patterns. Based on the information gathered in the background work; they were asked to keep the interruptions to minimum, and to avoid switching the main lights on unless it was necessary to do so. A short training session was provided on use of night observation lights and reduction of noise.

6.7.2 Optimisation of light-dark cycle

We aimed to achieve optimization of light-dark cycle by;

A) ensuring the disturbance at night is kept to a minimum as explained above,
B) ensuring the lights were off, and windows and doors fully obscured at night, if safe to do so,
C) Using light therapy boxes in the mornings.

Blue light therapy is the only treatment that is able rapidly to shift the biological clock. Blue-light therapy can be used in treatment of sleep disorders, circadian rhythm disorders, dementia and seasonal affective disorders. Light treatment can be provided with light therapy lamps are specifically manufactured to provide blue light.

Bright light administration start time was agreed as 8am which is the time of shift changeover between night and day nursing staff to prevent night staff's exposure to bright light.

Figure 6-8: Philips Energy Light Lamp

We also considered use of eye shields at night to minimize light exposure at night. However, as the illumination levels in the patients' rooms were within the recommended levels, eye shields were not used. Other factors that influenced our decision were a wish to avoid additional work load of donning and doffing of patient eye shields in the evening and morning by nursing staff and a wish to avoid risk of unintentional but prolonged use of eye shields in the morning hours which could negatively affect exposure to bright day light and blue light therapy in the mornings.

6.7.3 Pharmacological agents

6.7.3.1 Melatonin

Melatonin is licenced for treatment of sleep onset insomnia and delayed phase syndrome for adults over 55 years of age in the UK. The recommended clinical dose for this indication is 2 mg modified-release tablets once daily 1-2 hours before bed time for up to 13 weeks. It should be avoided in patients with autoimmune disease, hepatic impairment, pregnancy and breast feeding. A liquid preparation for paediatric use is also available for off licence and research purposes.

In the early research of 1970s and 1980s it was shown that up to 300 mg of melatonin daily was administered without any toxicity in humans. Therefore, it was concluded that melatonin can be used in clinical trials with safety. Most

trials however, shown that much smaller doses of melatonin can induce phase shifts in humans.

Melatonin is usually given 1-2 hours before usual bedtime around between 8 and 10pm. The patients with DOC usually have poor sitting tolerance and they are usually in bed before 6pm in the afternoon. Evening dose of their prescribed medications usually given between 6 and 8 pm and they may have visitors until 8pm. The main lights in their rooms are turned off after 8pm. Therefore, in order to synchronize environmental cues, light-dark cycle of their environment and sleep-wake cycle, melatonin administration time was set as 6pm in the evenings.

Acute administration of melatonin acutely lowers the body temperature- in contrast to acute elevation of body temperature with bright light. Therefore, combination treatment with bright light and melatonin would be more effective in the management of circadian rhythm disorders.

6.7.3.2 Caffeine

Caffeine, the third and final interventional component used in this study, is a weak psycho-stimulant. In addition to this it has circadian effects, especially on melatonin synthesis in humans, inhibiting melatonin production due to its adenosine receptor antagonist properties (Ribeiro and Sebastião, 2010, Shilo et al., 2002, Wright et al., 1997). Caffeine increases levels of wakefulness and counteracts deteriorations in task performance related to sleep deprivation (Snel and Lorist, 2011). It has been shown that subjects who suffer from jet lag achieve resynchronization of their circadian rhythm earlier than placebo group controls when given caffeine to restore wakefulness and melatonin to encourage sleep (Pierard et al., 2001, Beaumont et al., 2004). However, as with other regulators of circadian rhythm the timing of caffeine administration is important. For example, if caffeine is consumed late in the day it will negatively affect night sleep resulting in daytime sleepiness the next day.

Caffeine was chosen for its stimulant properties as well as its effect on melatonin secretion. It has no significant side effects. The timing of caffeine administration was set to be in the early hours of day in order to maximize its effect during the day but prevent its possible carry over effect to night time. As the research participants were not used to consuming caffeinated products since their brain injury, a prescription schedule which provided gradual increase of caffeine given was designed.

6.8 CHAPTER SUMMARY

Methods development chapter of this thesis shows that designing an interventional study is challenging, as the researchers need to carefully consider number of ethical and methodological issues. There are only few studies which examined effect of intervention in PDOC patients, especially when given over a period of weeks rather than days. In addition to this, as the literature review chapter previously indicated, there are limited number of literature examined the circadian rhythms in PDOC at baseline. Only one study examined the effect of light treatment in six PDOC patients for one week (Blume et al., 2017). Although, it was found that bright light treatment could boost circadian rhythmicity in some PDOC patients whether their sleep is also improved or not, is unknown. Therefore, we needed to design a study which investigated the effect of interventions (optimization of sleep environment, blue light, caffeine and melatonin treatment) on sleep and circadian rhythms which was given over weeks. In addition to this, we designed a robust methodology which examined possible fluctuations of sleep and circadian rhythms at the baseline in order to reduce possibility of false positive results. Furthermore, we wanted to test if the circadian rhythm changes after intervention maintained or not. The following chapter provides detailed information on methods used in this PhD research study.

Chapter 7 METHODS

The main aim of this research project is to investigate effect of light, melatonin and caffeine treatment on circadian rhythm and sleep of patients with PDOC. However, in the first instance, we needed to know how their circadian rhythm and sleep were at their baseline. Therefore, this study was performed in 2 stages - baseline assessment of sleep and circadian rhythm followed by the interventional study, as outlined in Table 7.1.

There has been no formal calculation of sample size. For this study we estimated that only one or two participants at a time can be studied due to the complex nature and timeline of the study which required participants to be enrolled in the study for 12 to 14 weeks. This meant that a maximum of 10 participants could be included in the study per year. Therefore, we estimated that over two years of data collection, a sample size of 20 would be reasonable and achievable. We recruited 17 patients with PDOC in the baseline studies using convenience sampling. Twelve of these 17 patients then were enrolled in the interventional study. Ten of the 12 patients were able to complete the interventional study.

For the patients who were participated in the baseline stage only, the study duration was one week. For the 10 patients who completed the interventional study, the study duration was 13 weeks.

Table 7-1: Study time points

<table>
<tr><th>Week 1</th><th>Week 5</th><th>Week 6-9</th><th>Week 10</th><th>Week 11</th><th>Week 13</th></tr>
<tr><td>BAEP, EEG</td><td rowspan="2">Baseline-2 Investigations</td><td rowspan="2">Intervention</td><td>Intervention</td><td rowspan="2">24 Hour saliva collection for melatonin</td><td rowspan="2">24 Hour saliva collection for Melatonin</td></tr>
<tr><td>Baseline-1 investigations</td><td>Post-Intervention Investigations</td></tr>
</table>

The baseline investigations included BAEP, 30-minute EEG, CRS-R, 24-hour PSG and 48-hour saliva melatonin levels for all patients (n=17). We also aimed to collect body temperature measurements data as a supplementary circadian rhythm marker.

The patients who proceeded for the interventions also received ERP studies using auditory oddball experiments for mismatch negativity responses and subject's own name paradigms. All tests with the exclusion of BAEP and 30-minute EEG were repeated 4 weeks later to see if there was any significant fluctuation of sleep, circadian rhythm, bedside clinical assessments, mismatch negativity and subject's own names responses on neurophysiological tests. The same set of tests were repeated once more, during the last week of interventions, to evaluate the effect of interventions given on sleep, circadian rhythm and consciousness levels. Once the interventions stopped, further saliva melatonin measurements were performed 3 days later and 3 weeks later, to see if the effect of interventions on circadian rhythm was maintained.

This chapter aims to give detailed information on the methods used in this research.

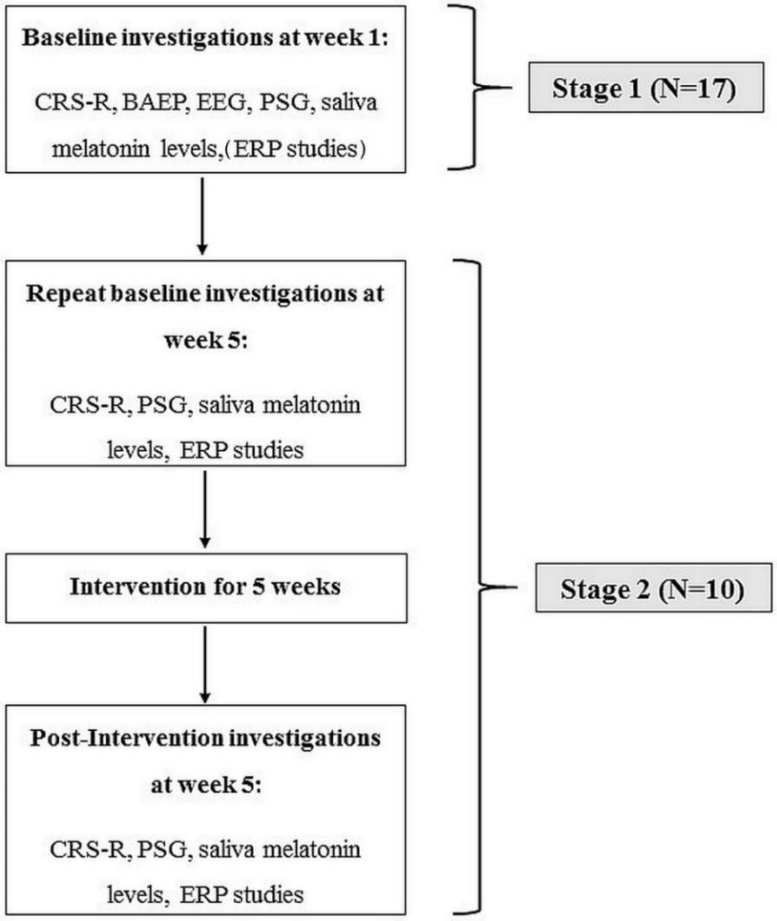


Figure 7-1: Study protocol

7.1 BASELINE METHODS

7.1.1 Setting and patients

This study was conducted in rehabilitation and long-term care units of Royal Hospital for Neuro-disability (RHN), Putney, London. The rehabilitation unit admits patients with PDOC for a period of 3 to 6 months to provide specialist diagnostic assessments, disability management and rehabilitation. The long-term care units of the hospital provide specialist nursing care for people with profound brain injuries.

Patients are usually admitted to hospital's neuro-rehabilitation unit from acute healthcare settings. During this admission, the patients may not be in a medically stable condition. They often require treatment for spasticity,

involuntary movements, dysautonomia and seizures; specialist investigations such as EEG, computerized tomography and/or magnetic resonance imaging of brain; and even surgical procedures such as cranioplasty and ventriculo-peritoneal shunt insertion for treatment of hydrocephalus. Their feeding regimen is being optimized and if possible decannulation of tracheostomy is attempted using weaning protocols. Only once medical stability is achieved, patients undergo assessments of consciousness and the diagnosis is confirmed. The clinical care, assessments and rehabilitation are provided by multi-disciplinary teams led by consultant doctors in rehabilitation medicine.

Following assessment and disability management at the neuro-rehabilitation unit, patients are usually discharged to specialist long term care settings which include specialist nursing home section of the RHN. The majority of patients in the specialist nursing home of RHN had been discharged from its neuro-rehabilitation unit. While they are residents in the specialist nursing home their medical stability is maintained by specialist nursing care, medical care provided by general practitioners with input from neuro-rehabilitation consultants as well as, neurology, neuro-surgery and other specialists as required.

In the specialist nursing home, the patients are given regular sensory stimuli which are provided in sensory rooms and are exposed to art and music therapy sessions as well as to regular social events. They are provided with maintenance level therapy input to maintain good posture and to prevent secondary complications such as development of contractures and pressure sores. Specialist nursing care of the patients with disorders of consciousness entails management of artificial feeding and hydration via enteric tubes; management of tracheostomies if present; postural management with use of specialist support equipment, regular re-positioning as well as bladder and bowels management. Patients are subject to further assessments if a change in their consciousness state is suspected.

The inclusion criteria were:

- patients with diagnosis of VS or MCS made by expert clinicians using validated assessment tools with robust psychometrics such as SMART and CRS-R;
- being medically stable,
- predicted to remain in RHN for the duration of the study;
- intact brain stem-auditory evoked potentials in at least one ear.

Exclusion criteria for the study were:

- uncertain diagnosis or diagnosis made without the use of validated assessment tools;
- PDOC secondary to progressive neurological condition;
- medical instability;
- oral hypersensitivity;
- very large skull defects;
- BAEP indicating hearing impairment in both ears;
- excessive involuntary movements or agitation;
- due to be discharged from RHN and thus not likely to complete study
- Baseline EEG showing frequent/ ongoing seizure activity of brain.

In order to identify potential candidates for the study, case notes of all the patients with disorder of consciousness were screened for diagnostic information and predicted length of stay at the RHN. Following this, a second stage face to face screening was performed to identify patients with medical instability, oral hypersensitivity, excessive involuntary movements and large skull defects. Patients who fit the above criteria underwent BAEP and EEG.

A total of 17 patients (7 female, aged 30-73) with PDOC (5 VS/ UWS, 12 MCS) were included in the baseline studies which aimed to investigate the state of circadian rhythms and sleep patterns in these patients. Eight of the 17 patients had suffered from anoxic brain injury. Other aetiological causes for the brain

injury included trauma (n=3), cerebrovascular accident (CVA) (n=3), mixed pathology (n=2) and vasculitis (n=1).

12 of the 17 patients who were residents in the long-term care unit, and therefore likely to remain at RHN for the duration of the study, were included in the interventional study. Out of 12, one of the residents died due to newly diagnosed malignancy and another was excluded due to excessive movement artefacts observed on baseline EEG/ PSG data, which rendered neuro-physiological tests unsatisfactory.

Time since the brain injury varied between 6 months and 9 years. At the time of study all patients were medically stable and established medication regimes. Seven out of 17 patients were being fed during the day, and the remaining during evening/night hours. All patients required turning/ repositioning every 4-6 hours to prevent pressure sores. Five of the patients were in a shared room where two or three other patients with PDOC also stayed. The remaining 12 patients were in single rooms.

7.1.2 Investigations to examine clinical status of patients

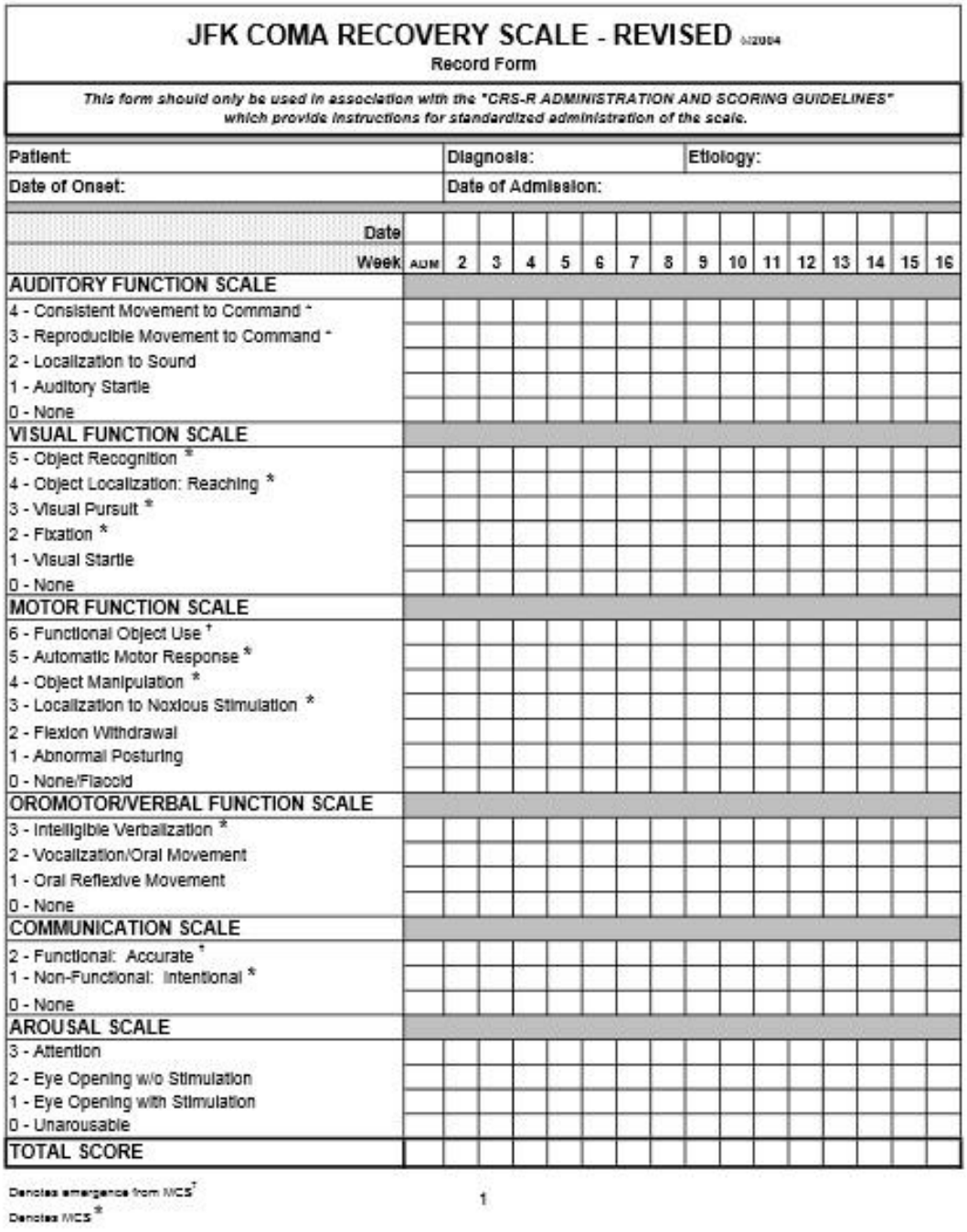
Electronic and paper based clinical files were reviewed for all patients. Demographic variables (age and sex), cause of brain injury (e.g. traumatic, anoxic, subarachnoid haemorrhage, ischaemic stroke), time from brain injury to diagnostic assessment at the neuro-rehabilitation unit and the outcomes of the initial diagnostic assessments were recorded on Excel (Microsoft Office 365, 2016). Significant clinical episodes, results of brain imaging studies, medications, feeding regime were documented.

7.1.2.1 Coma Recovery Scale- Revised assessments

All patients who were included in the study were re-assessed using CRS-R at the beginning of each investigations week by two clinicians who were experienced in clinical assessments of patients with disorders of consciousness for confirmation of diagnosis.

The re-assessments were undertaken in a quiet, well lit room while patients were in the sitting position. CRS-R was chosen as the method of assessment as it is a quick and reliable assessment tool for screening purposes. CRS-R includes all the modalities of the SMART assessment with the exclusion of the gustatory and olfactory sensory stimulation techniques.

One of the investigators was not involved in the initial review of case notes and was blinded to the initial diagnosis of the patients. CRS-R scoring sheets were completed upon consensus of both clinicians on patients' responses during the assessment. In a few occasions, where there was disagreement between the assessors on the responses elicited, the CRS-R scores were recorded for the lower assessment. For example, if one of the clinicians did not agree on the presence of a consistent movement to command, this was scored as "not present".



JFK COMA RECOVERY SCALE - REVISED 5/2004

Record Form

This form should only be used in association with the "CRS-R ADMINISTRATION AND SCORING GUIDELINES" which provide instructions for standardized administration of the scale.

Patient:	Diagnosis:	Etiology:
Date of Onset:	Date of Admission:	

	Date															
	Week	ADM	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
AUDITORY FUNCTION SCALE																	
4 - Consistent Movement to Command *																	
3 - Reproducible Movement to Command *																	
2 - Localization to Sound																	
1 - Auditory Startle																	
0 - None																	
VISUAL FUNCTION SCALE																	
5 - Object Recognition *																	
4 - Object Localization: Reaching *																	
3 - Visual Pursuit *																	
2 - Fixation *																	
1 - Visual Startle																	
0 - None																	
MOTOR FUNCTION SCALE																	
6 - Functional Object Use †																	
5 - Automatic Motor Response *																	
4 - Object Manipulation *																	
3 - Localization to Noxious Stimulation *																	
2 - Flexion Withdrawal																	
1 - Abnormal Posturing																	
0 - None/Flaccid																	
OROMOTOR/VERBAL FUNCTION SCALE																	
3 - Intelligible Verbalization *																	
2 - Vocalization/Oral Movement																	
1 - Oral Reflexive Movement																	
0 - None																	
COMMUNICATION SCALE																	
2 - Functional: Accurate †																	
1 - Non-Functional: Intentional *																	
0 - None																	
AROUSAL SCALE																	
3 - Attention																	
2 - Eye Opening w/o Stimulation																	
1 - Eye Opening with Stimulation																	
0 - Unarousable																	
TOTAL SCORE																	

Denotes emergence from MCS†

Denotes MCS*

1

Figure 7-2: Coma Recovery Scale-Revised Scoring Sheet

7.1.2.2 Brainstem auditory evoked potentials

Brainstem Auditory Evoked Potentials (BAEP) are useful in assessing peripheral auditory nerve, auditory portion of cranial nerve VIII (CN VIII) and auditory pathways in the brainstem as they provide closely correlated information between specific auditory waveforms and structures in the brain stem (Daube and Rubin, 2009). They occur within the first 10 milliseconds of stimulus. Patients with severe brain injuries and PDOC may have had an additional injury to auditory pathways which might have been missed or not assessed at the acute stages of their illness. Therefore, prior to using any auditory event related potential investigations to detect signs of conscious information processing or any changes in the brain activities in reaction to given stimuli (simple tones, names etc.), it is important to make sure that auditory pathways are functionally intact at least on one side. Also, any hearing impairment may lead to false negative outcomes in clinical assessments as all structured assessment clinical tools use auditory stimuli to elicit responses. Therefore, we performed BAEP testing for our research participants at the start of study.

To correctly interpret our BAEP results, and later on auditory stimuli ERP investigations results, a brief summary of how auditory pathways are structured and the BAEP waves are generated will be given here.

The auditory system begins with the peripheral auditory apparatus, where the pressure changes from vibrations generated by sound waves are sensed by hair cells in the organ of corti (Blum and Rutkove, 2007). The hair cells then communicate with bipolar neurons of cochlea which then project to the pontomedullary junction via the auditory portion of CN VIII. Wave 1 of the BAEP is generated by the distal action potential of the CN VIII and seen as a negative potential at the ipsilateral ear electrode which appears about 1.5 milliseconds after the stimulus. To reliably assess the central auditory conduction wave 1 needs to be present but will only be present ipsilaterally. The axons of cochlear nerve then enter the medulla and project to dorsal and

ventral cochlear nuclei where the Wave II of the BAEP is generated. After this point, the input from cochlear nuclei is transmitted to ipsilateral superior olivary nucleus where Wave III is produced, but also to the contralateral superior olivary complex. Therefore, superior olivary nuclei receive auditory inputs from both sides. Signals then projects proximally to lateral lemniscus (Wave IV), inferior colliculus (Wave V) and finally to medical geniculate nuclei of thalami and superior temporal gyri (Waves VI and VII).

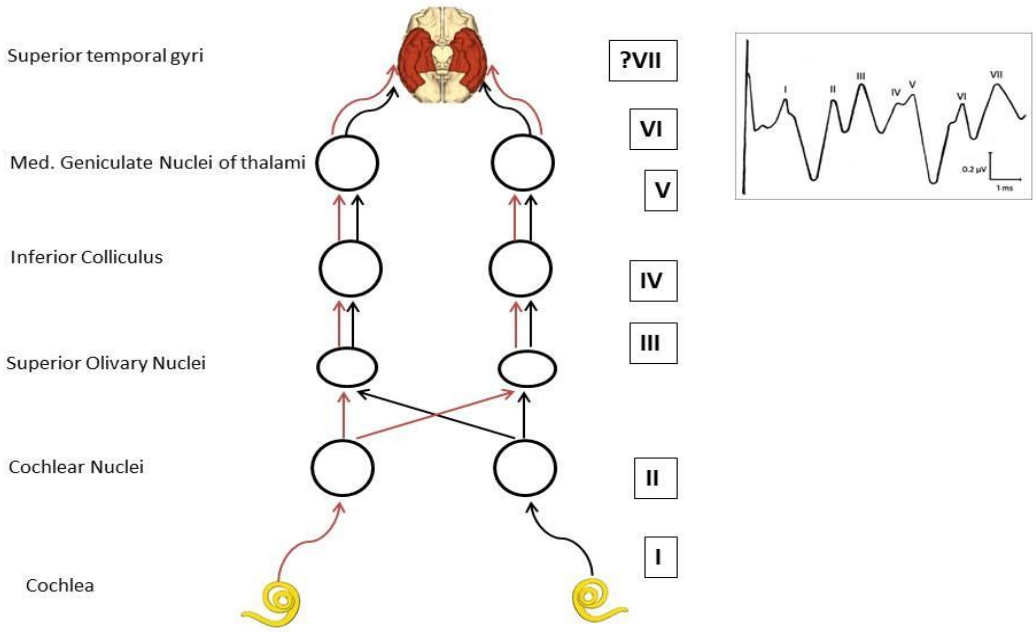


Figure 7-3: BAEP pathways

Clinically absolute latency values and inter-wave intervals need to be evaluated and the normal values for these parameters are given in table 7-2.

Table 7-2: Brainstem auditory evoked potentials normal values (Chiappa, 1982, Chiappa, 1983, Misulis et al., 2007)

	Male	Female
Wave I	2.10 ms	2.10 ms
I-III	2.55 ms	2.40 ms
III-V	2.35 ms	2.20 ms
I-V	4.60 ms	4.45 ms
V/I AMP	0.5	0.5
I-V inter-ear	0.5 ms	0.5 ms

The most common BAEP abnormality in patients with disorders of central nervous system is prolongation of I-V interpeak latency. Absence of all waveforms, decreased V/I amplitude ratio and preservation of wave-I with poorly formed II-V waves are also seen in central nervous system disorders (Daube and Rubin, 2009).

It is important to note that the inputs from the both ears project to the central structures through superior olivary nucleus where the ipsi- and contra-lateral signals cross-over. Therefore, the pathways above superior olivary nuclei carry signals from both ears to lateral lemniscus, inferior colliculus, medial geniculate nucleus and finally to primary auditory cortex. Primary auditory cortex is in the transverse temporal gyri of Heschl in the superior temporal gyrus, where Brodmann Area 41 (primary area where auditory input received) and Area 42 (auditory association area) are located (see figure 7-4). Then, further connections are made to the secondary auditory areas to interpret auditory stimuli.

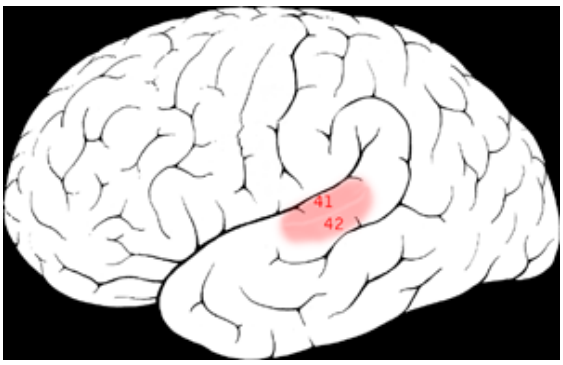


Figure 7-4: Brodmann areas 41 & 42

Another important information to note is that lateralization exists in the brain cortex. However, this does not mean that only one hemisphere participates in processing, but one side plays a more significant role than the other. For example, processing of speech mainly occurs in the left hemisphere and processing of musical and environmental stimuli in the right hemisphere as with other emotionally significant auditory stimuli.

The BAEP was measured from each subject using Dantec KeyPoint EMG/NCS/EP system incorporating a 4-channel amplifier (Natus Medical Inc, USA). Stimuli were delivered monoaural stimulation of each ear via headphones. The stimulus frequency was 10Hz using alternating polarity clicks (condensation/ rarefaction). The hearing threshold could not be ascertained in our research participants as they were unable to communicate, so the assumption of sensory hearing level was determined at 10dB. The stimulus intensity was 80dB. The click stimulation was square wave pulse of duration 100µs. The epoch duration was 10msec. To obtain the BAEP 1500 epochs were averaged.

Peak latency measurements of I, III and V, amplitude measurements of waves I and V, and inter-peak interval calculations of I-III and III-V were performed.

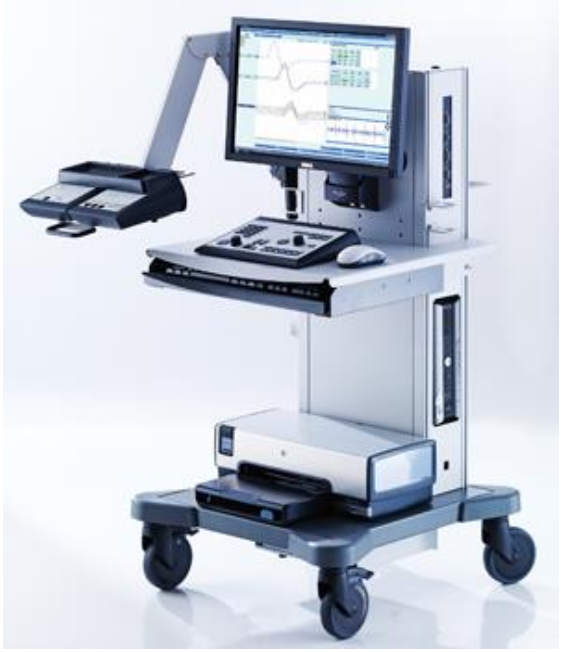


Figure 7-5: Dantec KeyPoint 4 Workstation

7.1.2.3 Baseline EEG

Baseline EEG investigations were performed to assess electro-physiological features of brain and to exclude non-clinical status epilepticus.

7.1.2.3.1 EEG data acquisition

The EEG was recorded through the application of single use Ag/AgCl electrodes (Unimed Electrodes Ltd, UK) applied to the scalp in accordance with the 10-20 system of electrode placement (Klem et al., 1999). EEG electrodes applied were F4, F3, C4, C3, T4, T3, P4, P3, O2, O1, Fz, Cz, Pz, A2 and A1. EOG was recorded from electrodes positioned over the right and left outer canthi. Submental EMG was recorded from three electrodes applied (i) midline, 1cm above the inferior edge of the mandible, (ii) 2cm below the inferior edge of the mandible and 2cm to the right of the midline, (iii) 2cm below the inferior edge of the mandible and 2cm to the left of midline. Bipolar surface EMG was measured from a pair of electrodes applied to the right and left tibialis anterior muscles using a belly-tendon montage (Perotto, 1994).

All electrode impedances were kept to below 5kΩ. Acquisition of the EEG signals was conducted with an EEG system incorporating a 50-channel amplifier, Desktop PC and integrated Digital Signal Processing board, with Neuroworks Acquisition Software (Natus Neuroworks version 7.1) installed onto a windows XP operating system (XLTEK, Optima Medical, Guilford, Surrey, UK). The EEG signals were online acquired with the common average reference montage (Goldman, 1950), band pass filtered between 0.5-70Hz, and sampled at a rate of 256Hz without mains suppression.

Figure 7-6: Xltek EEG Acquisition System

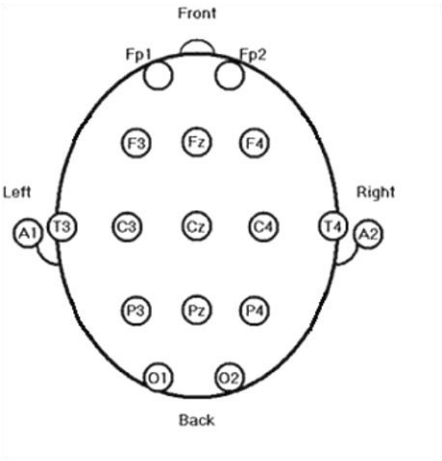


Figure 7-7: EEG Electrode placement based on 10-20 system

7.1.2.3.2 Artefact rejection

Visual inspection of the EEG data was conducted using profile reader software. Any sections of recording contaminated by EMG (e.g. spontaneous movements, facial grimacing, yawning), ECG and eye blink artefacts, particularly affecting the electrodes of interest were identified. Sections of contaminated recording deemed irrecoverable were excluded. Recordings of suitable quality were exported in European Data Format (EDF) for further processing for artefact rejection, using independent component analysis (ICA) algorithms within the EEGLab toolbox (Delorme and Makeig, 2004). ICA algorithms enable the isolation of artificially generated EEG sources with the assumption that the EEG sources of non-cerebral origin will not be reliably phase locked. The automated version of the informax ICA algorithms, runica, was used as a MATLAB function from within the EEGLab toolbox. The decomposed ICA EEG data were visualised within EEGLab, and the sources representing artefactual contaminants of EMG and mains interference were excluded.

7.1.2.3.3 Reporting and classification of EEG

EEGs were reported by a Senior Clinical Neuro-physiologist (LJ) based on the recommendations of American Clinical Neurophysiology Society guidelines (Tatum et al., 2016). EEG reports included description of dominant and non-dominant background electrocerebral activity including characteristics of waveforms, reactivity to external stimuli, degrees of symmetry, rhythmicity, amplitude, location, and any epileptiform discharges. Any significant/ unexpected abnormalities such as epileptiform activities were reported to the main researcher (KY) immediately who then liaised with the patient's usual clinicians to initiate treatment and/ or dose adjustment of anti-epileptic medications.

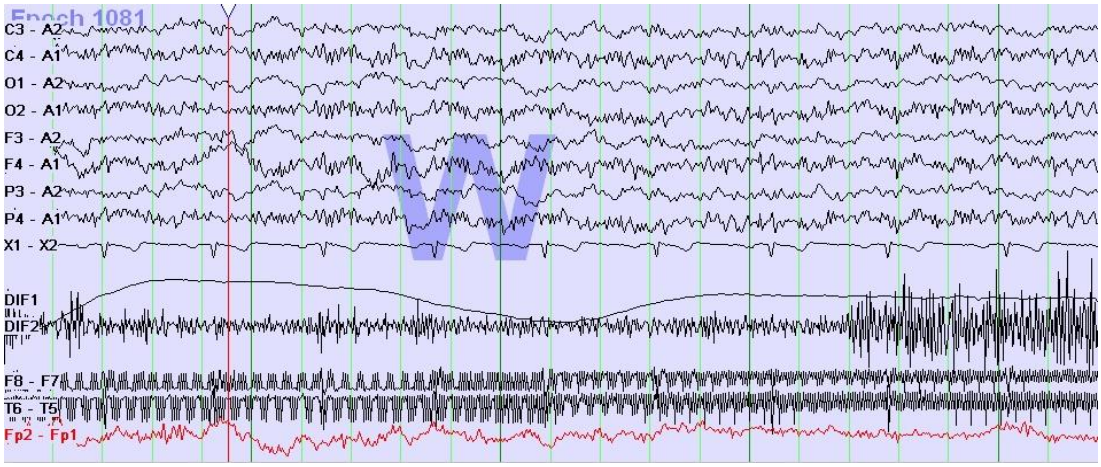


Figure 7-8: Typical resting state EEG of patients with PDOC: Characterized by diffuse polymorphic delta activity at 1.0-2.5Hz between 10-20µV (low amplitude record).

7.1.2.4 Polysomnography/ Sleep studies

24-hour polysomnography recordings were obtained from 15 out of 17 patients who participated in the baseline studies by the Senior Clinical Neuro-physiologist (LJ). Recordings were started around midday, following 30-minute baseline EEG recording. Electrodes were taken out after a 24-hour PSG recording the next day. Patients were checked over for any skin reaction at each electrode site.

7.1.2.4.1 PSG data acquisition

PSG data was recorded using the standard physiologic parameters of Electroencephalography (EEG), Electrooculography (EOG), Electromyography (EMG) and Electrocardiography (ECG). Respiratory parameters of chest and abdominal movements were also recorded.

The PSG EEG data was acquired via Ag/AgCl electrodes (Unimed electrodes Ltd, UK) applied in accordance with the 10-20 system of electrode placement. The electrode positions utilised were F4, F3, C4, C3, T4, T3, P4, P3, O2, O1, Fz, Cz, Pz, A2 and A1.

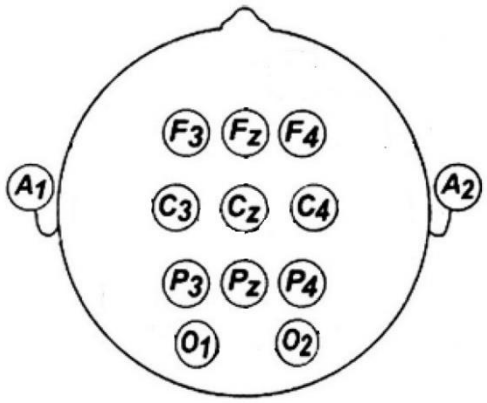


Figure 7-9: PSG Scalp electrode placement

Measurement of EOG was achieved by applying electrodes over the right (1cm above) and left (1cm below) outer canthi (AASM, 2007). Submental surface EMG was also measured the same as described for the EEG recordings. Surface EMG taken from the right and left tibialis anterior in a belly-tendon montage. A lead I ECG was also applied. All electrode impedances were kept below 5kΩ. Respiratory effort was measured using inductive Plethysmography Effort Sensor (Sleep Sense SLP Inc, USA) by positioning the effort belt around the chest at the level of the zyphoid process.

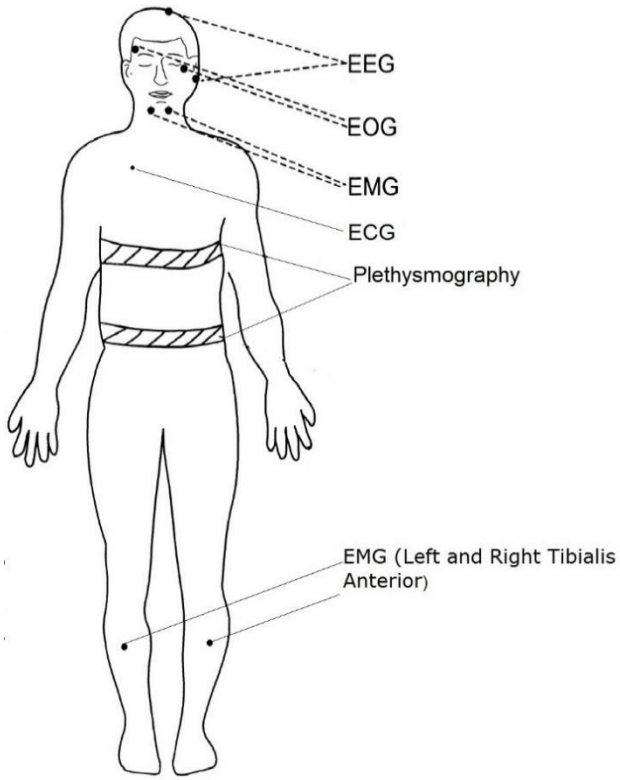


Figure 7-10: PSG Electrode Placement

The instrumentation used consisted of a Video-EEG monitoring system (XLTEK, Optima Medical, Guilford, UK) in conjunction the Trex HD ambulatory EEG headbox (XLTEK, Optima Medical, Guilford, UK) which incorporated polygraphic inputs for the measurement of additional physiologic parameters.

Figure 7-11: Xltek Trex HD Ambulatory System Headbox

The sampling rate for acquisition of all signals was 256Hz. Filter settings for the online acquisition of the EEG and ECG signals was 0.5-30Hz, sensitivity was 70uV/cm. The filter settings employed for EMG signals was 10-100Hz. Sensitivity settings for submental EMG was 20uV/cm and 100uV/cm for EMG taken from tibialis anterior. Respiratory effort filter settings and sensitivity settings were 0.5-30Hz and 0.5mV respectively.

During the PSG recording nursing staff were asked to fill in activity log where any event which may have attributed to awakenings and/ or artefacts were recorded. Examples of such activities include, re-positioning in bed, giving medications, routine checks.

Raw PSG data was loaded to computer using Xltek data transfer cable and made available for data analysis and reporting by our neuro-physiologist (LJ).

7.1.2.4.2 Analysis of PSG data

Sleep staging was done using visual inspection method adopted from AASM 2007. We reviewed 8 channels of EEG over 30sec epochs, 3 channels of EMG, one channel ECG and one channel of EOG. Information obtained via nursing activity monitoring sheet were entered on PSG data. This helped us to identify influence of external events which are not in control of subjects on EEG data and sleep events. Sections of recording contaminated by EMG (e.g. spontaneous movements, facial grimacing, yawning), ECG and eye blink artefacts, particularly affecting the electrodes of interest were identified. Sections of contaminated recording deemed irrecoverable were excluded.

Once the broad EEG patterns identified, detailed visual inspection and micro-structure assessment of sleep recording were used to identify sleep stages independently by two experienced neurophysiologists who were blinded to the stage of the study that the patients were in at the time of the reporting.

The following criteria was established for sleep stage scoring in DOC patients based on the characteristics of baseline EEG features and criteria used in previously published work (Cologan, 2013).

Table 7-3: Sleep staging criteria developed for DOC patients

Sleep Stages	EEG Characteristics in Normal individuals	EEG characteristics in DOC patients
Stage 1	Beginning of sleep, slow eye movements and overall muscle relaxation	Absence of eye blinks as well as markers of other sleep stages
Stage 2	Presence of spindles (12-15 Hz, 10-50 μV, 0.5-2 s) and κ-complexes	Presence of "slow" spindles can be seen in the 6-9 Hz frequency range
Stage 3/4	High amplitude (>75 μV) and slow frequency (0.5-2 Hz) activity dominating ≥20% of the 30-s epoch	Proportion of delta activity >20% of ongoing record with higher amplitudes, lack of muscle artefacts and eye blinks, -/+ rolling of eyes
REM	Muscular atonia, lack of spindles and slow wave sleep, presence of rapid eye movements, predominant theta rhythm	Muscular atonia, lack of spindles and presence of rapid eye movements

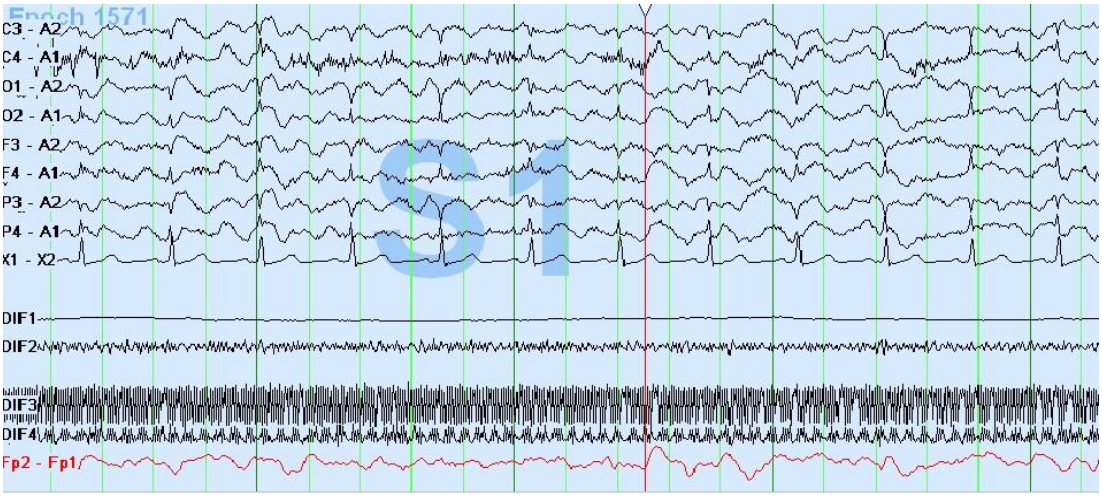


Figure 7-12: Stage 1 sleep. Characterized by absence of eye blinks as well as markers of other sleep stages

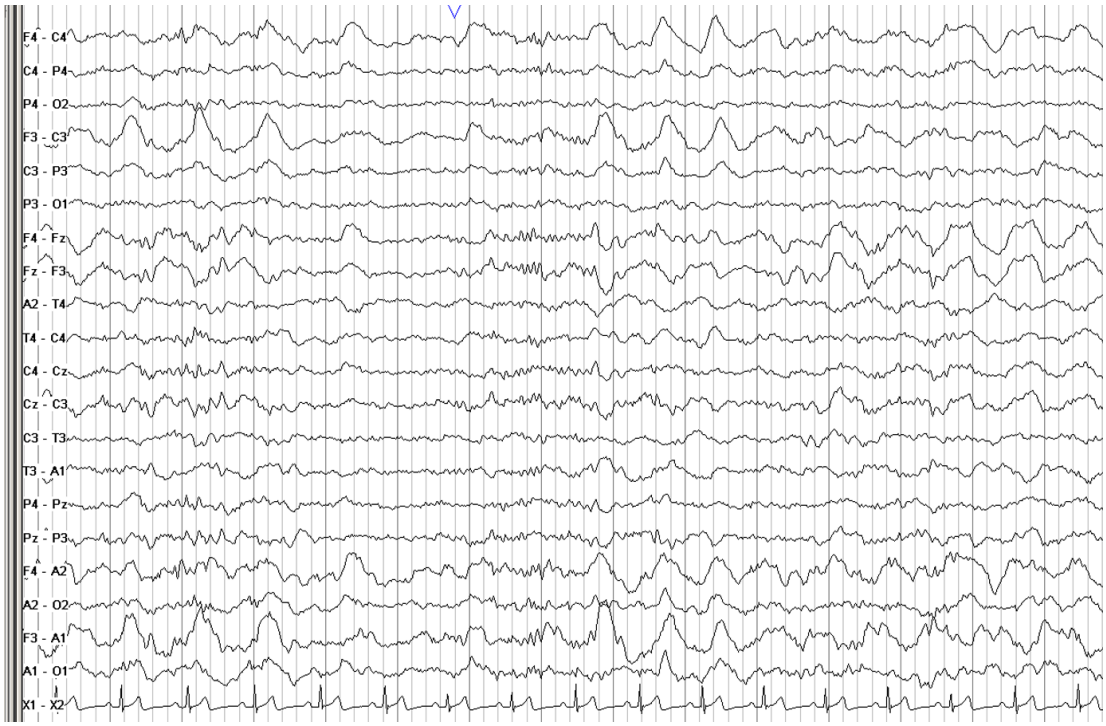


Figure 7-13: Slow spindles seen in PSG of a patient in minimally conscious state

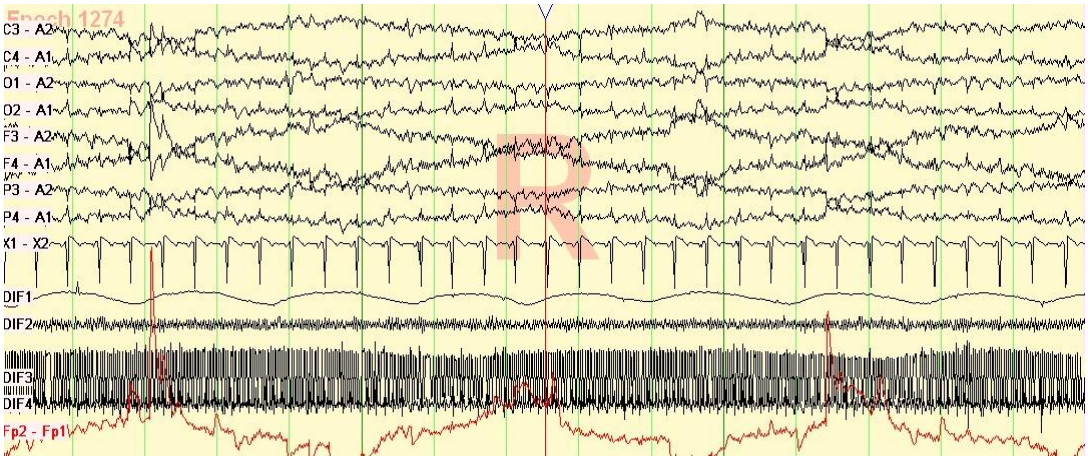


Figure 7-14: REM: Identified by muscular atonia, lack of spindles and presence of roving rapid eye movements

7.1.2.4.3 Evaluation of sleep using sleep parameters

The following sleep parameters were computed in order to be used for evaluating sleep both quantitatively and qualitatively (Pressman, 2000):

1. Study Duration: This represents the total recording time which the amount of time that the recording equipment remains activated. This is the time between the “First Lights off Time” and “Last Lights on time” as recorded by the neuro-physiologist.
2. Total Sleep Time: Total Sleep Time is the total amount of sleep time scored during the study. This is computed by adding Sleep Stage 1 + Stage 2 + Stage 3&4 + REM sleep. Low total sleep time is usually indicative of nonrestorative sleep and may contribute to daytime sleepiness.
3. Sleep Efficiency: Sleep efficiency represents the percentage of total sleep time actually spent in sleep. This is typically computed by (Total sleep time/ Time in bed) X 100. It gives an overall sense of how the patient slept but makes no distinction between frequent, brief periods of awakenings from long periods of awakenings. Therefore, it needs to be evaluated with other sleep parameters in order to get a better picture of sleep quality.

4. Total wake time: This is the amount of wake time during the study. It is computed from the total of all epochs scored as wake using the sleep scoring criteria employed.
5. Sleep latency: This is the time in minutes from lights out to sleep stage 1.
6. Number of awakenings/ arousals: These are the events scored during any stage of sleep if there is an abrupt shift of EEG frequency including alpha, theta, and/or frequencies > 16 Hz (but not spindles) that lasts at least 3 seconds, with at least 10 seconds of stable sleep preceding the change.

Table 7-4: Example of sleep parameters computed for one of the participants

Sleep Statistics			
Study Date:	aa/bb/cccc	Study Duration:	1717.2
Study ID:	XXX	First Lights Off Time:	12:06:52 PM
Time in Bed:	1644.8	Last Lights On Time:	03:31:41 PM
Total Sleep Time:	69.5	Sleep Efficiency:	4.2%
Sleep Onset:	10:08:54 PM	Total Awakenings: 7	Index: 6.0
Sleep Latency:	602.0	REM Onset:	Not detected
REM Latency:	N/A	Final Wake Onset:	04:56:54 AM
Final Wake Latency:	408.0	AHI:	N/A
PLM Index:	0.0	Minimum OSat:	<1%
Average OSat:	Not measured	WASO:	338.5
Number of Epochs:	3331	Unscored Epochs:	-

Sleep efficiency, time spent and % sleep time in identified sleep stages (stages 1, 2, 3/ 4, REM) were also reported.

Table 7-5: Sample sleep stage distribution for one of the research participants.

Stages	Time	% Sleep Time	% Time in Bed	% Sleep Period Time
WAKE	1576.0	-	95.8%	82.9%
Stage 1	67.0	96.4%	4.1%	16.4%
Stage 2	2.5	3.6%	0.2%	0.6%
Stage 3	0.0	0.0%	0.0%	0.0%
Stage 4	0.0	0.0%	0.0%	0.0%
REM	0.0	0.0%	0.0%	0.0%
MT (movement time)	0.0	0.0%	0.0%	0.0%
UNS	0.0	-	0.0%	0.0%
NREM (1+2+3+4)	69.5	100.0%	4.2%	17.0%

7.1.3 Assessment of circadian rhythm

7.1.3.1 Melatonin as a marker of circadian rhythmicity

Saliva melatonin samples were collected using Salimetrics paediatric oral swabs (Salimetrics, USA) over 48 hours during. The samples were collected every 4 hours with a shift of 2 hours on collection times in the second half.

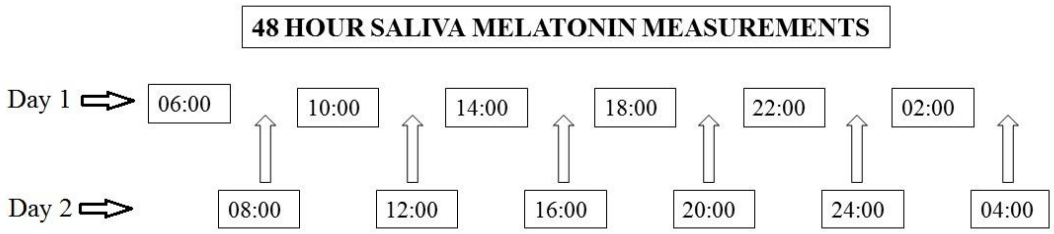


Figure 7-15: Saliva melatonin collection protocol.

After collection, the samples were frozen and later shipped for melatonin levels measurement using radioimmunoassay analysis at the laboratories of Stockgrand Ltd. The assay characteristics were as follows: limit of detection =

0.9 pg/ml, quality controls low = 3.6 pg/ml +/- 0.4 CV = 10.7%; medium = 26.1 pg/ml +/- 2.3 CV = 8.9%; High = 54.6 pg/ml +/- 4.9 CV = 9.0%.

7.1.3.2 Body temperature as a measure of circadian rhythmicity

Ibuttons were previously used to obtain continuous skin temperature measurements from patients in vegetative state (Bekinschtein et al., 2009b). In our study, we have attached DS1921H High resolution Thermochrone ibuttons (Maxim Integrated, USA) to chest wall using hypafix surgical tape for 72 hours. Ibuttons were set to record temperature every 30 seconds.

7.2 INTERVENTIONAL STUDY

7.2.1 Study design

For the 10 patients who proceeded to the interventional study, EEG, BAEP, CRS-R, PSG, saliva melatonin and body temperature measurements were already performed at baseline. In addition to this, event related potential studies were performed using MMN and Subject's Own Name (SON) paradigms within the same week. All investigations were repeated four weeks later in order to detect any fluctuation that may occur without any intervention. This was followed by five-week period of interventions which were consisted of melatonin in the evening, blue light treatment and caffeine in the morning. On the fifth week of intervention period all investigations were repeated once more to detect and improvement of consciousness/ brain functions.

During the intervention, 8am and 8pm saliva samples were collected to confirm presence of high and low saliva melatonin levels. A 24-hour saliva collection were performed 3 days after cessation of intervention to find out whether the circadian rhythm of melatonin secretion was entrained and once again two to three weeks after to see if this was maintained or not. Total number of saliva melatonin samples collected from each patient was 38 (12 at baseline-1, 12 at baseline-2, 2 during interventions, 6 three days after the interventions and 6 three weeks after the interventions). See Figure 7-16: Saliva collection time points throughout the study.

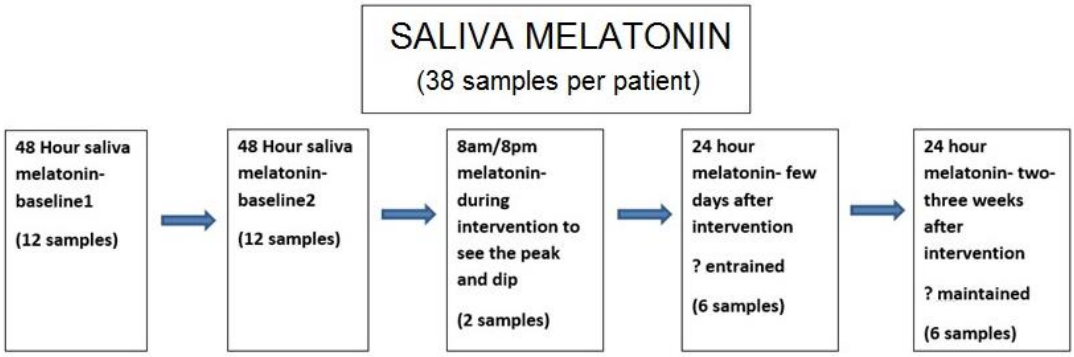


Figure 7-16: Saliva collection time points throughout the study

7.2.2 EEG, BAEP, CRS-R, PSG, Melatonin and Body Temperature Measurements

These investigations were performed as explained in the section 7.1.

7.2.3 Event related potentials (MMN and SON paradigms)

7.2.3.1 Data acquisition

All experiments were performed while patients were sitting in their own wheelchair in a quiet room. EEG activity was recorded using 64-channel EEG amplifier (ANT-Neuro, Netherlands) and shielded Waveguard EEG cap. To optimize signal quality, the impedance at electrodes was kept below 5 kΩ. Sampling rate was 1024 Hz and two of the electrodes were used to record electro-oculogram (EOG).

Two experiments were used to obtain event related potential component mismatch negativity: standard frequency oddball paradigm and 'subject's own name' paradigm. Patients were provided with 5-10 minutes rest time between the experiments.

During the MMN paradigm the patients were presented with 1000 repetitions of two types of pure tone delivered binaurally through headphones at an intensity of 70dB. The standard tone was set at 1000Hz (80%) and the oddball

or deviant at 1200Hz (20%). The duration for each stimulus was 60ms (rise time 10ms) with an inter-stimulus interval of 500ms.

In the subject's own name paradigm, three different types of stimuli were used:1) subject's own name; 2) four examples of other peoples' names; and, 3) four examples of time reversed names. Other names were chosen from a pool of spoken names that were not deemed significant to patients by their relatives. Some of the other name stimuli were time reversed to create unintelligible stimuli which preserve their acoustic complexity and some voice identity. The names were recorded by a native English speaker male person unknown to them and edited for quality and length (range 400-800ms), time-reversed and amplified using Praat software version 5.3.23 (Boersma, 2012). Inter-stimulus interval was 1200ms. The stimuli were delivered binaurally via headphones.

7.2.3.2 Pre-processing of data on SPM 12

ERP data pre-processing was performed offline using Statistical Parametric Mapping (SPM12, Institute of Neurology, UCL) software. The following steps took place in the pre-processing of EEG data as outlined in the SPM 12 manual (published by Functional Imaging Laboratory Group, UCL, 2013);

1. Conversion of EEG Data
   This is the first step of the analysis where native machine-dependant format EEG data is converted to a MATLAB based, common SPM format. The conversion of native EEG data produces two SPM files: *mat file which contains data structure and *dat file which contains the EEG data.
2. Preparation of the data and batch inputs
   This step involves review of the trials, selecting subset channels (EEG, EOG) which produces 'channelselection.mat file.

Trial definition was performed by specifying the time window (in ms) to be cut around triggers and the number of conditions. The conditions are then labelled such as “standard” or “rare”.

Montaging stage allows one to specify custom montages. A simple way to specify some common montages is via ‘re-reference’ option. In our pre-processing re-referencing was done by selecting all channels. This step produces ‘…avref.mat’ file (average referenced file) batch input file. Following the montaging spm produces two files Mspmeeg_subject.mat and Mspmeeg_subject.dat files.

3. The next step is to derive the EOG channels by using the previously saved ’avref.mat’ file. At this step he HEOG channel is defined as the difference of EXG1 and EXG2 and VEOG as the difference of EXG2 and EXG3. Then, the montaged is saved as ’avref_eog.mat’ file.
4. Epoching the data (=spm_eeg_epochs)

   Epoching cuts out little chunks of continuous data and saves them as single trials. This step involves defining trials by defining the time window in peri-stimulus time, specifying the triggers and labelling the conditions. Peristimulus time window of -100ms to 400ms was used for our data. This step produces a trialdef.mat batch input file.
5. Filtering the data

   Continuous or epoched data can be filtered, over time with a low-, high-, stop-, or bandpass- filter. High-pass filter removes ultra-low frequencies close to DC, and a low-pass filter removes the high frequencies. SPM uses Butterworth filter to do this. In our pre-processing we used ‘high-pass’ with 0.5 as cut-off frequency and ‘low-pass’ with 20 cut-off frequency. The resulting filtered data is saved as fMspmeeg_subject_mat and fMspmeeg_subject_dat files.
6. Baseline correction

   This step subtracts the baseline from channel data where baseline period is set e.g. [-100 to 0 ms]
7. Down-sampling (spm_eeg_downsample)

This step is performed when data is acquired at a higher sampling rate (in our study this was 1024 Hz) than one needs for making inferences about low-frequency components. We chose sampling rate of 200 Hz at this stage. This step produces dfMspmeeg_subject.mat and dfMspmeeg_subject.dat files.

8. Filtering

   In our pre-processing we used 'low-pass' with 20 cut-off frequency. This step produces fdfMspmeeg_subject.mat and fdfMspmeeg_subject.dat files.

9. BERG correction of eye artefacts (In this method an accurate, head model-independent estimate of the spatial distribution of eye activity is obtained from calibration data containing systematic eye movements and blinks. Using the resulting spatial vectors together with the brain model, eye activity in EEG and event-related response data are estimated in the presence of overlapping brain activity and corrected.) (Berg and Scherg, 1994).

10. Epoching

    Previously produced trial definition file is used to epoch the data. The epoched data is then saves to files efdfMspmeeg_subject.mat and efdfMspmeeg_subject.dat files.

11. Artefact detection and rejection

    In our pre-processing we used 80 μV threshold value was used to detect and reject artefacts. This step creates aefdfMspmeeg_subject.mat and aefdfMspmeeg_subject.dat files.

12. Averaging (spm_eeg_average)

    Averaging of single trial data is a crucial step to obtain evoked (or induced) response. When averaging single trial data, single trials are averaged within condition. Robust averaging of data estimates weights, lying between 0 and 1, that indicate how artefactual a particular sample in a trial is. Later, when averaging to produce evoked responses, each sample is weighted by this number. Robust averaging is useful for

finding out what parts of the data were down-weighted and adjusting parameters if necessary. In our pre-processing, we used robust averaging by computing weights by condition as we had unequal number of trials for each condition. Following the robust averaging maefdfMspmeeg_subject.mat file is saved.

The 12 steps described above completes the pre-processing stage.

Following the pre-processing, 3D images were created for each trial, containing smoothed recorded potentials in scalp space (two dimensions, X and Y in mm) over peri-stimulus time in ms (z dimension).

7.2.4 Intervention techniques

Interventions included melatonin treatment in the evenings, blue light and caffeine treatments in the mornings in order to optimize circadian rhythmicity.

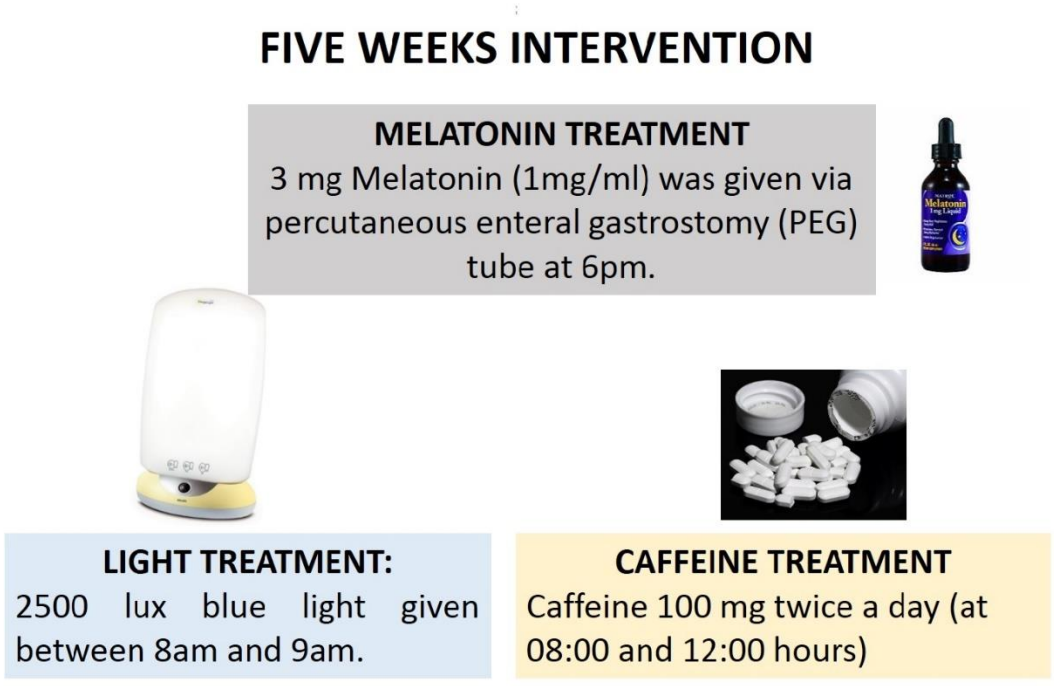


Figure 7-17: Intervention techniques used in the study

7.2.4.1 Melatonin treatment

Melatonin is normally given 1-2 hours before usual bedtime, between 8 and 10pm. The patients with PDOC typically have poor sitting tolerance and therefore need to be put in bed before 6pm in the afternoon. Evening dose of their prescribed medications given between 6 and 8 pm and they may have visitors until 8pm. The main lights in their rooms are turned off after 8pm. Therefore, in order to synchronize to the environmental cues and light-dark cycle of their environment, melatonin administration time was set as 6pm in the evenings.

In our study liquid 3 mg Melatonin (1mg/ml) was given via percutaneous enteral gastrostomy (PEG) tube at 6pm each night by nursing staff during their medication rounds.

During the interventions once only 8am and 8pm saliva melatonin measurements were performed to confirm presence of raised melatonin levels following administration and absorption of melatonin at 6pm; and decreasing levels of saliva melatonin at 6am, following its overnight metabolism.

7.2.4.2 Blue-light therapy

We used Philips Energy Light lamps (HF3308) to provide blue light treatment in the mornings. According to the manufacturer, these lamps mimic natural daylight by giving up to 10,000 lux at 20 cm and 2500 lux at 60 cm distance.

Prior to use in the study the lamps were tested in the laboratories of Health Protection Agency and it was reported back that luminance values place no restriction on use of lamps for therapy, up to 2 hours per day at distances of 20 cm or greater. At the bedside of patients, Tecpel 536 light meter/ RS232 data logger was used to measure illuminance levels in patients' eye level within one-meter distance which yielded illuminance levels between 1500 and 2500 lux.

Lamps were placed in close proximity to patients in their rooms. Average distance from patients' eye level to lamp was 60 cm. It was not possible to place the lamps closer than 50 cm due to width of the hospital beds and equipment/ furniture around the beds. Lamps were automatically controlled using a timer electrical plug in order to maintain consistency of treatment. The on time was set to 08:00 and off time to 09:00 every day. Earlier on time was avoided to prevent night nursing staffs' exposure to bright light at the end of their shifts. Day nursing staff were instructed to turn the patients towards the lamps at 08:00 hours and to make sure patients remained faced towards the light during the treatment.

7.2.4.3 Caffeine

We aimed to administer caffeine 100 mg twice a day (at 08:00 and 12:00 hours) as part of our intervention due to its stimulant and circadian rhythm effects. However, there is no licenced caffeine in the UK as a single agent. It is often added to analgesics to improve their efficacy.

ProPlus Caffeine tablets (Bayer plc.) are sold over the counter in high street shops for temporary relief of tiredness and to maintain mental alertness. It is commonly used by students. Each ProPlus tablet contains 50mg of caffeine which is equivalent to caffeine content of 100 mls instant coffee or regular tea. Maximum recommended caffeine intake per day is 400 mg regardless of the source.

Caffeine was introduced in gradual manner as PDOC patients are not given any caffeine containing feed or fluids normally. Initially 50mg Caffeine was given at 8am for three days, then increased to 50mg at 8am and midday for three days, then 100mg at 8am for three days, and finally 100mg twice a day which was the dose given for the rest of the intervention period.

Pro-plus tablets were crushed and dissolved according to usual nursing practice of giving tablets via feeding tube and after administration the tubes were flashed with water to avoid tube blockage.

7.2.5 Post-intervention investigations

On the fifth week of the interventions all clinical and neuro-physiological investigations (CRS-R, saliva melatonin, body temperature, PSG, ERPs) were repeated in order to assess effect of interventions on sleep, behavioural and neurophysiological indicators of awareness/ consciousness.

Three days after the cessation of interventions 4-hourly saliva melatonin samples were collected for 24 hours and once again three weeks after. The aim of these two further collections was to see if the circadian rhythmicity was improved and maintained.

7.3 STATISTICAL ANALYSIS

Descriptive statistical tests, means comparisons, ANOVA tests and reliability analyses were performed using SPSS software (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY).

7.3.1 Analysis of saliva melatonin data

7.3.1.1 Summary statistics

Raw melatonin data were normalized to the mean.

The following were performed prior to applying detailed statistical tests:

1. Normalized saliva melatonin data was plotted as a function of time
   - to explore whether an obvious rhythmicity could be recognized or not;
   - to make macroscopic/ qualitative analysis of noise;
   - to see if there is cycle-to-cycle variability or not
2. Examination of maximum (peak) melatonin values to see if maximum values reached around 02:00-03:00 h as a response to dark and minimum values reached between 14:00-15:00 h
3. Means comparison between diurnal (08:00-20:00h) and nocturnal (20:00-08:00h) values

7.3.1.2 Cosinor analysis

Raw saliva melatonin results were normalized to mean melatonin values and further analysed using single cosinor analysis software which was provided by Stockgrand UK laboratories. The software is an Excel based program where from values of circadian rhythm markers mesor, amplitude, acrophase and % rhythms are computed. Amplitude and acrophase 95% confidence limits and p-values are calculated. P-values < 0.05 are deemed to be statistically significant.

Cosinor Analysis is based on linear least squares regression model that is used to fit a sinusoidal curve to time-series data (Nelson et al., 1979). It involves representing the data span by best-fitting cosine function and is performed using the formula below (Muñoz-Hoyos et al., 1993):

$$Y_i = M + A \cos (wt + \varphi)$$

Y is the mean of the function at time *i*

M is the mesor (the mean level of the cosine curve)

A is the amplitude of the curve

w is the angular frequency of the curve

t is the time when point *i* was measured

φ is the acrophase (phase angle of the maximum values of the curve/ calculated peak time)

Parameters of the rhythm (mesor, amplitude, and acrophase) can be calculated from this equation. In other words cosinor analysis reduces the time series to three independent variables per subject: amplitude (level of melatonin in the blood at its peak in the 24-h cycle), acrophase (the time the peak occurs), and mesor (the mean level over the period)(Gibertini et al., 1999).

Percentage rhythm is the measure of the relative strength of a rhythm and is calculated by making direct comparison between the variability of the data points about the fitted curve. (Minors and Waterhouse, 1988) .

The percentage rhythm is calculated as:

Percentage rhythm= 100 X (Total Sum of Squares-Residual Sum of Squares)/Total Sum of Squares

In a perfect fit percentage rhythm is 100%. In a healthy person, this is expected to be around 90%.

T-test statistics were performed for pre- and post-intervention % rhythmicity values in order to investigate the effect of interventions on melatonin circadian rhythmicity.

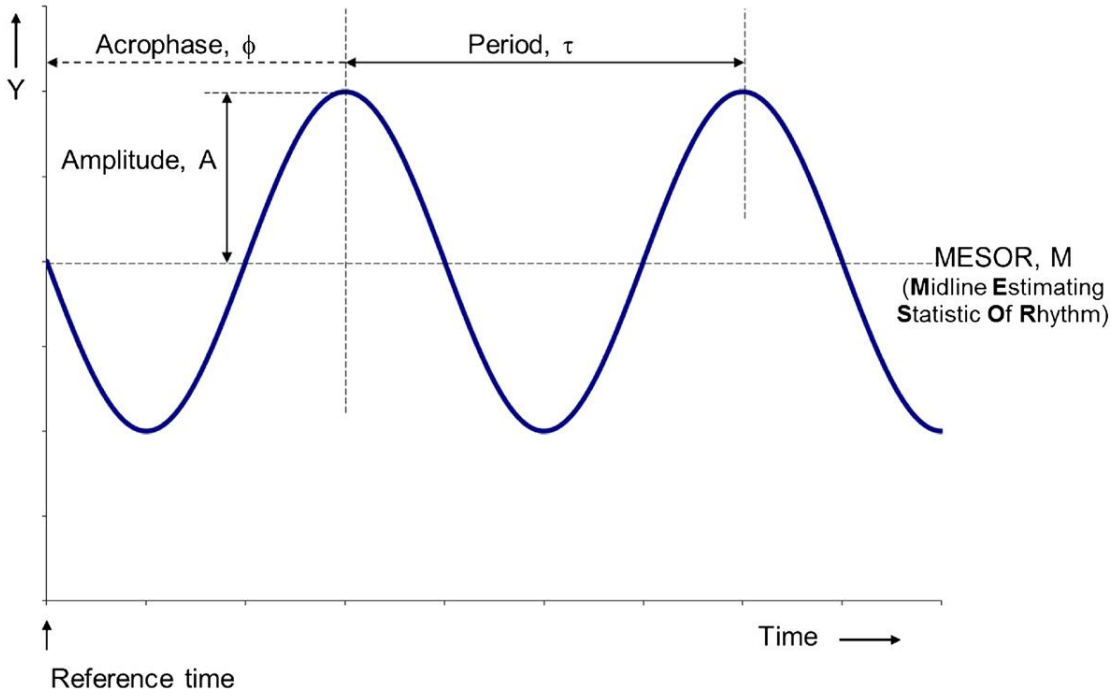


Figure 7-18: Definition of rhythm characteristics. The MESOR is a rhythm-adjusted mean; the double amplitude (2A) is a measure of the extent of predictable change within a cycle; the acrophase is a measure of the timing of overall high values recurring in each cycle, expressed in (negative) degrees in relation to a reference time set to 0°, with 360° equated to the period; and the period is the duration of one cycle. (modified from Cornelissen Theoretical Biology and Medical Modelling 2014, 11:16)

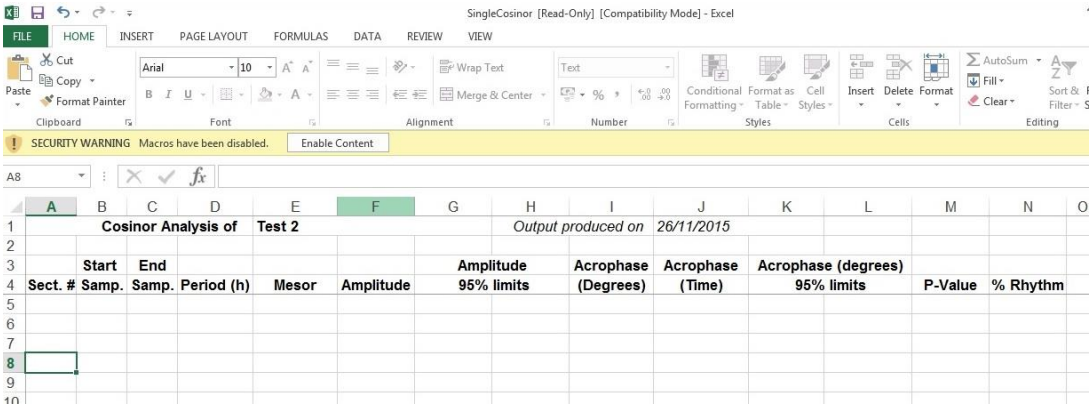


Figure 7-19: Cosinor analysis results output

7.3.2 Analysis of body temperature data

Body temperature data obtained via I-Button data loggers was transferred to a laptop PC using the connection kit supplied by the manufactured and data was initially reviewed on 1-wire software (Maxim Integrated, USA) and then analysed on Excel 2013 (Microsoft, USA). Summary statistics and cosinor analysis were performed as described above. T-test statistics were performed for pre- and post-intervention % rhythmicity values in order to investigate the effect of interventions on body temperature circadian rhythmicity.

7.3.3 Statistical analysis of PSG data

For the patients who were included in the interventional study General Linear Model ANOVA tests were performed to see if the data differed at three time points (baseline 1, baseline 2 and post-intervention) and pairwise comparisons were made. If the baseline 1 and 2 data were not significant at 0.05 level (CI=95%), these were collapsed together and means comparisons were made against post-intervention data.

As the PSGs were reported by two neurophysiologists who were blinded to each other, the PSG data obtained two-way mixed intra-class correlations (raters are chosen and subjects are random) were performed to assess inter-rater reliability. If the absolute agreement was at and above acceptable level

(>0.7, CI=95%), the data from both raters were collapsed together and t-test comparisons were made between pre-intervention and post-intervention results.

7.3.4 Analysis of ERP data

ERP data analysis was performed offline using Statistical Parametric Mapping (SPM12, Institute of Neurology, UCL) software. Following data pre-processing, 3D images were created for each trial, containing smoothed recorded potentials in scalp space (two dimensions, X and Y) over peri-stimulus time (z dimension).

We searched across the whole of scalp space but, limited the analysis to a fixed time window of interest 150-500 ms post-stimulus (by applying a small volume correction). After this mask was applied to the SPM we report voxels that survived a peak level value of $p< 0.001$ uncorrected.

Contrasts for each subject were created at the first level that were then taken up to the second level design matrix. Second level design matrix contrasts were defined to examine statistical differences between the responses before and after the intervention using -1 1 contrast for the oddball experiments, and 1 -1 contrast for the SON experiments.

Table 7-6: Contrasts applied for oddball and subject's own name experiments at the 1st level design matrices. (SON= Subject's own name)

ERP Test	Timepoints that the contrasts applied	1st level individual design matrix for 3 time-points
Oddball	Baseline experiments	-1 1 -1 1 0 0
	Post-intervention experiments	0 0 0 0 -1 1
SON	Baseline experiments (SON> OTHER)	1 -1 0 1 -1 0 0 0 0
	Post-intervention experiments (SON> OTHER)	0 0 0 0 0 0 1 -1 0

In order to extract the MMN values after the intervention, we went to the individual subjects' 1st level design matrix and searched for the peak MMN values closed to the group peak and extracted the beta values.

Chapter 8 RESULTS

This chapter aims to present the study results. The first section of the chapter will give the results of baseline investigations which provided insight into the sleep and circadian rhythms of our 17 participants with PDOC. This will be followed by the results of the interventional study which 10 patients received melatonin, blue light and caffeine with the aim of optimizing sleep and circadian rhythmicity.

8.1 BASELINE RESULTS

CRS-R, BAEP, EEG, PSG, saliva melatonin and body temperatures were performed as baseline investigations. 17 patients were enrolled in baseline assessments. Although we aimed to perform all tests for all patients, this was not possible due to missed appointments and occasions due to the practical difficulties performing the tests on this very severely disabled patient population.

Detailed demographic and clinical information which include outcomes of baseline BAEP and EEG investigations are summarized in Table 8-3. Medications that act on the central nervous system or known to cause drowsiness as a side effect are shown in bold and medications that may reduce melatonin levels and/ or production are shown in italic. None of the patients suffered from liver disease which may reduce melatonin metabolism.

8.1.1 Investigations to examine clinical status of patients

8.1.1.1 Coma Recovery Scale-Revised assessments

Patients' initial diagnoses were recorded based on the outcomes of multi-disciplinary team assessments using standardized clinical assessment tools (SMART, WHIM, CRS-R, MATADOC) upon their admission to the Royal Hospital for Neuro-disability. All patients who were long term care (LTC) residents in the specialist nursing home were re-assessed using CRS-Revised and their diagnosis were updated when applicable.

All clinical and demographic data are presented in table 8.2. The average CRS-R score value for our cohort of patients was 10.2. The number of patients with CRS-R≤10 (VS/ UWS and low MCS) was 8.

Three patients (patient 1, 6 and 8) were found to have had significant improvement of their consciousness and change of diagnosis- from VS to MCS, since their initial diagnostic assessments, over 3 to 5 years (Table 8.1).

Table 8-1: Diagnosis change in three patients

	Age/Gender	**Cause of Brain Injury**	**Time from brain injury to SMART diagnosis**	**Time since Brain Injury (years)**	**Diagnosis (SMART, WHIM)**	**CRS-R at the time of participation**
Patient-1	71/Female	SAH	13 months	5	VS	16
Patient-6	43/Female	CVA (Bleed)	11 months	3	VS	17
Patient-8	40/Female	TBI (DAI)	12 months	3	VS	11 (with visual pursuit and attention)

8.1.1.2 BAEP test results

We were able to obtain BAEP testing in 13 patients (one study difficult/ inconclusive, three not done due to missed appointments). While, seven out of 13 patients had completely normal results, the remaining six patients had some abnormality in their BAEP results. None of the patients had severe hearing impairment affecting both sides.

In all the six patients with abnormality of BAEP, the abnormalities were mainly localized to the right ear confirming additional brainstem pathology in addition to their known widespread central nervous system disease (see table 8-2).

Three of our interventional study participants (patient 2, 4 and 9), had absent wave 1 on right side.

Table 8-2: Observed BAEP abnormalities in six patients

Patient ID	Cause of Brain Injury	BAEP Abnormality
Patient 2	Anoxia	Right BAEP absent
Patient 4	CVA (bleed)	Right BAEP absent
Patient 6	CVA (bleed)	Right BAEP wave latencies delayed, waves poorly formed bilaterally
Patient 9	Vasculitis	Right BAEP absent
Patient 12	Anoxia	Right BAEP absent
Patient 14	CVA (bleed)	Right BAEP wave latencies delayed

8.1.1.3 Baseline EEG results

Baseline EEG outcomes were available on 16 patients. One patient had severe involuntary movements which resulted in very poor-quality EEG. All our patients who had baseline EEG had abnormal baseline EEG's: poorly responsive, low amplitude EEG with theta and delta waveforms dominance. One of our patients had copious amounts of beta activity on her EEG which was explained by prescribed benzodiazepine medications.

Many prognostic classifications of EEGs are based on the dominant waves (Synek, 1988, Young et al., 1997, Hockaday et al., 1965). Polymorphic delta dominance suggests a disruption of cerebral function. Therefore, it can be concluded that the slower the frequency, the more severe the abnormality especially when the reactivity/ responsiveness is also diminished. Although, such EEGs are expected in PDOC population dominant theta and delta waves pose an additional challenge when reporting stages 3/ 4 of the sleep EEGs which will be explored further in PSG sections of the thesis.

In addition, three patients with anoxic brain injury had frequent sharp waves/ spikes with no associated clinical seizure/ myoclonic activity. There has been a long-standing discussion whether the patients with sharp waves/ spikes require anti-epileptic treatment or not. While some clinicians favour treatment, others argue that these waves are due to nonepileptic encephalopathies and treatment with anti-convulsant is not indicated and may cause side effects. In our study, two patients with sharp waves/ spikes were already on clonazepam (patient 3 and 11) and the third patient (patient-2) was on triple anti-epileptics: clonazepam, valproate and levetiracetam. Both patient-3 and patient-11 had history of troublesome myoclonic jerks and patient-2 had history of myoclonic jerks as well as tonic-clonic seizures. As there was no correlation of EEG features with clinical presentation, we did not make a recommendation to alter these patients' anti-epileptic treatment regime.

Table 8-3: Patient demographic and clinical information

(F=female, M=male, BI= Brain injury, CVA= cerebrovascular accident, TBI= traumatic brain injury, SAH= subarachnoid haemorrhage, VP shunt= ventriculo-peritoneal shunt, R= right, L= left, AVM= arteriovenous malformation, LTC= Long-term care, BL= baseline, Dx= diagnosis, Tx=treatment, BAEP= brainstem auditory evoked potentials, α= alpha, β= beta, δ= delta, θ= theta) Note: The medications with sedating side effects are written in bold, and the one that affect melatonin production in italic.

ID/Age/ Gender/ Setting	Aetiololgy	Dx/ BL CRS-R	Experiment Condition	Time since BI	Feeding Regime	Medications	CT/MRI	BAEP	EEG BL
PT1/ 71/ F/ LTC	CVA	MCS/ 14	BL and Tx	5 years	Daytime	Co-careldopa, Levetiracetam, Lansoprazole	Extensive white matter low attenuation, atrophy, R frontal bleed, L occipital infarct, VP shunt	Normal	Polymorphic δ at 2-2.5 Hz at 20-30 μV with occasional β
PT2/ 58/ M/ LTC	Anoxia	MCS / 8	BL and Tx	5 Years	Daytime	Amiodarone, Frusemide, Ramipril, Valproate, Levetiracetam, Diltiazem, **Clonazepam**, Levothyroxine	Widespread ischaemic changes, loss of grey-white matter differentiation in cortex and basal ganglia	R absent, L present	Polymorphic δ activity at 1-2.5 Hz at 10-20 μV, some β, sharp waves/ spikes
PT3/ 36/ M/ LTC	Anoxia	VS/ 4	BL and Tx	2 years	Daytime	Lansoprazole, Metformin, Aspirin, Simvastatin, *Bisoprolol*, **Clonazepam**, Dantrolene, **Zopiclone**	Widespread ischaemic changes	Normal	frequent spike wave on low amplitude featureless background
PT4/ 52/ M/ LTC	CVA	MCS/ 10	BL and Tx	4 years	16:00-04:00	**Baclofen**, Dantrolene, **Gabapentin**, Valproate, Lansoprazole	R temporal AVM, intracerebral and subdural haemorrhage	R absent, L present	Asymmetric EEG, R: low amplitude featureless, L: θ and δ
PT5/ 30/ F/ LTC	TBI	MCS/ 13	BL and Tx	5 years	16:00-02:00	Lansoprazole, Hyoscine, **Baclofen, Gabapentin,** Citalopram	Multiple widespread petechial haemorrhage	Normal	Dominant δ activity at 1.5-2.0Hz, up to 50μV, ripples of θ
PT6/ 43 /F/ LTC	CVA	MCS/ 16	BL and Tx	3 years	18:00-02:00	Amlodipine, doxazocin, levetiracetam, oxybutinine, simvastatin	L temporoparietal stroke & haemorrhage	R delayed, L Normal	Asymmetric EEG, dominant δ and θ activity on the right

P7/ 73/ M/ LTC	CVA	MCS/ 12	BL and Tx	8 years	16:00-02:00	*Bisoprolol*, clopidogrel, **gabapentin**, warfarin	Bilateral ganglia infarcts, and infarction in the L occipital lobe	Normal	Diffuse θ, occasional δ, 1-2.5Hz
PT8/ 40/ F/ LTC	TBI	MCS/ 11	BL and Tx	3 years	Daytime	**Baclofen,** hyoscine, lansoprazole, modafinil	Multiple widespread petechial haemorrhage	Normal	Diffuse θ, occasional δ
P9/ 68/ M/ LTC	Vasculitis	VS/ 6	BL and Tx	2 years	16:00-04:00	*Bisoprolol*, **pregabalin**	Widespread necrotising leucoencephalopathy	R absent, L present	Diffuse δ 1-2,5Hz, occasional θ
PT10/ 66/ F/ LTC	Anoxia	MCS/ 11	BL and Tx	6 years	Daytime, bolus	**Clobazam, diazepam,** levetiracetam, mebeverine, **mirtazapine,** paracetamol, ramipril, **zopiclone,** amlodipine,	Extensive diffuse signal change in the L temporal and occipital lobes and parietal cortices consistent with ischaemia	Normal	Copious amounts of β activity seen at 15-17Hz
PT11/ 59/ F/ Rehab	Anoxia	VS/ 5	BL only	6 months	10:00-24:00	**Clonazepam, baclofen,** hyoscine patch, Dantrolene	Diffuse hyperintense signal at bilateral periventricular and deep white matters, basal ganglia and occipital gyri.	N/A	Poorly responsive EEG, frequent spikes, diffuse irregular δ activity at 2Hz
PT12/ 59/ F/ LTC	Anoxia	MCS/ 13	BL only (Enrolled for both)	8 years	Daytime	Lansoprazole, **Tizanidine,** laxatives	Global cerebral infarction and ischemia	R absent, L normal	Diffuse θ, occasional δ 1-2.5Hz
PT13/ 49/ M/ Rehab	Anoxia	VS/ 7	BL only	9 months	Daytime	**Baclofen,** Warfarin, Dantrolene, Levetiracetam, **Clonazepam**	Global hypoxic brain injury	N/A	θ activity at 5-6Hz, Occasional δ at 1.0-2.0Hz, Widespread β at 18-20Hz
PT14/ 55/ M/ Rehab	CVA (haem)	MCS/ 10	BL only	9 months	16:00-04:00	Folic acid, Lansoprazole, Levetiracetam, *Bisoprolol*, Thiamine, Amlodipine, Ramipril, Solvazin, **Baclofen**	L intracerebral haemorrhage, cerebral atrophy, ischaemic changes	R delayed, L absent	diffuse δ activity at 1.5-2.0Hz, θ activity at 5-6Hz over the central regions
PT15/ 60/ M/ Rehab	Anoxia	MCS/ 11	BL only	6 months	24:00-06:00 +bolus	Aspirin, Levetiracetam, **Pregabalin,** Simvastatin, Alendronic acid	Global hypoxic brain injury	N/A	Poorly responsive EEG, central-posterior θ, Widespread β, posterior δ

PT16/ 38/ M/ LTC	Anoxia	MCS/ 15	BL only (Enrolled for both)	4 years	16:00-02:00	**Baclofen, Clonazepam, Gabapentin,** Lansoprazole, Levetiracetam, **Lorazepam,** trihexyphenidyl	CT: no specific pathology	Inconclusive (difficult study)	N/A
PT17/ 53/ M/ Rehab	TBI	MCS/ 13	BL Only	7 months	16:00-04:00	**Baclofen**	Extensive oedema, large L parietal haematoma, contra-coup traumatic haemorrhage, SAH	Normal	β activity at 15-16Hz over both hemispheres. Occasional θ and α

8.1.2 Polysomnographic study results

One patient was excluded from PSG studies due to identification of frequent seizures on EEG and another due to worsening of involuntary movements. Therefore, 15 patients (7 female, 8 male), aged between 30 and 73 years, had 24-hour polysomnography. Seven of the 15 patients had suffered from anoxic brain injury which led to prolonged disorders of consciousness. Other aetiological causes for the brain injury included trauma (n=2), haemorrhagic stroke (n=4), ischaemic stroke (n=1), and central nervous system inflammatory disease/ vasculitis (Steven- Johnson Syndrome) (n=1).

A healthy adult with good sleep is expected to have sleep efficiency of 95%, to have few and brief nocturnal awakenings only (Hirshkowitz, 2004). Recommended total sleep time is 7 to 9 hours (Hirshkowitz et al., 2015). In adults, approximately 5% of the total sleep time is spent in stage 1, 50% in stage 2, 20% in stage 3 and stage 4, and the remaining 20% in REM (Pressman, 2000, Silber et al., 2007).

Basic review of hypnograms revealed that all patients had abnormal sleep patterns with one of the following conditions:

a) increased sleep activity during daytime with wakefulness at night (6/15),

b) increased sleep activity in both day and night time (2/15),

c) reduced sleep activity in both day and night time (5/15)

d) short sleep mostly at night (2/15).

Total recording time for the PSGs varied between 1247 and 1855 minutes. Therefore, Total Sleep Time (TST) and Sleep Efficiency (S.Eff.) values were normalized to 24-hour period. TST was reduced in all patients (Range:34-518mins, Mean:246 mins, SD:146.4) in comparison to recommended 7 to 9 hours of sleep for adults.

Two patients with traumatic brain injury had the least sleep efficiency (<%5) with 69.5 and 37.5 mins of sleep during PSG recording of 24 hours. Two patients with the most total sleep time and sleep efficiency however, had either increased sleep activity during day time and night time or increased sleep activity during day time. Both of these patients suffered from anoxic brain injury and had diagnosis of vegetative state (See Figure 8.1 for hypnograms of the patients with longest TST, and figure 8.2 for graphical representation of total sleep time in relation to CRS-R scores).

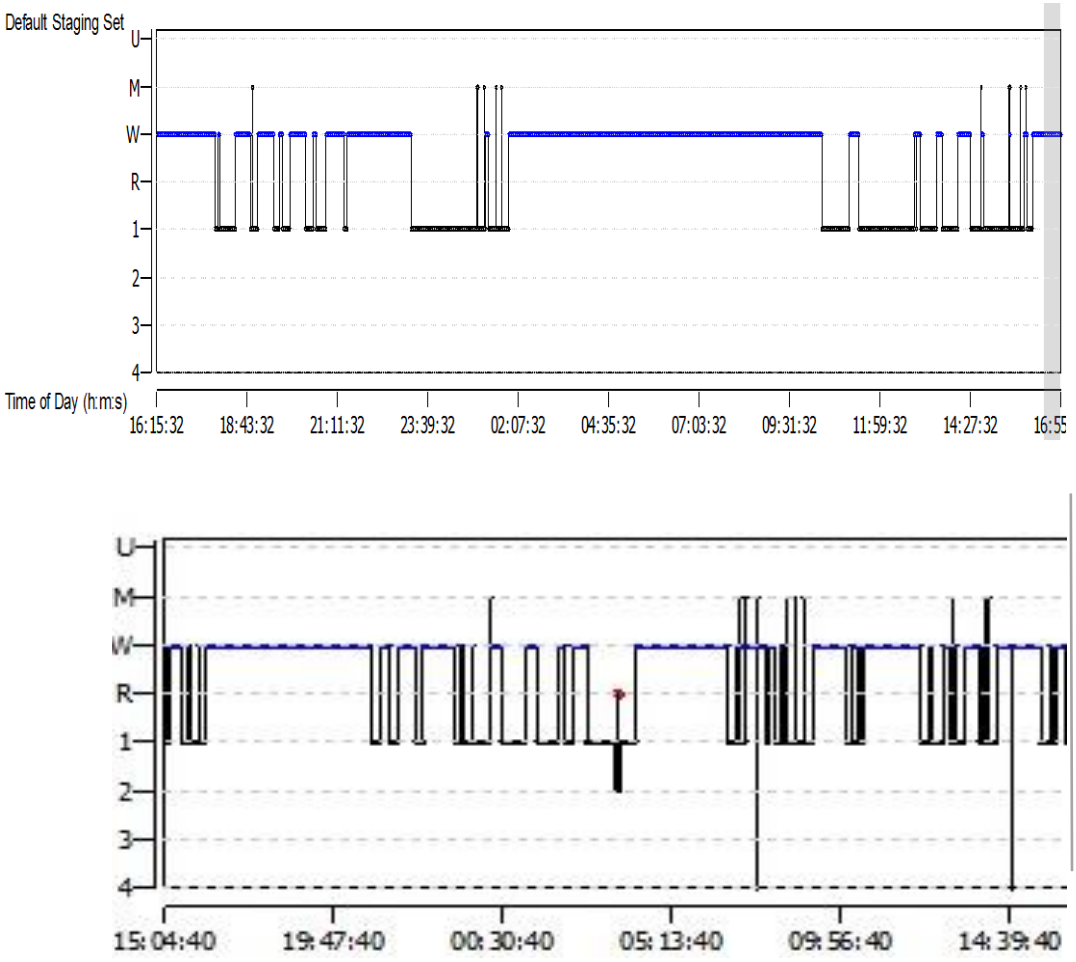


Figure 8-1: Hypnograms of two patients with highest sleep time and efficiency

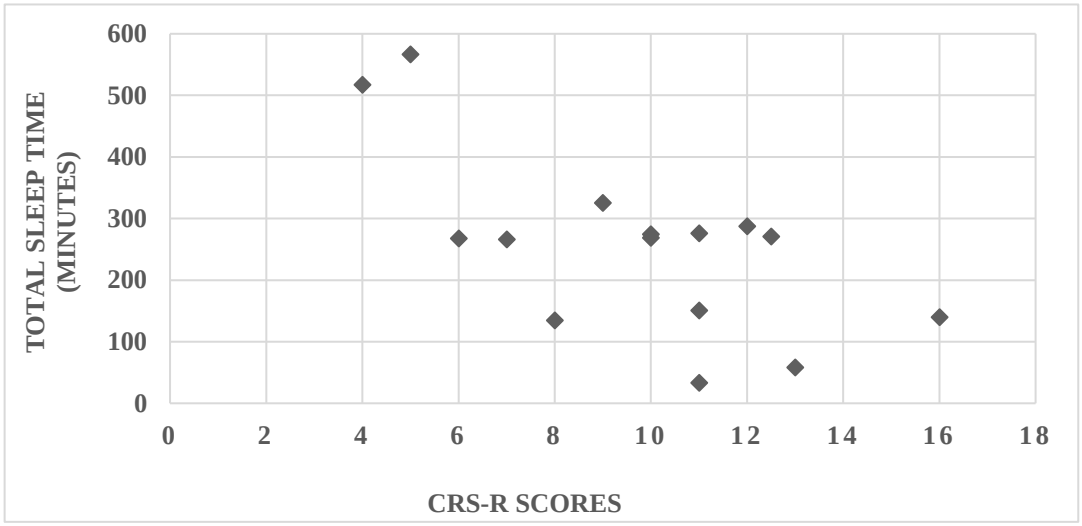


Figure 8-2: Graphical representation of total sleep time in relation to CRS-R scores

In our study, baseline sleep efficiency was very low (Mean= 18.1%, SD=8.45) and sleep was fragmented in all patients' PSGs with the number of spontaneous awakenings varying between 6 and 56 within a 24-hour period (Mean=16.5, SD= 10.8). None of the baseline PSGs contained sleep 3,4 stages and only 4 out of 15 patients, albeit very short, REM sleep (Table 8.4 and Figure 8.3, Figure 8.4).

Table 8-4: PSG findings of patients with PDOC. Total Sleep Time and Sleep Efficiency are normalized to 24-hour period. (Please note; in the table below, stage % may not add up to %100 due to movement time and presence of epochs that were not scorable)

Patient ID	Diagnosis	PSG Study Duration (mins)	Total Sleep Time (mins)	Sleep Efficiency (%)	Number of Awakenings	Stage 1 (% sleep time)	Stage 2 (% sleep time)	Stage 3 & 4 (% sleep time)	REM (% sleep time)
PT-1	MCS	1508	271	19.5	10	67.8	32.2	0	0
PT-2	MCS	1374	135	10.4	11	60.7	39.3	0	0
PT-3	VS	1480	518	36.4	21	99	0	0	0
PT-4	MCS	1691	269	17.3	19	94.8	2.8	0	2.4
PT-5	MCS	1717	58	3.7	7	96.4	3.6	0	0
PT-6	MCS	1855	140	11.7	6	100	0	0	0
PT-7	MCS	1484	288	21.2	22	58.3	39.2	0	0
PT-8	MCS	1610	34	2.2	11	82.7	17.3	0	0
PT-9	VS	1645	268	16.3	15	100	0	0	0
PT-10	MCS	1728	276	19.7	10	14.9	66.5	0	20.5
PT-11	VS	1423	567	20.2	27	97.1	1.8	0	0
PT-12	MCS	1483	326	23.1	18	84.8	0	0	14.5
PT-13	VS	1599	266	28.3	17	85.1	14.9	0	0
PT-14	MCS	1285	275	23.1	48	90.2	6.5	0	3.3
PT-15	MCS	1247	151	18.3	5	62.5	37.2	0	0
MEAN		1541.9	256	18.1	16.47	79.6	17.4	0	2.7
SD		171.4	146.4	8.75	10.83	23.4	20.6	0	6.2

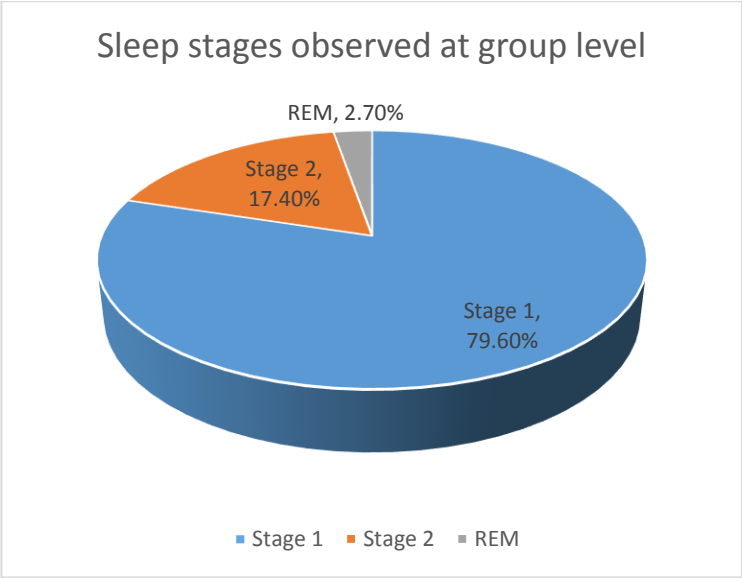


Figure 8-3: Sleep stages observed at group level on 24-hour polysomnography of 15 patients with PDOC

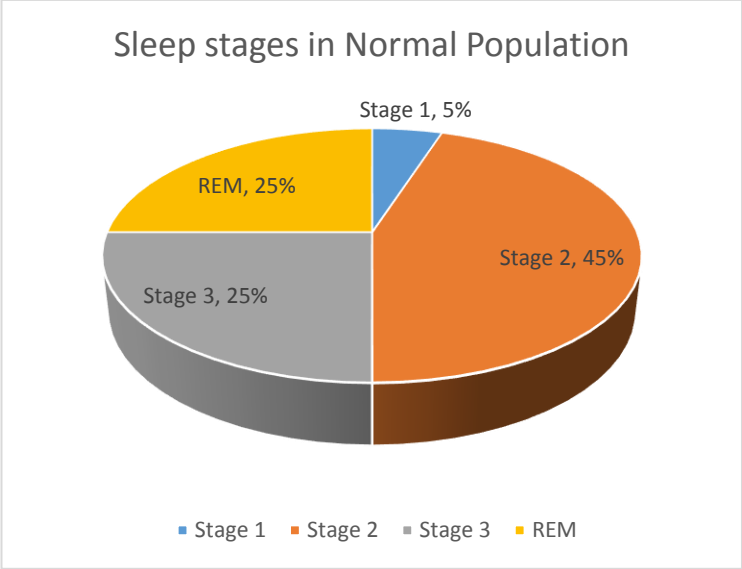


Figure 8-4: Sleep stages expected to be observed in normal subjects

Only 4 of the 15 patients had REM sleep in our study. Two of these patients had of anoxic brain injury and two had cerebrovascular event as cause of their

prolonged disorders of consciousness. All of the patients who had REM sleep were in minimally conscious state. 11 of the 15 patients had stage-2 sleep during time spent asleep. 3 patients had stage 1 sleep only. None of the patients showed presence of deep sleep (stage 3&4).

8.1.3 Circadian rhythm assessment results

8.1.3.1 Melatonin as a marker of circadian rhythm

48-hour saliva melatonin results were available for 15 patients. Out of the 180 samples collected within 48 hours 158 samples yielded results. Review of the melatonin plots showed that 6 out of 15 patients (1 VS, 5 MCS) had melatonin peak times occurring within the same two-hour window on consecutive 24-hour periods indicating that these patients may have a degree of preserved circadian rhythmicity. However, two of the patients had melatonin peaks at 7am and 11am.

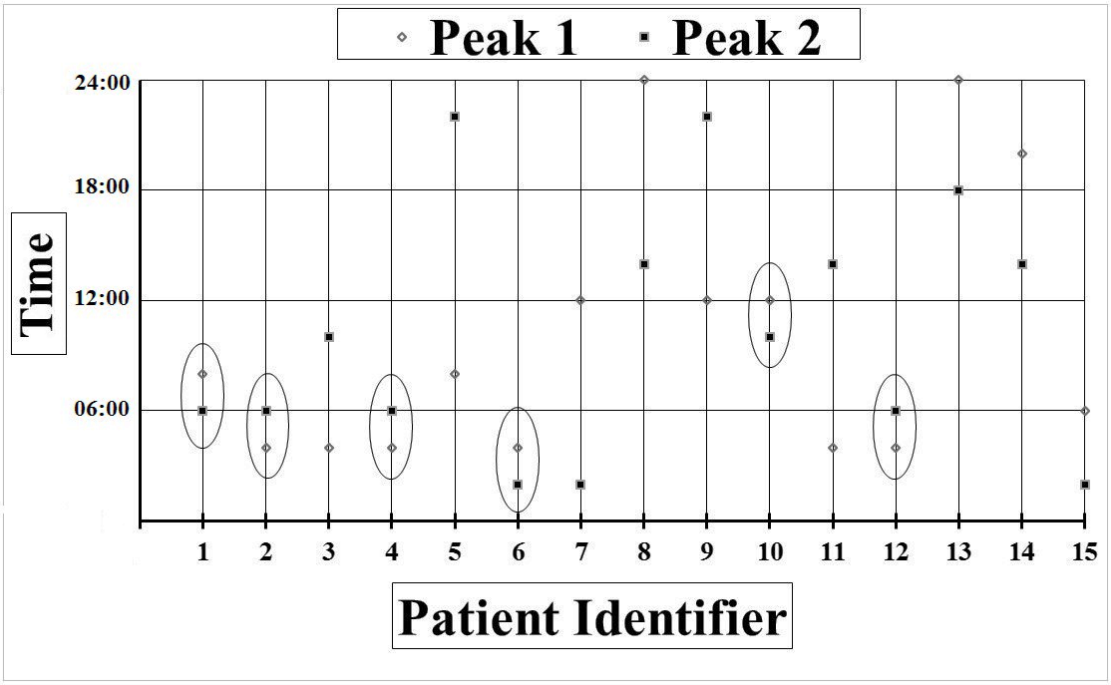


Figure 8-5: Peak times in each consecutive 24-hour period for each patient. Peak times occurring within the same 2-hour window are circled.

Daytime (8am to 8pm) melatonin mean was 84.42 (SD=29.4), and night (8pm to 8am) melatonin mean was 106.63 (SD=27.8). Means comparisons of day and night normalized melatonin values did not reach statistically significant values (p=0.07).

Cosinor analysis of saliva melatonin results revealed that averaged baseline % rhythmicity was low (Mean: 30.87%, Range: 10% - 66.4%, SD: 16.5) in comparison to a perfect fit scenario where percentage rhythm is 100% (Minors and Waterhouse, 1988). Although % Rhythmicity was higher in the patients who were fed during the day than in the patients who were fed at night; the difference between the two groups was not statistically significant $t(13)=1.27$ p=0.22 on the Two-Sample Assuming Unequal Variances t-test.

Table 8-5: Baseline sleep and melatonin rhythmicity results in order of Melatonin %Rhythm

Pt ID	TST	S. Eff	PSG Stages identified	Stage 1 %	Stage 2%	REM %	Most sleep occurred in;	Melatonin % Rhythm	Feeding Regime
PT7	288	21.2	1, 2	58.3	39.2	0	night	66.4	16:00-02:00
PT10	276	19.7	1, 2, REM	14.9	66.5	20.5	night	51.9	Daytime
PT11	567	20.2	1,2	97.1	1.8	0	day &night	49.8	Daytime
PT8	34	2.2	1, 2	82.7	17.3	0	night	46.5	Daytime
PT14	275	23.1	1,2, REM	90.2	6.5	3.3	night (early AM)	40.5	16:00-04:00
PT1	271	19.5	1, 2	67.8	32.2	0	night (early AM)	34.55	Daytime
PT12	326	23.1	1, REM	84.8	0	14.5	Night	32.9	Daytime
PT3	518	36.4	1	99	0	0	day &night	28.2	Daytime
PT9	268	16.3	1	100	0	0	day &night	23.45	16:00-04:00
PT17	N/A	N/A	N/A	N/A	N/A	N/A	N/A	19	16:00-04:00
PT4	269	17.3	1,2, REM	94.8	2.8	2.4	day &night	16.8	16:00-04:00
PT6	140	11.7	1, 2	100	0	0	day	16.55	18:00-02:00
PT2	135	10.4	1,2	60.7	39.3	0	day &night	13.5	Daytime
PT5	58	3.7	1, 2	96.4	3.6	0	night	12.9	16:00-02:00
PT16	N/A	N/A	N/A	N/A	N/A	N/A	N/A	10	16:00-02:00
PT13	266	28.3	1,2	85.1	14.9	0	Night	N/A	
PT15	151	18.3	1,2	62.5	37.2	0	night (early morning)	N/A	

PSG= Polysomnography, TST=Total Sleep Time (normalized to 24 hour), S.Eff= Sleep Efficiency (Normalized to 24 hour), REM= Rapid Eye Movement

Means comparisons were made between 8pm, midnight and 4am values to evaluate whether there was a melatonin response dark in patients whose melatonin results were available at these time points. The result of the paired t-test was statistically significant at group level between 8pm and midnight values. (See table 8.6)

Table 8-6: Melatonin response to dark at baseline (all patients)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	eight_pm - midnight	-9.223	10.073	2.794	-15.310	-3.136	-3.301	12	.006
Pair 2	eight_pm - four_am	-14.800	25.518	7.366	-31.013	1.413	-2.009	11	.070
Pair 3	midnight - four_am	-5.975	18.372	5.304	-17.648	5.698	-1.127	11	.284

The same test was applied for the patients in vegetative state and minimally conscious state subgroups to see if the response to dark differed between two diagnostic subgroups. While the patients in VS had no significant increase of melatonin with dark, the patients in MCS had (See table 8.7 and 8.8).

Table 8-7: Melatonin response to dark in VS

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	eight_pm - midnight	-5.220	6.750	3.019	-13.601	3.161	-1.729	4	.159
Pair 2	eight_pm - four_am	-8.600	19.322	8.641	-32.591	15.391	-.995	4	.376
Pair 3	midnight - four_am	-3.380	18.441	8.247	-26.278	19.518	-.410	4	.703

Table 8-8: Melatonin response to dark in MCS

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	eight_Pm - midnight	-11.725	11.371	4.020	-21.232	-2.218	-2.916	7	.022
Pair 2	eight_Pm - four_am	-19.229	29.832	11.276	-46.819	8.362	-1.705	6	.139
Pair 3	midnight - four_am	-7.829	19.557	7.392	-25.916	10.259	-1.059	6	.330

8.1.3.2 Body Temperature as a marker of circadian rhythmicity

Complete data sets for temperature measurements for 48 hours were available in eight patients yielding total of 768 measurements (measurement with i-button every 30 minutes).

Graphical representation of the mean temperature values revealed a similar pattern to distal body temperature rhythm of healthy people with increased values during night; instead of a similar pattern to core body temperature

rhythm of healthy people (Kräuchi, 2002). Please see figure 8.6, 8.7 and 8.8 for comparison.

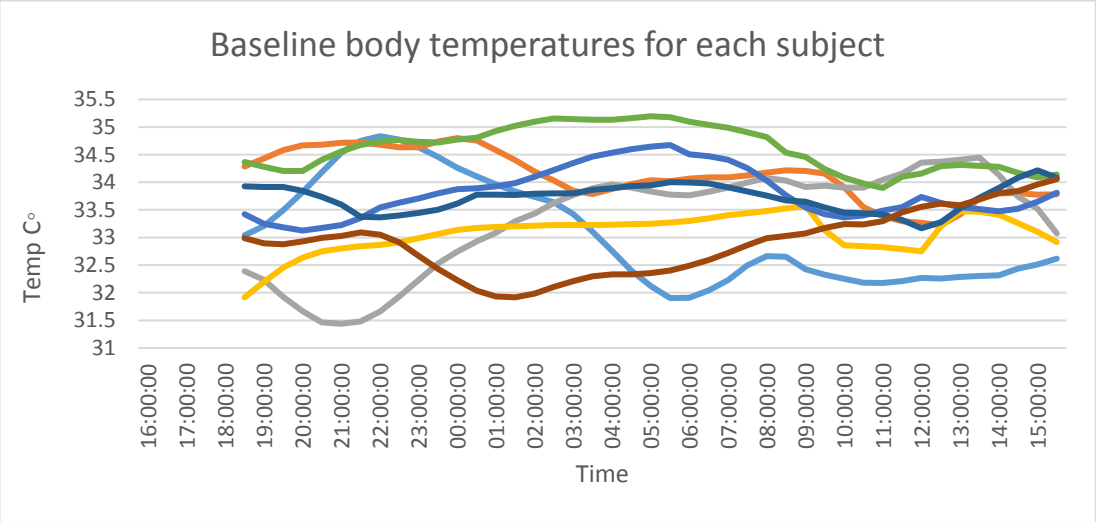


Figure 8-6: Body temperature data (moving averaged) for each subject n=8

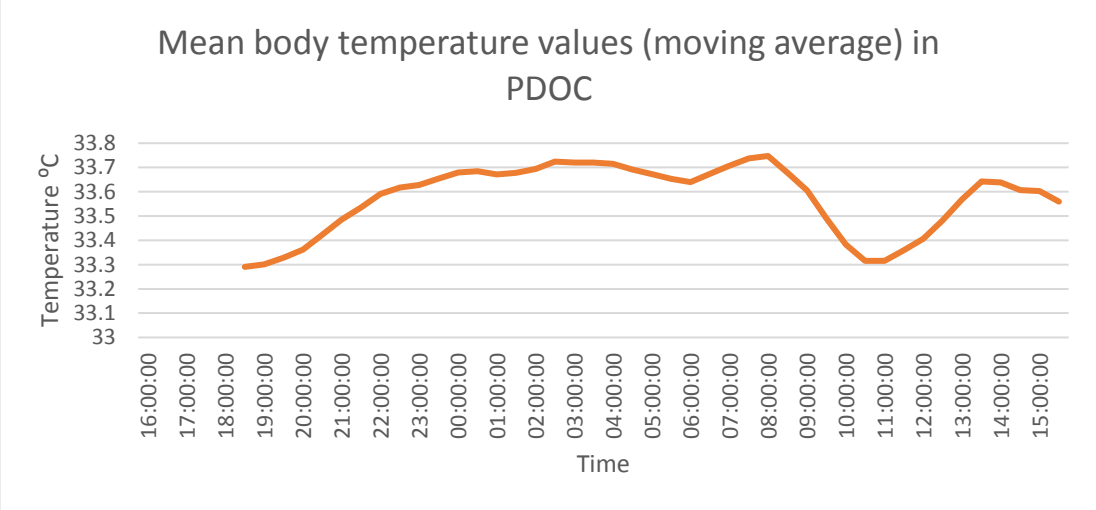


Figure 8-7: Body temperature measurements in PDOC (n=8)

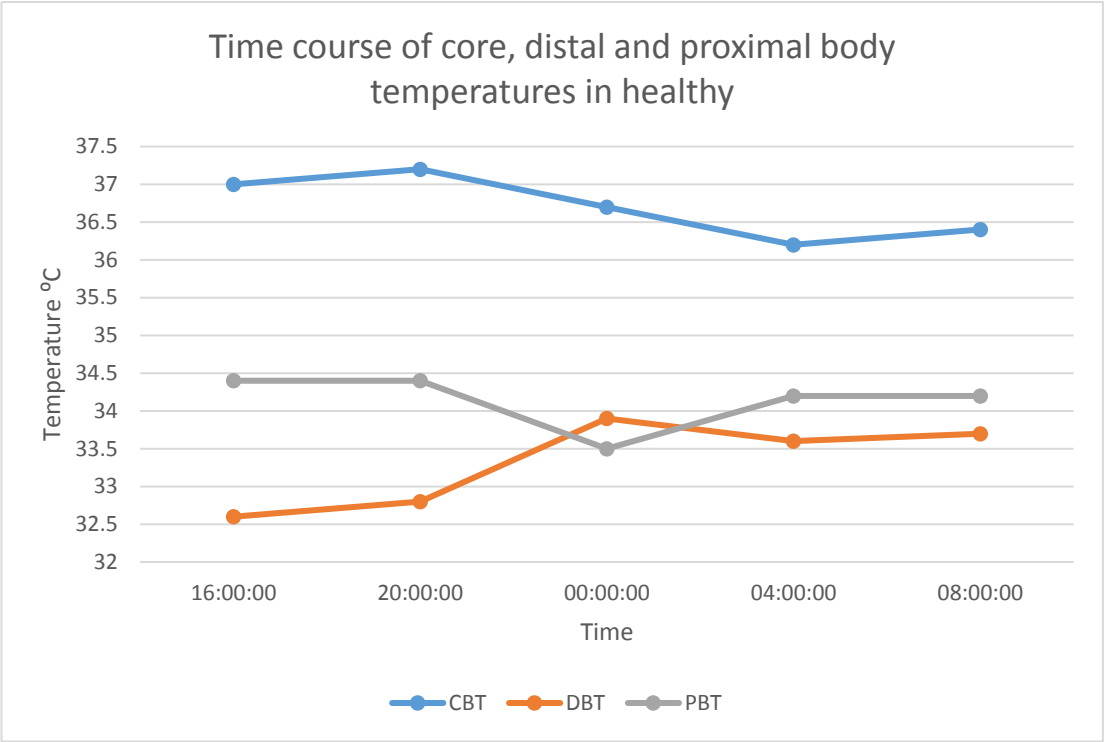


Figure 8-8: Body temperature measurements of healthy people (Adopted from Kräuchi, Clin Auton Res (2002) 12: 147–149)

Cosinor analysis of baseline body temperatures (period 23.5 to 24.5 hours) revealed low % rhythmicity. Mean % rhythm was 40.47% (Range= 2.1-84.4, SE= 5.1).

8.1.4 Summary of baseline investigations

- All patients had abnormal baseline EEG with low amplitude record characterized by diffuse polymorphic delta activity at 1.0-2.5Hz between 10-20µV.
- Our assessments showed that baseline circadian rhythmicity and sleep were abnormal in all patients.
- Cosinor analysis of saliva melatonin results revealed that averaged baseline %Melatonin rhythmicity was low (Mean= 31%, Range= 13% - 66.4%, SD= 18.4). Body temperature %rhythmicity was also low. Mean= 40.47% Range= 2.1-84.4, SE= 5.1).

- Quantity and quality of sleep was severely abnormal in patients with PDOC. Total sleep time was low (Range 35-523 mins, Mean= 251 mins, SD=139.8). Sleep efficiency was low (Mean= 18.1%, Range 3.7%-36.4%, SD= 8.45). None of the patients had slow wave sleep and only four patients had REM sleep.
- These results suggest that there is a need to attempt to normalize circadian rhythm and sleep of patients with PDOC, which may then lead to improvement of awareness/ consciousness levels.

8.2 INTERVENTIONAL STUDY RESULTS

This section of the thesis summarizes the results of the 5-weeks intervention which aimed to optimize the circadian rhythm and sleep with melatonin at night, caffeine and light treatment in the mornings with the aim of optimizing circadian rhythm and sleep. We also wanted to explore that whether the interventions led to improvement of consciousness/ brain functions measured using either clinical assessment tools (CRS-) and/ or neurophysiological investigations (on MMN and SON experiments).

Detailed clinical information on interventional study participants can be found in Appendix 1.

8.2.1 Side Effects and Tolerability of Interventions

One of the patients (patient 12) who was initially enrolled in the interventional study developed behavioural responses which were unusual for her (agitation, grimacing during personal care and manual handling suggesting patient might had been experiencing pain) whilst she was in the second week of interventions. This prompted medical reviews and investigations. She was very quickly diagnosed with advanced intra-abdominal malignancy. She was then transferred to another healthcare facility near to her family to receive palliative care. She died few weeks later.

None of the 10 patients who completed the interventional study had any side effects. Nursing staff and families of patients reported no difficulty in giving care and/or interacting with patients during intervention.

8.2.2 Effect of intervention on circadian rhythm

8.2.2.1 Melatonin

General Linear Model pairwise comparisons between baseline 1 and baseline 2 conditions were not significant for melatonin rhythms. However, comparisons between both baseline measurements and post-intervention measures were significant. This indicates that, melatonin circadian rhythms were stable at the baseline tests which were performed one month apart from each other without any change to patient's conditions; and, the change following the intervention was not due to natural fluctuations in these measures. Hence, the data from baseline conditions and post-intervention conditions were collapsed together and t-test statistics were performed to find out if the improvements were significant.

Increase in %Melatonin Rhythm following intervention was statistically significant (p=0.012) (see table 8.9 and 8.10 for details). Improvement in % melatonin rhythm was maintained even three weeks after cessation of intervention as general linear model pairwise comparison between 3-days and 3-weeks after the intervention was not significant (*sig*=0.888). Plotting of peak times revealed that while prior to intervention only 2 patients had melatonin peaks in dark, after the intervention 7 patients had their melatonin peaks in dark (figure 8.9).

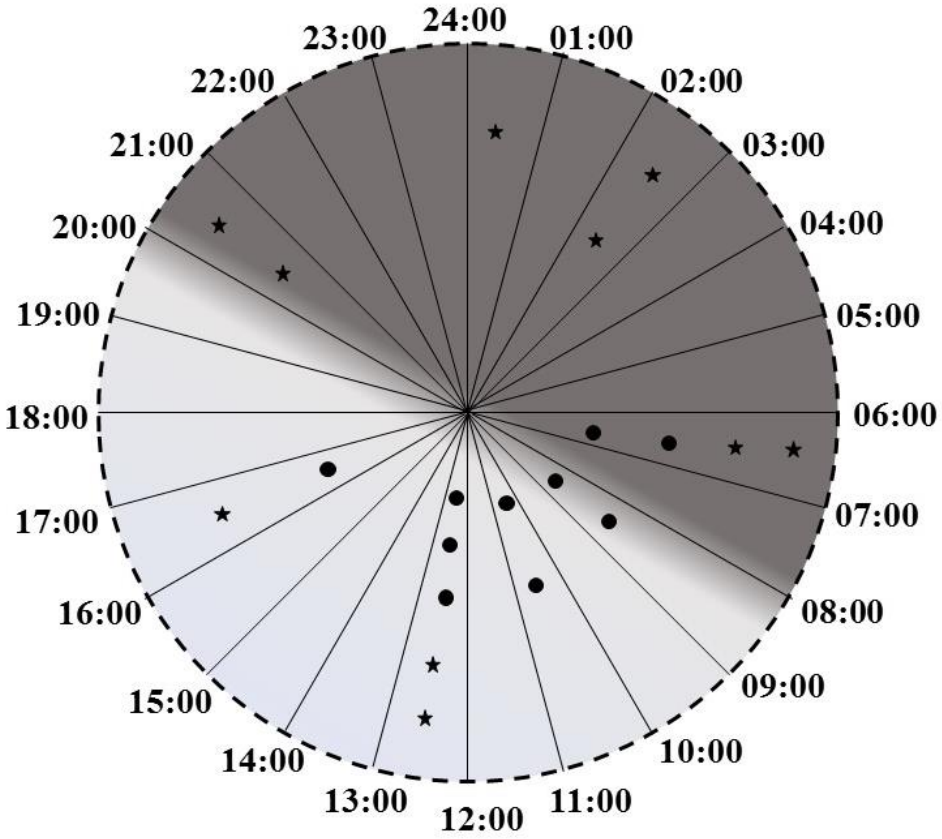


Figure 8-9: Melatonin peak times before the intervention (circles) and after the intervention (stars).

Table 8-9: Descriptive statistics of saliva melatonin % rhythmicity results at baseline1 (BL1), baseline 2 (BL2), post-intervention 1 (PI1) and post-intervention 2 (PI2) time points.

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
BL1%rhythm	10	11.20	92.20	37.7000	24.25586
BL2%rhythm	10	7.40	60.60	24.4500	19.08328
PI1%rhythm	10	.00	96.80	54.3500	26.98478
PI2%rhythm	10	2.40	100.00	51.8500	37.29934
Valid N (listwise)	10				

Table 8-10: Means comparison (paired samples t-test) results for baseline and post-intervention saliva melatonin % rhythmicity results

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	BL1&2AveRhythm - PI1&2AveRhythm	-22.02500	22.22358	7.02771	-37.92279	-6.12721	-3.134	9	.012

8.2.2.2 Body Temperature

Cosinor analysis of body temperature values showed that the patients with PDOC maintained degree of rhythmicity at baseline (period 23.5- 24.5 hours). However, the value was low. Baseline body temperatures % rhythmicity mean value was 40.47 (Range= 2.1-84.4, SE= 5.1).

The post-intervention mean body temperature % rhythmicity was lower than the baseline (Mean= 36.44, SE= 4.94, Range= 7.1-90.3), however this was not statistically significant at the paired samples two-tailed t-test (p=0.57).

8.2.3 Sleep studies

General Linear Model pairwise comparisons between baseline 1 and baseline 2 conditions were not significant for any of the PSG parameters. As the PSG studies were independently reported by two different neuro-physiologists who were also blinded to patient's experimental conditions and to each other, intra-class correlations were performed to see if there was an absolute agreement between them. It was found that there was excellent agreement for total sleep time (TST), time spent in sleep at night, sleep efficiency and REM stage

scorings. Therefore, all baseline and all post-intervention data for these parameters were collapsed together and means comparisons were made to see if the improvements observed were statistically significant. Although improvement of all 4 parameters were observed the improvement following the intervention did not reach statistically significant levels (table 8.11).

Table 8-11: 24-hour Polysomnography parameter changes. TST= Total Sleep Time, SE= Sleep Efficiency, REM= Rapid Eye Movement

Sleep parameter	Range	Mean	SD	P value
Baseline Night Sleep	78.4- 204.3 mins	145 mins	39.7	.596
Post-Intervention Night Sleep	23.5-334.3 mins	168 mins	112	
Baseline REM	0-31.9 mins	5.8 mins	11.3	.145
Post-Intervention REM	0-76.5 mins	22 mins	33.5	
Baseline TST	71.1-439.7 mins	236.3 mins	119.3	.420
Post-Intervention TST	56.3-627.0 mins	286.7 mins	197.2	
Baseline SE	4.5-29.3	15.6	7.8	.413
Post-intervention SE	3.6-44.5	19.3	13.9	

8.2.4 Behavioural assessments

General Linear Model pairwise comparisons between baseline 1 and baseline 2 conditions were not significant for behavioural measures. However, comparisons between both baseline measurements and post-intervention measures were significant. This indicates that, CRS-R scores were stable at the baseline tests which were performed one month apart from each other

without any change to patient's conditions; and, the change following the intervention was not due to natural fluctuations in these measures. Hence, the data from two baseline conditions (two time points) were collapsed together and compared with post-intervention (one time point) results to find out if the improvements were significant.

Table 8-12: CRS-R Scores Pre- and Post- intervention for each patient

Subject	CRS-R Average Values pre-intervention	CRS-R Values post-intervention
Patient 1	12.5	15
Patient 2	8	9
Patient 3	4	4
Patient 4	10	10
Patient 5	13	18
Patient 6	16.5	16
Patient 7	12	15
Patient 8	11.5	14
Patient 9	6	6
Patient 10	10.5	11

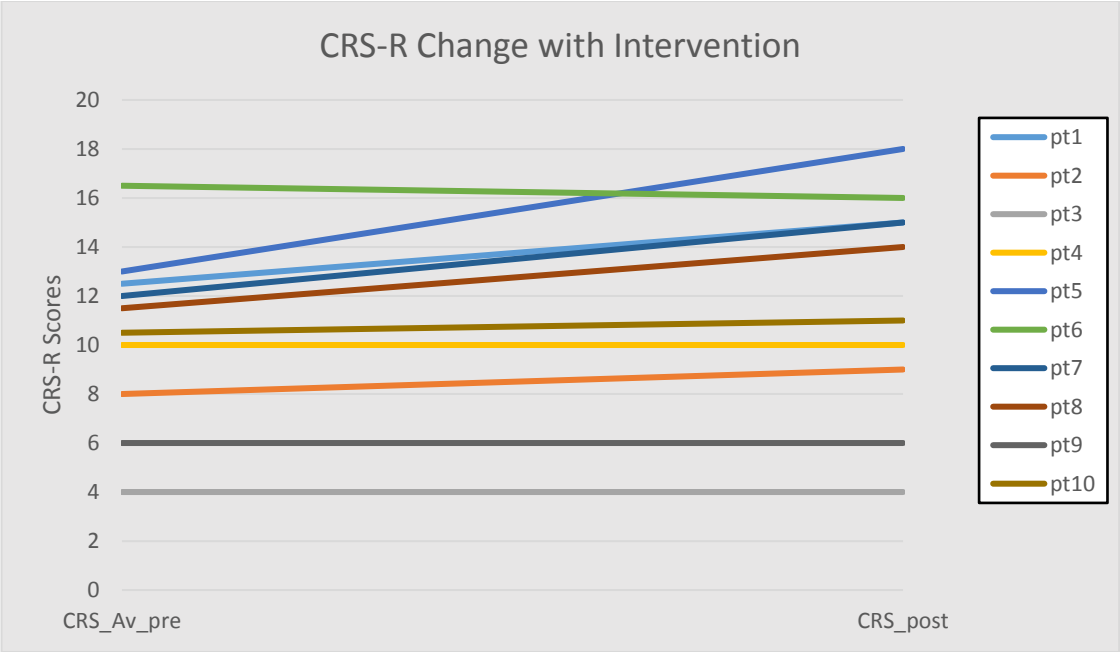


Figure 8-10: Graphical representation of Pre and Post intervention CRS-R scores

7 patients had improvement of CRS-R scores with intervention. Wilcoxon signed ranks test revealed statistically significant improvement of CRS-R scores with the intervention; both between CRS-R baseline averaged data and post-intervention (p=0.034) and, between baseline-2 and post-intervention which represents the change between the two closest time points before and after the intervention (p=0.027).

8.2.5 ERP results

All the patients who had improvement of clinical scores (7/10) also had statistically significant improvement of neurophysiological responses on MMN and SON experiments (p=0.001).

At the group level we identified two peaks where the MMN increased after therapy. These were in the right frontal region and left occipital region. In the SON experiment we identified two peaks response to stimuli amplitude increased with therapy. These were both located at the left parietal region (see Figure 8-11: Statistical parametric mapping group t-test results for oddball and subject's own name experiments for pre-intervention and post-intervention conditions. MMN=mismatch negativity, SON= Subject’s own name).

Table 8.13 and 8.14 gives beta values (μV) for each subject during each MMN and SON experiments. (negative-going ERP responses are in minus values).

Table 8-13: ERP magnitude beta values for MMN experiments

MMN Beta values from nearest maximum location to group mean x=34mm, y=56mm, z=396ms														
	x (mm)	y (mm)	z (ms)	TP1_odd-β	TP1_standard_β	TP1_MMN	TP2_odd-β	TP2_standard_β	TP2_MMN	TP3_odd-β	TP1_standard_β	TP3_MMN	MMN-pre	MMN-post
pt1	38	61	371	0.8737	-0.6837	1.5574	2.9956	-2.1709	5.1665	2.0657	2.6283	-0.5626	**3.362**	**-0.563**
pt2	-30	61	389	1.2135	-0.1921	1.4056	0.6208	0.5643	0.0565	-3.319	0.7715	-4.0905	**0.731**	**-4.091**
pt3	38	34	398	14.3254	0.1797	14.1457	0.0462	0.0481	-0.0019	1.865	1.5357	0.3293	**7.072**	**0.329**
pt4	17	2	387	3.2224	0.1029	3.1195	0.0745	-0.7573	0.8318	-1.1454	0.2477	-1.3931	**1.976**	**-1.393**
pt5	38	-73	381	0.7162	-0.2974	1.0136	0.9784	-0.5453	1.5237	-1.5769	-0.3195	-1.2574	**1.269**	**-1.257**
pt6	-26	61	395	1.6772	0.16	1.5172	0.1368	-0.1179	0.2547	-2.8165	0.0003	-2.8168	**0.886**	**-2.817**
pt7	43	29	396	0.1661	-1.5561	1.7222	-0.1619	0.7094	-0.8713	-15.8518	-3.3752	-12.4766	**0.425**	**-12.477**
pt8	-9	2	309	0.1973	-0.106	0.3033	-0.5738	-0.2987	-0.2751	-0.7567	0.508	-1.2647	**0.014**	**-1.265**
pt9	-4	13	152	2.2739	0.1611	2.1128	-0.1993	-0.1326	-0.0667	-1.6999	0.5503	-2.2502	**1.023**	**-2.250**
pt10	44	33	328	1.4837	0.4567	1.027	4.5328	-0.4684	5.0012	15.8029	0.1855	15.6174	**3.014**	**15.617**

Table 8-14: ERP magnitude beta values for SON experiments

SON Beta Values from Nearest max location to group mean (x=-30mm, y=-68mm, z=186ms)														
	x (mm)	y (mm)	z (ms)	TP1-SON-β	TP1-Other-β	TP1_diff	TP2-SON-β	TP2-Other-β	TP2_diff	TP3-SON-β	TP3-Other-β	TP3_diff	SON_ Pre_ Ave	SON_ Post
pt1	-17	-19	229	-0.7417	-0.7361	3.7361	-1.4064	-1.2684	-0.138	-2.5306	-1.2135	-1.3171	**1.799**	**-1.317**
pt2	4	-36	164	10.0167	0.5585	2.4415	0.7839	0.4182	0.3657	0.0328	-0.0677	0.1005	**1.404**	**0.101**
pt3	-30	-52	172	0.608	0.1738	2.8262	0.8021	0.2045	0.5976	-0.5609	1.2384	-1.7993	**1.712**	**-1.799**
pt4	47	-62	252	1.7719	-2.5525	5.5525	5.7944	-2.0605	7.8549	-0.3245	-0.8132	0.4887	**6.704**	**0.489**
pt5	-60	-30	182	0.2894	0.2738	2.7262	5.7614	0.2027	5.5587	0.9811	1.4387	-0.4576	**4.142**	**-0.458**
pt6	13	-57	152	1.3171	-0.351	3.351	2.3274	-0.2161	2.5435	-0.707	0.3843	-1.0913	**2.947**	**-1.091**
pt7	60	-30	180	1.46	-2.3314	5.3314	0.6247	0.4014	0.2233	-3.8914	3.2333	-7.1247	**2.777**	**-7.125**
pt8	-4	-19	178	0.0796	0.0975	2.9025	0.3446	0.1601	0.1845	-1.2567	-0.464	-0.7927	**1.544**	**-0.793**
pt9	38	34	398	2.3922	-0.9823	3.9823	0.8565	0.7399	0.1166	0.9615	0.5466	0.4149	**2.049**	**0.415**
pt10	-17	-62	197	0.089	-0.1051	3.1051	0.9813	-1.2516	2.2329	-2.6455	0.1565	-2.802	**2.669**	**-2.802**

ODDBALL EXPERIMENTS (MMN INCREASE)

MMN Increase Peak Level Statistics at Group Level						
T	Z	P uncorr	mm	mm	ms	MMN Amplitude Post-intervention
6.54	3.23	0.001	34	56	398	-2.8 µV
6.12	3.14	0.001	-13	-95	357	-1.4 µV

SUBJECT'S OWN NAME EXPERIMENTS (SON vs OTHER NAMES)

Peak Level Statistics at Group Level						
T	Z	P uncorr	mm	mm	ms	Response to SON Amplitude post-intervention
9.30	3.92	0.000	-30	-68	186	1.6 µV
5.13	3.07	0.001	-17	-79	188	9.8 µV

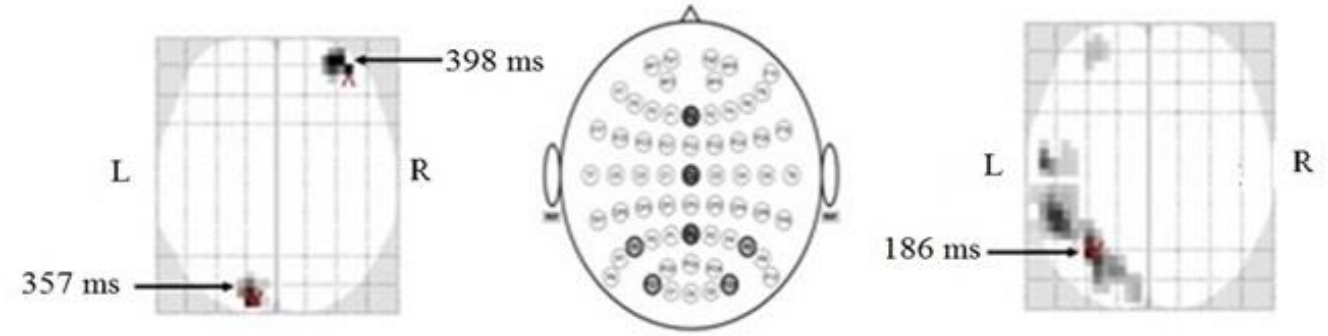


Figure 8-11: Statistical parametric mapping group t-test results for oddball and subject's own name experiments for pre-intervention and post-intervention conditions. MMN=mismatch negativity, SON= Subject's own name

8.3 CHAPTER SUMMARY

Our baseline work showed that sleep and circadian rhythms were severely deranged in PDOC. However, we were able to improve sleep and circadian rhythms with only five weeks of intervention which comprised of melatonin treatment at night, caffeine and blue light treatments in the morning.

With the international study we were able to show that following circadian rhythm intervention there was a significant improvement in %Melatonin rhythmicity (p=0.012).

Although, improvements in individual PSG parameters did not reach statistically significant levels, qualitative and quantitative improvement of sleep were evident for majority of patients as shown in figure 8.11.

Most significantly, 7 patients had improvement of CRS-R scores with intervention which was statistically significant (p=0.034).

Individually, those patients who responded well to the intervention also showed improvements in their functional brain imaging assessments (p=0.001).

The improvements in sleep quality (more complex sleep patterns and presence of REM sleep), sleep quantity (Total and night time sleep time), circadian rhythm (melatonin % rhythm), clinical presentation (CRS-R changes) and ERP effect magnitudes during the mismatch negativity and subject's own name experiments are given in table 8.15 for each subject. Correlation statistics revealed that improvements in melatonin rhythm and sleep time (total and night time) and, improvements in both event related potential tests were significantly correlated (See table 8.16). However, there was no significant correlation between these measures and behavioural measures in this sample of 10 subjects with disorders of conscious ness who are two to eight years post brain injury.

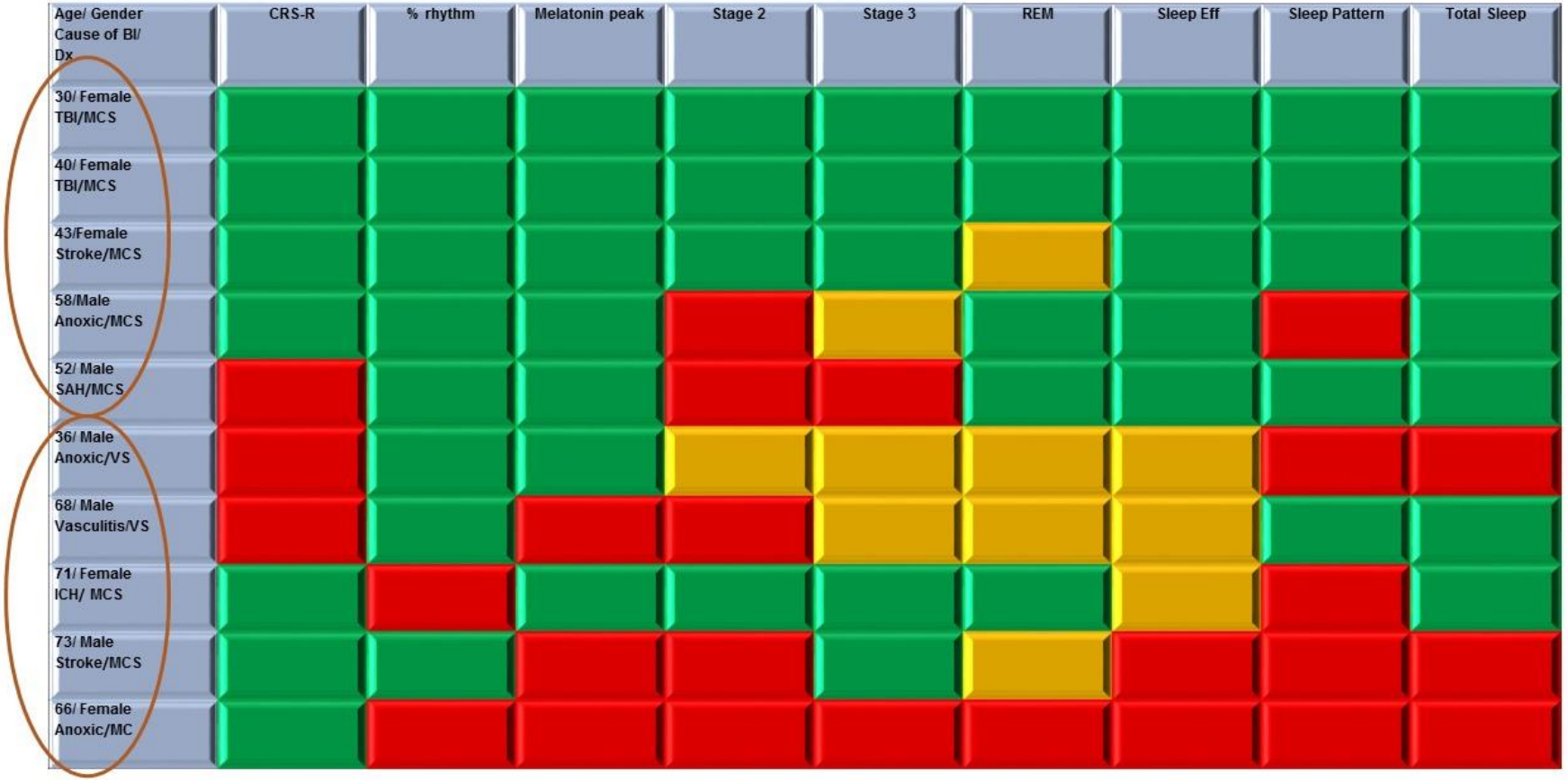


Figure 8-12: Changes with intervention on the parameters examined. Green represents clear and significant improvement, orange some minor improvement and red no improvement

Table 8-15: Changes in sleep quality, sleep quantity, circadian rhythm, clinical outcomes and event related potentials with intervention. Sleep pattern complexity scores are based on sleep pattern coding as defined by Valente: 1 =monophasic, 2 =cyclic alternating, 3 =rudimentary, 4= NREM, 5= REM (Valente et al., 2002)

PATIENT CHARACTERISTICS			EFFECT ON SLEEP QUANTITY		EFFECT ON SLEEP QUALITY		EFFECT ON CIRCADIAN RHYTHM	EFFECT ON CLINICAL OUTCOMES	EFFECT ON ERP MAGNITUDE (µV values from nearest maximum location to group mean)			
Pt ID	Sex/Age/ Diagnosis/ Aetiology	Years since brain injury	TST change (mins)	Night Sleep change (mins)	REM amount change (mins)	Sleep pattern complexity change	% Melatonin Rhythm change	CRS-R Change	MMN x=34mm y=56mm z=396ms		SON x=-30mm y=-68mm z=186ms	
									Pre	Post	Pre	Post
Pt-1	F/ 71/ MCS/ SAH	5	+14.5	-37.75	+0.5	4 → 5	-0.6	+2.5	2.441	-5.617	1.799	-1.317
Pt-2	M/ 58/ MCS-/ Anoxia	5	+74.25	+68.38	+76.5	3 → 5	+41.45	+1	0.964	-4.443	1.404	0.101
Pt-3	M/ 36/ VS/ Anoxia	2	-59	+29.25	0	2 → 2	+13.35	0	0.553	-1.577	1.712	-1.799
Pt-4	M/ 52/ MCS/ SAH	4	+192.5	+180.63	+37.63	5 → 5	+45.55	0	4.121	-1.268	6.704	0.489
Pt-5	F/ 30/ MCS/ TBI	5	+138.25	+158.13	+64.75	2 → 5	+29.4	+5	0.201	-3.287	4.142	-0.458
Pt-6	F/ 43/ MCS/ Stroke	3	+349	+204.38	0	3 → 4	+63.8	-0.5	2.531	-3.249	2.947	-1.091
Pt-7	M/ 73/ MCS/ Stroke	8	-163.25	-114.50	0	4 → 4	+17.1	+3	-0.099	-14.994	2.777	-7.125
Pt-8	F/ 40/ MCS/ TBI	3	+66.75	-96.50	4.125	3 → 5	+2.85	+2.5	0.158	-1.437	1.544	-0.793
Pt-9	M/ 68/ VS/ Vasculitis	6	+39.25	-16.00	0	2 → 2	+9.25	0	1.023	-2.250	2.049	0.415
Pt-10	F/ 66/ MCS/ Anoxia	6	-266	-153.63	-21.25	3 → 2	-1.9	+0.5	-1.657	-3.058	2.669	-2.802

Table 8-16: Pearson correlations for changes occurred following intervention showing that melatonin rhythm increase was correlated with sleep time increase (total and night time) and ERP effect magnitude changes for both MMN and SON were also correlated with each other.

		TST increase	CRS-R Increase	Melatonin rhythm change	Night TST increase	REM Increase	ERP Effect Magnitude for MMN	ERP Effect Magnitude for SON
TST increase	Pearson Corr.	1	-.132	.775**	.866**	.426	-.099	-.324
	Sig. (2-tailed)		.716	.008	.001	.220	.786	.360
	N	10	10	10	10	10	10	10
CRS-R increase	Pearson Corr.	-.132	1	-.270	-.152	.339	.272	.230
	Sig. (2-tailed)	.716		.451	.676	.338	.448	.522
	N	10	10	10	10	10	10	10
Melatonin rhythm change	Pearson Corr.	.775**	-.270	1	.866**	.508	.148	.055
	Sig. (2-tailed)	.008	.451		.001	.134	.684	.881
	N	10	10	10	10	10	10	10
Night TST increase	Pearson Corr.	.866**	-.152	.866**	1	.580	-.094	-.141
	Sig. (2-tailed)	.001	.676	.001		.079	.796	.698
	N	10	10	10	10	10	10	10
REM Increase	Pearson Corr.	.426	.339	.508	.580	1	-.010	-.213
	Sig. (2-tailed)	.220	.338	.134	.079		.979	.554
	N	10	10	10	10	10	10	10
ERP Effect Magnitude for MMN	Pearson Corr.	-.099	.272	.148	-.094	-.010	1	.651*
	Sig. (2-tailed)	.786	.448	.684	.796	.979		.042
	N	10	10	10	10	10	10	10
ERP Effect Magnitude for SON	Pearson Corr.	-.324	.230	.055	-.141	-.213	.651*	1
	Sig. (2-tailed)	.360	.522	.881	.698	.554	.042	
	N	10	10	10	10	10	10	10

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Chapter 9 DISCUSSION

Acquired brain injury is a worldwide and an important public health problem with a significant impact on people with brain injuries themselves, others involved in the care of them, and in wider societies. Although stroke and trauma are the most common causes, it can be due to infection, inflammatory conditions, tumours, and degenerative diseases of the brain. It is a silent epidemic all over the world with very severe consequences. Disorders of consciousness (vegetative and minimally conscious states) are the most severe forms of acquired brain injury which are not scientifically well-understood, currently with no treatment available for, and therefore creates a significant medical, ethical and legal conundrum. People with disorders of consciousness can live many years with good medical and nursing care but with very minimal chance of recovery of brain functions. The level of care they need is intensive, specialist, expensive and therefore scarce, even in the most modern countries of the world.

In the introduction chapters of this thesis, I summarized the key information on disorders of consciousness, sleep and circadian rhythms in general; reviewed the literature to explore what is known about the sleep and circadian rhythms of people with DOC. It became clear that, a handful of studies showed sleep and circadian rhythms are possibly impaired in DOC. This was not a surprise for us, because in the Royal Hospital for Neuro-disability, Putney, London which is a big centre caring for people with DOC for many years, we often observed abnormal sleep-wake patterns in this population. What we needed to do now was, to conduct a robust study to explore sleep and circadian rhythmicity in this population and to design a treatment protocol. The methods development and methods chapters of this thesis summarized how I achieved this.

The main aim of this research was to explore effect of circadian rhythm and sleep optimization on brain functions of people with PDOC. We first

investigated circadian rhythms of salivary melatonin and sleep of patients with PDOC and found that both processes were severely impaired. Then, we made an attempt to optimize circadian rhythm by giving melatonin at night, caffeine and blue light in the morning. Our results showed that, this simple and inexpensive intervention led to improvement of sleep quality and/or quantity in most patients, which then translated to improvement in bedside consciousness assessments and on neurophysiological paradigms we tested. This is a very significant outcome as it indicated that as scientists and clinicians we need to go back to the very basics, try to correct what is abnormal at the physiological and biological levels first. Sleep after all, one of the most important events in our lives and without good sleep we cannot function.

In these final chapters of the thesis, I will discuss strengths and weaknesses of my research, what I learnt while working of this study, and what I would do differently in the future.

9.1 SETTING and PATIENT POPULATION

This research was undertaken at the RHN, which is a specialist centre in diagnosis and management of PDOC in Putney, London, UK. The RHN has been caring for people with severe brain injuries since 1854 and, it houses the largest PDOC assessment and rehabilitation unit in the United Kingdom. Almost half of the long-term nursing care beds are occupied by people with PDOC.

In my research, 17 patients with PDOC took part in the baseline investigations and ten of those proceeded to interventional study.

In the baseline investigations cohort, 5 of the patients were from Level 1 rehabilitation unit and the remaining were from long-term care wards. While the patients in the rehabilitation unit had a limited length of stay (3-4 months) and at early stages of PDOC (6 to 9 months post brain injury), the patients from long-term care wards had no limitation for length of stay and were in

chronic stages of PDOC (2 to 8 years after brain injury). The interventional study cohort also had the advantage of being on stable medication regimen. They were well-known by the staff and their families who were extremely familiar with their usual repertoire of behaviours, sleep-wake patterns and usual arousal levels. Therefore, it can be safely said that any measured effect from interventions were not attributable to changes in the medications and were not due to their usual behavioural fluctuations.

Diagnosis of PDOC is challenging and unfortunately misdiagnosis of PDOC remains to be a concern. Up to 40% misdiagnosis rate (mainly patients being diagnosed with VS while they retain a degree of consciousness or diagnosed as MCS when emerged or uncertain diagnosis) has been reported in several papers when the diagnosis is made by non-specialist clinicians and when structured assessments tools such as CRS-R and SMART were not used (Schnakers et al., 2009, Majerus et al., 2005, Seel et al., 2010, Andrews et al., 1996).

All our research participants were assessed, diagnosed and monitored at the RHN where all clinicians are trained and experienced in assessment and management of PDOC. For the diagnosis and monitoring standardized clinical assessment tools (SMART, WHIM, CRS-R, MATADOC) are used routinely. All cases are reviewed at the multi-disciplinary team meetings regularly (once a week in rehabilitation unit and once a month in long-term care wards) and any change in the patient's status is recorded and actions are planned such as detailed assessment, trial with communication aids and further investigations. Therefore, chance of misdiagnosis in our research cohort was minimal.

All the patients who participated in the study were medically stable. The most common aetiology for PDOC was anoxic brain injury (8 out of 17 participants). The second common cause was CVA (5/ 17). We only had 3 patients with TBI and one patient with vasculitis due to Stevens-Johnson Syndrome.

13 out of 17 patients who participated in our research had diagnosis of MCS and their CRS-R total scores varied between 8 and 16. Three of MCS patients were meeting the low MCS criteria where basic conscious behaviours such as visual tracking, localization to noxious stimulus, object manipulation were present but higher-level responses such as object localization and reproducible movement to command were absent.

Almost all patients were on a medication which have a sedating side effect, but these medications were essential to treat their secondary complications such as spasticity, myoclonic jerks and seizures. Interestingly, two of the patients were on zopiclone in the evenings to treat insomnia and one patient was on long-term modafinil to combat daytime somnolence. We did not make any changes to their routine medications with the exception of two cases: one patient had increase of anti-epileptic dose due to frequent epileptiform discharges seen on EEG and for another patient modafinil was stopped at the time of enrolment to study to avoid interference with our interventions. In the latter case, there was two weeks of wash-out period after stopping modafinil and taking part in the research. There was no significant difference on patient's arousal levels after stopping the modafinil.

As all our patients were beyond the acute/ subacute stages of PDOC, the brain imaging studies were not repeated as they were not likely to provide additional information which could have been useful in interpretation of our results. At the RHN, brain imaging such as CT and MRI cannot be performed on site. If these tests are needed patients need to go to another hospital. However, the imaging on the CDs and reports are available. Therefore, we recorded findings of already performed brain imaging for our patients. All patients with anoxia and the patient with vasculitis had widespread ischaemic changes in their brain scans. Only one CVA was due to infarct and the remaining four were due to bleeding. Two of the TBI patients had changes in their brain scans in keeping with diagnosis of diffuse axonal injury.

In summary, our setting and cohort of patients who participated in the study provided following strengths:

1. Patients had a robust initial diagnostic work-up and therefore, chance of misdiagnosis was minimal,
2. Patients were in their familiar environment throughout the study and continued with their routine care,
3. Patients who involved in the interventional study were medically stable and were on established medication regimes,
4. Staff were very familiar with patients' usual repertoire of behaviours and were able to alert us if there was any change.

On the other hand, the following were the limitations of the study arising from setting and patient related issues:

1. Patients were in the chronic stages of their illness and therefore, the effect of the intervention on circadian rhythm optimization might have been less than what we would have seen in more acute stages,
2. Our setting did not have any facilities to repeat structural imaging,
3. The patients were on several medications which could influence circadian rhythm and sleep.

9.2 BASELINE INVESTIGATIONS

9.2.1 Sleep at baseline

Our baseline polysomnographic studies show that sleep-wake patterns of our patients (n=15) were deranged. Some had short episodes of sleep spread over 24-hour period; some was awake at night and asleep at daytime; some slept mostly at night but for a short while; and some slept day and night time. The variation between the sleep-wake patterns of patients cannot be explained by the external factors as all patients were in a similar environment where daily routines of the staff are very similar at evening/ night-times. However, there was slight difference of daily routine between patients in rehabilitation unit

(n=4) and in long term care wards (n=11). The patients in rehabilitation unit received daily therapy input including consciousness assessments, occupational therapy and physiotherapy sessions. The patients in long-term care wards on the other hand received minimal therapy input but passively involved in many other activities such as attending music concerts, film screenings, church services and hydrotherapy sessions. Other than this all patients, regardless of where they were located, went to bed in the late afternoon/ early evening hours, lights went off between 8 and 10 pm with minimal disruption until 8-9 am. Their day then started with personal care followed by hoisting into wheelchair. Although this is an institutionalized routine mainly dictated by staff change and medication administration times, it was generally same for all patients. Nevertheless, especially for the patients who sleep mostly during the day and keep awake at night-time, there is a danger of not recognizing some conscious behaviours which may have been displayed when they are awake at night, or not providing adequate social interaction during their waking hours.

At the group level the TST values were significantly reduced (mean= 246 minutes). This is well below the recommended total sleep time of 7 to 9 hours at night. However, the range was wide: 34 minutes to 518 minutes over a 24-hour period. Most interestingly, the most amount of sleep (day and night time) in two VS patients (patient-3 and patient-11) with anoxic brain injury and with the two lowest CRS-R scores and the least amount of sleep was observed in two TBI patients (patient-5 and patient-8).

In the two anoxic brain injury/ VS patients with high TST, the common electrophysiological feature was presence of frequent waves/ spikes on their baseline EEGs which were of very low amplitude/ featureless and poorly reactive. Another common clinical feature between the two patients was being on Clonazepam twice a day to treat myoclonus. The possible causes of the sleep disturbance/ low arousal levels in these patients include;

- damage to ascending arousal system at the hypothalamic/ thalamic level during anoxic event,
- frequent sharp waves/ spikes reflecting an 'epileptogenic' potential or even if nonepileptic, somewhat causing excitotoxic effects on the brain,
- side effect of Clonazepam.

The two of the TBI patients who had the least TST and sleep efficiency values, on the other hand were in MCS. Both patients' brain imaging studies were suggestive of diffuse axonal injury. Neither of the patients were on antiepileptic medications.

On the background of reduced TST and sleep efficiency, the number of awakenings were very high (mean=16.47, SD=10.83) suggesting that our patients had very fragmented sleep despite the fact they slept very little in a 24-hour period.

Review of sleep stages in PSGs showed that our patients spent majority of their eye-closed (appearing to be asleep) times in stage-1 which is a state of drowsiness. Furthermore, three of the patients had only stage-1 sleep. As mentioned before, stage-1 sleep is nor perceived as proper sleep by normal subjects and often described as catnapping/ dozing/ nodding off.

Previous PSG studies in PDOC suggested that sleep spindles were more common in TBI patients than in non-traumatic brain injury patients, more common in MCS than in VS, and indicated favourable prognosis. As our stage-2 scoring criteria was observation of spindle activity, all patients (11/15) who had stage-2 sleep scored by default had spindles on their PSGs re-confirming that spindles are more common in MCS than in VS (see table 9-1). We had only two patients with TBI and both had spindles. However, due to low numbers of TBI patients in our cohort, it is not possible to comment on frequency of spindles in relation to cause of brain injury.

Table 9-1: Sleep spindles in VS/ MCS patients

	VS (n=4)	MCS (n=11)
Spindles +	2	9
Spindles -	2	2

On the other hand, the four VS patients had an interesting divide in relation to spindle activity on their PSGs and the time since their brain injury. While the two VS patients who did not have any spindles were long-term care patients and two years after their brain injuries (patient-3 and patient-9), the other two who had spindles were in rehabilitation unit and only six and nine months after their brain injuries (patient-11 and patient-13). Unfortunately, both patients were discharged to other healthcare/ nursing home facilities following their rehabilitation and therefore, we don't know if they had late recovery of consciousness and emerged from VS later or not.

Another striking result of polysomnographic studies was the deficiency of REM sleep and non-existence of slow wave sleep at the baseline. Our review of literature PSG findings in PDOC suggested that REM sleep is more common in MCS than in VS (see chapter 4). This was re-iterated in our study as all four patients who had REM sleep were in MCS. However, the amount of REM was less than normal (Mean=10.2%, Range=2.4% to 20.5%). Normally 20-30% of sleep is spent in REM which is crucial for dreaming, memory consolidation and learning.

Identifying slow wave sleep is particularly difficult in PDOC patients as their background EEG is dominated by delta or theta waveforms which would indicate deep sleep in healthy individuals according to AASM criteria. Currently, there is no consensus on the method of sleep staging in this patient group. While some studies suggest that manual sleep scoring is possible,

some suggest this is almost impossible mainly due to general slowing of EEG, topographically contradicting “sleep” patterns and artefacts in VS and MCS and recommends development of automated EEG signal analysis combined with machine learning techniques (Wielek et al., 2018, Malinowska et al., 2013). Nevertheless, both manual and automated methods are still not very well established, and more research is needed on the subject. We opted for the manual scoring and our neurophysiologists were able to define a criteria for slow wave/ stage 3 sleep for PDOC based on detailed examination of many PSGs from PDOC patients and based on previously published work (Cologan et al., 2013). To be able to score stage 3, we needed to see higher amounts of delta activity (>20% of ongoing record) in higher amplitudes, accompanied by lack of muscle artefacts and eye blinks. All our PSGs were scored independently by two experienced neuro-physiologists which confirmed lack of slow wave sleep in our patients. As explained in the introduction chapters of this thesis, slow wave sleep plays an important role in strengthening memory circuits, consolidation of memories and motor learning.

In summary, the baseline PSGs of our patients showed that not only the amount of sleep was fragmented and reduced, but the quality of the sleep they had was also very poor. Chronic sleep deprivation coupled with poor quality sleep is likely to cause further damage to consciousness levels or at least negatively impact on recovery of brain functions in PDOC.

9.2.2 Circadian Rhythm Assessments

Patients with very severe brain injuries as in PDOC population are likely to have an irregular sleep/ wake rhythm circadian sleep disorder. Circadian rhythms were previously studied in PDOC population. A wide range of circadian parameters were examined including body temperature, blood pressure, heart rate, cortisol levels, prolactin levels, and even urinary excretion of electrolytes. All these studies showed a variety of outcomes i.e. preserved circadian rhythmicity of hormones alongside of diminished circadian rhythmicity of physiological markers and vice versa. Nevertheless, it was

pointed out that there is a difference in circadian rhythm regulation between VS and MCS patients and circadian rhythms can be used as a marker of prognosis as they reflect hypothalamic and midbrain functions (Bekinschtein et al., 2009b).

Only one study examined nocturnal melatonin regulation in six traumatic VS patients (Guaraldi et al., 2014). In the latter study, it was shown that VS patients had no significant increase of nocturnal plasma melatonin levels and they failed to supress melatonin levels when exposed to blue light. In this study the focus was on night-time variation of melatonin only. Day melatonin levels and melatonin peak times were not examined. Therefore, it does not give information on presence/ absence of melatonin circadian rhythm, and if present on the characteristics of it.

Our study showed that circadian rhythmicity of melatonin was severely impaired in PDOC patients as evidenced with abnormal melatonin peak times, poor differentiation of day/ night time melatonin levels and low % rhythmicity values.

There is no normal range for melatonin levels. In each person, melatonin levels change in a rhythm throughout the 24-hour period. Its levels go down when person expose to bright light in the morning and remains low until dark in the evening. When the environment is dark, melatonin levels start to rise and peak between 2am and 4am.

In our study, we measured saliva melatonin levels every four hours over two circadian cycles (48 hours). The saliva collection times were shifted by two hours in the second 24-hour period. Therefore, if the patient had a regular melatonin rhythm but a slight change in peak times, we would still be able to capture this in the second 24-hour period. Our results showed that only a small number of patients (six out of 15), had melatonin peak times occurring within the same two-hour window on the second 24-hour period. Even, by extending

the expected melatonin peak time window to midnight to 6am, we found that two of the peaks in this group occurred outside of this.

Five of six patients who had melatonin peak times within the same two-hour on consecutive 24-hour periods were in MCS. None of these patients were on beta-blocker medication which is known to inhibit melatonin production (Arendt et al., 1985, Wetterberg, 1978, Cowen et al., 1983). In the remaining 11 patients who did not have re-occurring of melatonin peaks within the same two-hour period however, four patients were on beta-blocker (bisoprolol) treatment. Therefore, it is not possible to comment whether the beta-blocker treatment negatively affected melatonin productions and supressed melatonin peaks on these four patients or not.

It was previously shown that aging has an adverse effect on melatonin production- possibly due to degeneration of the retina-SCN-pineal axis (Tan et al., 2018, Pandi-Perumal et al., 2005, Wu and Swaab, 2005, Sharma et al., 1989). In our study there was no clear correlation between age of the subjects and melatonin rhythm peak times (six patients aged between 36-71 versus nine patients aged between 30-73). Again, it is not possible to comment on influence of age on melatonin levels due to small sample size.

When the comparison between the mean diurnal and nocturnal values made we noted that the nocturnal values were higher than the diurnal values, but the difference was not statistically significant. However, there was evidence of melatonin rise in response to dark (8pm to midnight) with statistically significant t-test results at the group level which proved to be driven by the melatonin value changes of MCS patients- again, suggesting that MCS patients have better circadian rhythm reserves than VS patients.

Cosinor analysis of melatonin data showed that melatonin % rhythmicity was very low in our patient group (mean 30.87%, SD: 16.5). In fact, 13 out of 15 patients had % rhythm of less than 50%. When we re-ordered our patient list

according to their melatonin % rhythm results we noticed that most of the patients with higher melatonin % rhythm values were being fed during daytime. Although the difference between the day-time and night-time feeding groups was not statistically significant (p=0.22), this observation is important in PDOC clinical practice where the clinicians decide on timing of feeding via enteric tubes (clinically assisted hydration and nutrition).

Although light is the most prominent synchronizer for the mammalian central biological clock which resides in the SCN, we now know that there are many other circadian molecular clocks in the peripheral tissues such as adipose tissue and digestive system as evidenced by presence of clock genes. Animal studies showed that there are peripheral clocks in liver, pancreas, stomach and intestines (Stenvers et al., 2012, Balsalobre et al., 1998, Damiola et al., 2000, Hoogerwerf et al., 2010, Bostwick et al., 2010, Hoogerwerf et al., 2007). A literature review performed by Stenvers et al. (2012) outlines that not only these peripheral clocks have close working relationship with SCN, they also appear to be synchronized by energy metabolism and hormones that are released upon feeding.

Two research studies performed on people with VS indicated that enteral tube feeding can affect circadian rhythm. Saito et al. showed that VS patients who fed continuously, did not show any consistent cortisol circadian rhythm. The patients who were being fed at night had cortisol peak but at a wrong time (at 16:00 hours), and the ones who were being fed diurnally had appropriately timed cortisol peaks at 08:00 hours (Saito et al., 1989). Similarly, the same group studied body temperature and urinary excretions of water, sodium and potassium on 18 VS patients and showed that in the diurnal feeding group, there was a clear body temperature rhythm with a peak at 20:00 hours, whose pattern was similar to the well-established body temperature rhythm in normal subjects. While the nocturnal feeding group showed an altered temperature rhythm (the peak at 04:00 hours), the continuous feeding group did not show any consistent body temperature rhythms (Nishimura et al., 1992).

The schedule of enteric feed administration is mainly determined by practical issues. At the RHN, most rehabilitation unit patients are fed at night, so they can attend therapy and assessment sessions freely. It is often argued by nursing staff that, there is an increased risk of clinical incidents (i.e. forgetting to re-connect feed, wrongly setting up the pump rate by non-trained staff) when feeding is interrupted and re-started frequently to enable attendance to therapy sessions. Such incidents can have serious consequences if the patients are diabetic or are on medications such as hormone or enzyme replacement therapies. Patients who are residents in the long-term care wards however, can be fed during the day as there are less interruptions to feeding. In our study, even the patients who had their feed started in the afternoon, were being fed until 2am or 4am.

While, the above concerns are valid, it can be argued that clinical needs of the patients should take priority over practicalities. Although, more research is required, nocturnal feeding appears to have negative effect on circadian rhythms. From recent research in humans we know that circadian rhythm abnormalities are associated with immunodeficiency, impaired cognitive performance, cardiovascular illness, obesity, diabetes and even cancer (Gery and Koeffler, 2010, Lange et al., 2010, Gale et al., 2011, Dominguez-Rodriguez et al., 2010, Schmidt et al., 2007). Simple measures such as general training of therapy staff on feeding systems and placing warning signs by the bedside of patients may help to reduce the clinical incidents while enabling feeding during daytime and helping to optimize circadian rhythmicity.

Final parameter used to assess circadian rhythmicity in our study was body temperature measurements using I-buttons. Two previous studies used I-buttons on PDOC population to assess circadian rhythmicity (Bekinschtein et al., 2009b, Blume et al., 2017). While Bekinschtein et al. found only two out of five patients had preserved circadian rhythmicity of body temperature, Blume et al. detected circadian rhythms in all their 18 patients. Our study showed that although we measured proximal body temperatures, mean temperature values

of our patients resembled distal body temperature fluctuations of healthy people with rise in evening hours and dip in daytime hours. In addition to this, cosinor analysis revealed low % rhythmicity (mean=40.47%) in our cohort (n=8).

The methodological differences between the three studies may explain the slightly contradicting results. First, it is not clear from Bekinschtein study that where the I-buttons were placed on the body, but regardless of where the measurements were from, they collected continuous data over two weeks. Blume et al. chose the distal–proximal skin temperature gradient as a proxy to core body temperature and showed presence of rhythmicity in all their patients. However, detailed examination of peak values in their study show a spread over 24 hours with some patients reaching maximum CBT values at night or morning hours. Our study on the other hand used proximal skin temperatures over 48-hours.

Body temperature regulation depends on heat production and heat loss. Heat loss is mainly regulated by thermoregulatory distal skin flow and is affected by the temperature of environment. Our background work (Yelden et al., 2015) showed that the room temperatures were above recommended levels in our patient rooms. In the same study, it was noted that nursing staff in our rehabilitation unit believed that patients with profound brain injuries were not able to regulate their own body temperatures and therefore, needed warmer than recommended bedside environment. Further research on body temperature regulation of patients with PDOC is necessary to correctly interpret results of circadian rhythm studies when body temperatures are used as a marker of circadian rhythmicity.

9.3 INTERVENTIONAL STUDY

The aim of the interventions which consisted of melatonin in the evening, caffeine and blue light treatment in the morning was to entrain circadian rhythm

of ten PDOC patients. By achievement of realignment between the circadian rhythm, timing of sleep and wakefulness, and environmental time, the PDOC patients may be able to maintain wakefulness during daytime and have a good quality consolidated sleep at night.

We hypothesized that optimizing circadian rhythmicity will help to improve sleep quality and/or quantity which then will help to improve brain functions of patients. To detect any improvement in brain functions/ consciousness levels we used CRS-R as a bedside clinical assessment tool, and two different ERP paradigms as neurophysiological assessment tools.

In order to detect any fluctuations at the baseline we repeated all baseline investigations twice- four weeks apart. These were; 4-hourly saliva melatonin levels for 48-hours, and body temperatures as markers of circadian rhythms, PSG to study sleep quality and quantity, and MMN & SON ERP paradigms to assess brain electrical responses to stimuli. All of these tests were repeated after 5 weeks of intervention, while the patients were still receiving melatonin, caffeine and blue light treatment. Saliva melatonin measurements were repeated two more times after cessation of interventions: three days and three weeks after to see if any improvement of circadian rhythmicity was maintained or not. Here, the outcomes of the interventional study will be discussed.

9.3.1 Intervention to entrain circadian rhythm in PDOC

There is no standard dose, duration or treatment plan available for circadian rhythm entrainment. We chose to use a battery of interventions to optimize circadian rhythm in our ten PDOC patients rather than using a single agent or adding them on a step-wise manner. Although this precludes the information on which agent is more effective than the others, our main aim was to show that circadian rhythm is responsive to entrainment and once the circadian rhythm is entrained, this leads to improvement of consciousness.

PDOC is a result of catastrophic brain injury which leads to pathological functioning of many brain networks for a duration of months and years. A single agent used for a short-while may be sufficient to entrain circadian rhythm in any other patient population or pathological condition. For example, a few days of melatonin and/or light treatment may be sufficient to alleviate the symptoms of jet-lag sleep disorder or even to treat sleep phase disorders (Bjorvatn and Pallesen, 2009, Arendt and Skene, 2005, Duffy and Wright, 2005, Chang et al., 2012). On the other hand, it was shown that patients with Alzheimer's disease benefitted from melatonin treatment of much longer durations (Brusco et al., 1999, Brusco et al., 2000, Cardinali et al., 2012, Jean-Louis et al., 1998).

Our baseline study suggests that patients with PDOC suffer from irregular circadian sleep-wake disorder which is evidenced by erratic sleep-wake patterns coupled with circadian rhythm abnormalities. Irregular sleep-wake is mostly seen in patients with neurological disorders and thought to be due to neuronal damage within the SCN and hypothalamus, contributed by inadequate environmental light and lack of physical activity. For this condition a combination approach which include melatonin and bright light treatment is recommended (Smith et al., 2008). Taking into account of above and the severity of brain injury in PDOC patients, it is reasonable to try interventions which consist of combination of chronobiotic agents of reasonably long duration (weeks rather than days).

In our study melatonin and bright light treatment were used as chronobiotic agents and caffeine was used as an adjuvant as it inhibits melatonin production and has neuro-stimulating effects by blocking adenosin receptors. We also made sure that patients are exposed to adequate daylight during the day and night-time environment kept dark and quiet as much as possible. We were not able to increase physical activity levels due to disability levels of our patients and practical limitations in a long-term care environment. As the people with PDOC are at high risk of acute medical complications it was decided that five weeks of intervention duration would allow sufficient time for entrainment and

at the same time minimizing risk of false-negative results due to medical instability or drop out due to illness.

9.3.2 Changes to circadian rhythmicity with intervention

Our baseline investigations showed that circadian rhythm of melatonin was impaired, and this did not fluctuate significantly. With the intervention however, there was statistically significant improvement of melatonin % rhythmicity and melatonin peak times. This suggests that, although impaired, the circadian rhythms in PDOC is responsive to entrainment. We were also able to show that the improvement of melatonin & rhythmicity was maintained even three weeks after the cessation of treatment with melatonin, caffeine and bright light.

Failure to entrain circadian rhythms were observed in blind people but also in people who suffer from advanced or delayed phase disorders especially when patients have no light perception and social/ environmental time and biological time are misaligned (Duffy and Wright, 2005). On the contrary, some blind people were observed to entrain and this was thought to be due to strong enough non-photic cues such as exercise, scheduled meal-times and social cues (Mistlberger and Skene, 2005).

Upon close examination of the patient who had improvement of circadian rhythmicity, we observed that older patients (>60 years age) with non-traumatic brain injuries did not have much improvement of melatonin % rhythm or peak times with intervention in comparison to younger patients. It is quite possible that older patients need longer duration of treatment in higher doses due to additional age-related degeneration of circadian system and further reduced melatonin production. As we did not have any prior reference point, all our patients received the same dose and duration of light, melatonin and caffeine. However, it makes sense to take into consideration of additional age-related changes when designing future studies and adjust interventions.

We were not able to show any improvement of body temperature % rhythmicity which suggests that body temperature regulation in PDOC patients is somewhat complicated- possibly due to impairment of autonomic thermoregulatory mechanisms which need to be investigated further. With an attempt to boost body temperature circadian rhythmicity Blume et al. piloted bright light stimulation on eight PDOC patients and found that although results were not statistically significant at the group level, three of the patients showed signs of improvement of body temperature circadian rhythms as well as diagnosis change (VS to MCS), as evidenced by shift in body temperature peak times and improvement of CRS-R scores (Blume et al., 2017). This re-iterates the need to explore circadian rhythm optimization in this particular patient group as there is a correlation between the consciousness state and intactness of the circadian rhythm, and more importantly, improvement of circadian rhythm is associated with clinical improvement.

9.3.3 Changes to sleep with intervention

24-hour PSGs were performed three times for each subject during the study: twice at the baseline (4 weeks apart) and once more in the fifth week of interventions. This enabled us to see if there were any significant fluctuations of qualitative and quantitative sleep data in PDOC patients or not. Due to previously outlined challenges of scoring sleep stages in PDOC population, two different neuro-physiologists, who did not know which stage of the study the PSGs were from, reported each PSG independently. By using intra-class correlations, we were able to show that there was an excellent agreement between the two neuro-physiologists. This suggest that, although difficult, visual sleep staging can be performed by using the staging criteria we employed in this patient group.

The changes on PSG parameters following interventions were interesting. Although none of the sleep parameters improvement reached statistically significant levels at the group level, we observed a variety of changes. Some patients slept longer as evidenced with increased total sleep time and sleep

efficiency, some slept deeper as evidenced by less stage 1 sleep with increased spindles and/or increased slow wave sleep, some had more REM sleep and some had better sleep-wake patterns. Please see Appendix 2 for hypnograms and sleep stage pie-charts of patients at baseline 1, baseline 2 and post-intervention.

It is difficult to draw conclusions on why such variety of changes were observed in the sleep of these ten people with PDOC. The heterogeneity in mode of brain injury, anatomical variations of brain damage, age, medications and even pre-morbid sleep habits all influence outcomes of circadian rhythm interventional studies like ours. Unless a large group of patients with same/ very similar pathophysiological characteristics and clinical features studied, it will be very challenging to conclude. However, based on the previous research on healthy and other pathological conditions, one can attempt to speculate.

It was previously reported that exogenous melatonin which was administered during daytime led to increased sleep efficiency in young healthy volunteers (Hughes and Badia, 1997). However, timing of melatonin administration in certain conditions dictate the outcomes due to its phase advancing effects. In some subjects it may even cause increased fragmentation of sleep or early sleep off-set in the second half of sleep (Dijk and Cajochen, 1997). This may be particularly important in older patients where tendency to wake up in early morning hours is prominent. It was suggested that older patients may require administration of melatonin in the second half of the sleep episode. In our study, we did not see much improvement of sleep-wake patterns in older patients. Indeed, two of the patients (pt-7 who was 73 years old, and pt-10 who was 66 years old) had even worsening of their sleep efficiency with intervention. On the other hand, younger patients had much more consolidated sleep with intervention.

Several studies examined the effect of melatonin on sleep architecture. Improvement of symptoms of REM sleep behaviour disorder which is a

parasomnia and presents itself with violent behaviours during REM sleep was observed with exogenous melatonin (McGrane et al., 2015). Increase of stage 2 sleep and REM were reported in other patient groups following melatonin administration (Kunz et al., 2004, Fischer et al., 2003).

In addition to known circadian, mild hypnotic and sleep regulatory effects of melatonin, we need to consider the effect of caffeine and blue light which were given in our study to inhibit melatonin secretion during the day and, also to increase daytime arousal levels due to their stimulating effects. Caffeine stops urge to sleep by counteracting adenosine.

Adenosine regulates sleep inhibiting and sleep initiating regions of the brain. Adenosine levels build up during the day and peak usually 12 to 16 hours of being awake in people with normal circadian rhythm. This creates the sleep pressure (desire to sleep). Caffeine binds adenosine receptors and inactivates them. Therefore, despite building up of adenosine levels, brain does not get the desire to sleep. This continues as long as there are enough caffeine molecules binding to the adenosine receptors. Once the caffeine is metabolized, the adenosine freely binds its own receptors which creates high sleep pressure (Walker). (See figure 9-3). By giving caffeine as part of the intervention, we might have facilitated increased alertness/ less urge to sleep during daytime and increased sleep pressure at night. It is possible that this might consequently have led to production of more consolidated sleep and increased slow wave activity.

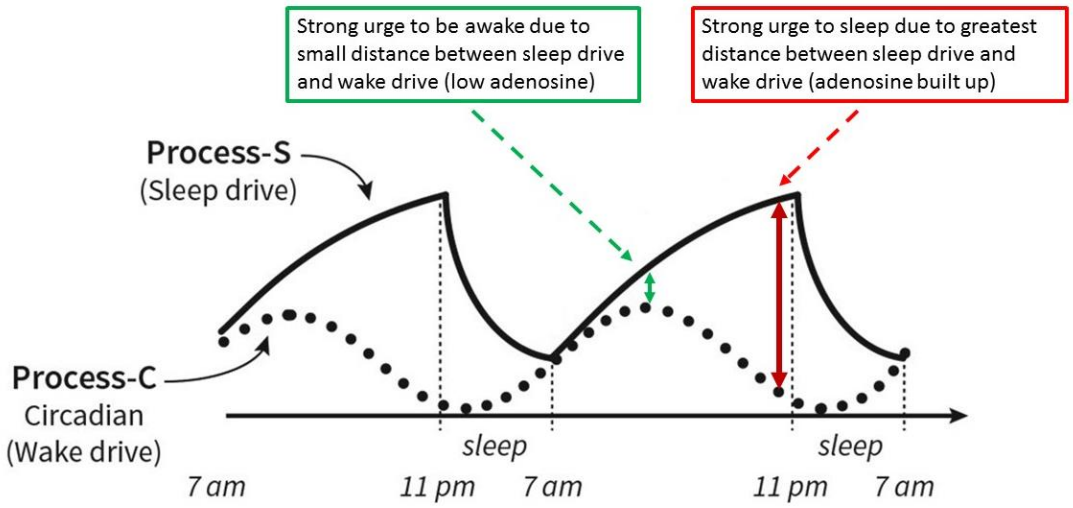


Figure 9-1: The interactions between sleep drive and circadian wake drive. (Modified from "Why we sleep? The new science of sleep and dreams. Matthew Walker, 2017)

9.3.4 Improvement of awareness/ consciousness levels with intervention

A statistically significant increase of CRS-R scores was the most important outcome of this study however due to small sample size the results of this study should be interpreted with caution. As majority of our patients were in MCS some fluctuation in the consciousness levels are expected. The feedback given (subjectively) by the nursing and healthcare assistants were that the patients appeared more alert when they were awake during the intervention. Repeated CRS-R assessments showed that, the younger MCS patients had the most apparent change in their repertoire of behaviours in response to standard stimuli. This was in parallel with the improvements in sleep and circadian rhythmicity. The results of this research suggest that despite being in the chronic stage of their illness and, even years after brain injury, PDOC patients may benefit from optimization of circadian rhythmicity.

While the positive results seen in younger MCS patients are very exciting, the neutral or negative results obtained in VS and older MCS patients are

scientifically also very significant, as gaining insight into their situations may help to solve some of many puzzles of disorders of consciousness. Here, I will be discussing them further.

The two VS patients, who already had longer baseline TST than the others (albeit stage 1 sleep), showed improvement of melatonin % rhythmicity and peak times but they did not show any improvement of CRS-R scores or sleep quality/ stages. This situation raises further questions which need to be explored in future research: Is the brain damage so severe that despite positive reaction of the circadian system, other brain networks are not able to activate? Or, do the VS patients need longer duration or higher doses of intervention to generate higher level brain responses?

Another set of interesting results were in the older patients who did not have any significant improvement of circadian rhythmicity and even worsening of sleep with intervention. Despite this, these patients still became very animated during intervention and had improvement of their CRS-R scores. Outside of formal assessments with CRS-R, they were observed to display behaviours which were unusual for them, such as looking at television screen directly, pulling on bedside rails and nodding head with music. It is likely that these patients are more resistant to circadian rhythm changes due to natural degeneration of circadian system with age, but more sensitive to stimulating effects of blue light and especially caffeine. Do they need melatonin at higher dose than we gave them? Does the timing of melatonin administration need to be changed to later at night, or even given twice- once in the evening and once more middle of the night? Do we need to give them caffeine in a smaller dose- may be once in the morning only to avoid sleep disruption at night?

The results of the MMN and SON event related potential studies confirmed that the clinical improvements that we observed were associated with event related potential improvements too. This is an encouraging outcome which indicates that in PDOC patients brain responses, either to changes in trains of

meaningless stimuli (tone MMN) or personally meaningful stimuli (SON) sensory stimuli, can also improve in response to circadian rhythm optimization allowing it to be assessed quantitatively. Although clinical assessment tools such as CRS-R are still the gold standard for diagnosis, neurophysiological tests such as ERPs can help to track responses to any given treatment more objectively and allow more reliable quantification of changes. To my knowledge this is the first study which examined changes to brain's electrophysiological activity as a response to clinical treatment in PDOC population.

As summarized in chapter 2 of this thesis, ERPs help us to examine information processing and are designed to measure brain responses to different types of stimuli. On the other hand, challenges associated with event related potential studies in this patient group were previously reported (Kotchoubey, 2017). Due to very abnormal EEGs at the baseline and lack of obvious clinical/ behavioural feedback inherent to the disorders of consciousness, it is difficult to interpret time-locked responses in this patient group. Even in the less severe brain injured patients cognitive processing speed and reaction times are expected to be delayed. Therefore, one can argue that both temporal and topographical features of ERP responses would be atypical in DOC and therefore, outcomes of the ERP studies will need to be interpreted cautiously.

MMN responses usually appear as a frontocentral negative wave with slight right preponderance (Morlet and Fischer, 2014). MMN is an indicator of pre-attentive sensory memory process and shown to help predicting outcomes in coma and other disorders of consciousness states. On the other hand, MMN can also modulated by attention and therefore, it can be a useful tool for assessing potential to regain conscious brain functions (Holler et al., 2011). In a longitudinal study Wijnen et al. showed that MMN amplitudes increased with recovery of consciousness while VS patients were transitioning to MCS (Wijnen et al., 2007). It was suggested that this may be an indicator of

consolidation of the neural networks even before clinical observation of behavioural responses.

Own name is one of the most powerful auditory stimuli which captures the attention of the named person and can elicit a robust electrophysiological response, mainly in the right hemisphere of the brain due to its self-relevance and emotional content (del Giudice et al., 2014). By using the SON, Qin et al. showed that presence of MMN carried a prognostic value in recovery from VS to MCS (Qin et al., 2008). Several other studies indicated that SON may have value in differentiating VS from MCS as well as in assisting to predict recovery (Schnakers et al., 2008, Risetti et al., 2013, Hauger et al., 2015).

In this study we looked for the increase in MMN and SON amplitude with intervention, which both showed statistically significant results. The MMN responses observed in our study were later than usually observed in healthy people (350ms to 400ms time window as opposed to 160ms to 220ms) and topographically resembled the normal MMN responses (frontal with right preponderance). Response to SON were mainly located in left hemisphere, but almost resembling an MMN response within the 150ms-200ms window. This is not that surprising as most ERP studies conducted on PDOC patients show some temporal and topographical abnormalities of ERP components especially in the MCS patients. The functional meaning of this is not clear and undoubtedly more research needs to be conducted in this area. It is also important to note that even in some healthy people ERP responses are not always present and early ERP responses- like in our study, do not prove that the patients are consciously processing information. However, they indicate that the neural networks which required for actual conscious information processing (measured with task-related or late latency evoked responses) are intact.

Simply based on the results of neurophysiological testing, it is not possible to conclude that optimization of circadian rhythm and/ or improvement of sleep

led to higher levels of conscious processing in our patients. This study was not designed to examine this. However, what this study shows is that, the patients who had clinical improvement also had better electrophysiological responses to auditory stimuli, which require some form of higher order processing (supra-segmental processing in the case of MMN and semantic meaning in the case of SON), indicating that hierarchical processing by these patients' neural networks is was likely improved by optimization of their circadian rhythm, sleep and arousal.

9.4 Methodological considerations

This study was designed to explore effects of circadian rhythm optimization on sleep and brain functions of patients with PDOC. The first two years of the PhD work was spent on setting up the laboratory and developing methods. Several pieces of equipment, sample collections for melatonin measurements and electrophysiological recordings techniques were trialled to find ways of collecting most robust data, without causing any discomfort or harm to patients.

Due to measuring many modalities to assess circadian rhythmicity, sleep, behavioural and neurophysiological assessments twice at the baseline and again after the interventions, each patient spent minimum of 13 weeks in the study. Although for many pathological conditions this is not a very long-time frame, for PDOC patients it can be, as patients with PDOC are at high risk of developing infections, other medical complications and death. Therefore, the intervention time was decided as five weeks. Although circadian rhythms can be re-synchronized much quicker in otherwise healthy subjects in conditions such as jet-lag and mild phase delay circadian sleep disorder, there was no clear guideline on how long this would take in patients with severe brain damage. It is possible that the five weeks intervention was not long enough to see optimum effect. Nevertheless, it was long enough to show some effect which led to positive clinical outcomes.

There are two methods of studying circadian rhythm (Minors and Waterhouse, 1981):

1) In a longitudinal study design where one or a few subjects are studied over many cycles
2) In a transverse study design where many subjects are studies over one or few cycles.

Minors and Waterhouse recommend that both methods have their own strength and weaknesses and in an ideal study both designs should be used. While longitudinal study design allows assessment of reproducibility of a rhythm for that individual, the transverse rhythm allows assessment of the reproducibility of a rhythm between individuals. In our baseline study we had two cycles (two 24-hour periods) to examine melatonin circadian rhythm of 15 patients. Saliva collection was scheduled for every four hours. It was not practical to increase number of cycles further due to practical difficulties. Therefore, an additional marker of circadian rhythmicity- body temperature measurements were included in the study.

Although, we aimed to collect body temperature data for a minimum of seven days, we only had complete data for 48 hours. We often found the I-buttons loose on the bed or not in contact with skin properly after shower and personal care of patients.

At the RHN, every effort is made to mimic patients' pre-morbid life-styles and habits. There are no hospital gowns, so patients wear their own night clothes at night, get washed in the morning and dressed in day clothes. Then, if there is no medical contra-indication they are hoisted to their own wheelchairs. Most patients get their hair done at the hospital's hairdresser and some female patients even get to put on make-up. At the end of daily activities, and once the allowed sitting out time is reached, they are hoisted back to bed for personal care and clothes change. When in bed, most patients are re-

positioned every four hours and often there are additional personal care episodes if the patients need it. Such an active daily routine and frequent manual handling activities prevented data collection with I-buttons to assess circadian rhythm assessments over many cycles. When the advantages and disadvantages weighted, it was decided that it was better to compromise on data collection, than to interrupt daily routine of patients which potentially could have affected their rhythms and sleep negatively.

Similarly, with polysomnographic investigations, the initial aim was to obtain PSG data for at least 72 hours. Again, due to practical issues explained above and scalp irritation observed, PSG was limited to 24-hours.

ERP studies were easier to perform than circadian rhythm assessments and PSG studies. When designing the paradigms, we decided to utilize simple and passive ERP experiments only. If I was to repeat this work in the future, I would also include at least two cognitive ERP experiments such as task-relevant paradigms (P3b components) and language-related paradigms (N400 components for semantic violations).

With regards to sample size, inclusion of 17 patients for baseline investigations was sufficient to conclude that patients with PDOC have abnormal circadian rhythms and sleep. However, this was not a large enough number to make sub-group analysis when the patients were of different ages, had a variety of aetiologies and a wide range of time passed since their brain injuries.

Similarly, for the interventional study, I was able to show that an attempt to optimize circadian rhythmicity was a worthwhile exercise as the majority of patients had improvement of their circadian rhythmicity, sleep, behavioural and neurophysiological markers. However, I was not able to expand the results analysis to a variety of responses observed in patient sub-groups. This made it clear that, although piloting the treatment in 10 patients yielded promising

results in this very specific area of neuroscience, future studies are warranted, and larger sample sizes are needed.

Chapter 10 CONCLUSIONS AND FUTURE DIRECTIONS

This PhD study makes the following contributions to the scientific body of evidence in PDOC;

- Sleep and circadian rhythms are severely deranged in PDOC.
- Optimization of circadian rhythms and sleep with melatonin, caffeine and blue light treatment led to improvement of all physiological parameters measured.
- And, most importantly, this simple and inexpensive treatment protocol led to improvement of awareness/ consciousness levels as evidenced by improvement of CRS-R scores and supported by enhanced amplitudes of event-related potential responses in MMN and SON experiments.

It is important to note that the participants of this research study received only five weeks of treatment and they were chronic PDOC patients whose circadian rhythms and sleeps run abnormally for many months and years. It is very likely that if optimization of circadian rhythms was achieved in earlier stages of the DOC, maybe even when patients are still in an intensive care environment, and this was maintained during rehabilitation and long-term care, the clinical outcomes would be much better.

Based on the outcomes of this PhD research study, I finally conclude that future research studies are warranted to further explore the effects of circadian rhythm and sleep optimization in PDOC patients. The future work should include;

a) Bigger cohort of patients to perform in depth analysis of results obtained in different sub-groups i.e. traumatic vs. non-traumatic brain injuries, young patients vs. older patients,

b) A larger sample size to further explore the relationship between feeding times and sleep/ circadian rhythm optimization,
c) Future studies should be multi-centric to enable inclusion of patients from acute, sub-acute and chronic disorders of consciousness stages,
d) A wide collaborative work should be considered to include expertise of neuro-rehabilitation clinicians, chronobiologists, neurophysiologists and neuroscientists to achieve robust methodology and data analysis in this very complex scientific subject.

APPENDIX 1: DETAILED CLINICAL INFORMATION FOR INTERVENTIONAL STUDY PARTICIPANTS

The clinical features of the participants are summarized below:

	Age/Gender	Cause of Brain Injury	Time from brain injury to SMART diagnosis	Time since Brain Injury (years)	Diagnosis (SMART, WHIM)	CRS-R Highest Score at the time of participation
Patient-1	71/Female	SAH	13 months	5	VS	16
Patient-2	58/Male	Anoxic	12 months	5	VS	8
Patient-3	37/Male	Anoxic	6 months	2	VS	7
Patient-4	51/Male	CVA(Bleed)	18 months	4	MCS	11
Patient-5	30/Female	TBI (DAI)	5 years	7	MCS	17
Patient-6	43/Female	CVA(Bleed)	11 months	3	VS	17
Patient-7	73/Male	CVA	18 months	8	MCS	12
Patient-8	40/Female	TBI (DAI)	12 months	3	VS	11
Patient-9	68/Male	Vasculitis	13 months	2	VS	5
Patient-10	66/Female	Anoxic	38 months	6	VS	9

10.1 Patient-1

Clinical Background: Patient-1 is a 70 year old woman who suffered from right frontal intracranial bleeding secondary to thrombolysis treatment for pulmonary embolism in 2008 while undergoing treatment for tuberculosis meningitis. She later on developed hydrocephalus which required ventriculo-peritoneal (VP) shunt insertion. She received initial rehabilitation at the Brain Injury Services of the Royal Hospital for Neuro-disability and underwent SMART and WHIM assessments in 2009. Both assessments were completed 13 months post brain injury. Her diagnosis was vegetative state. She had a period of two weeks where she showed increased levels of alertness and single word replies to simple questions following VP shunt insertion. However, these responses disappeared, and neuro-surgical opinion was that there was no VP shunt malfunction to explain this. She remained in vegetative state with occasional and very brief periods of increased alertness and responsiveness while with close family members. She was then transferred to a long term care ward at RHN for provision of specialist nursing home care.

Brain Imaging: CT Brain, 2009- dilatation of the ventricular system in comparison with the degree of sulcal prominence in keeping with a communicating hydrocephalus, together with extensive white matter low attenuation which could reflect transependymal oedema.

Medications at the time of study: Co-Careldopa, Levetiracetam, Lansoprazole, Ramipril, Laxatives, Hyoscine Patch, Dalteparin. No PRN sedatives were given to patient at the time of study.

Feeding Regime: Daytime via PEG.

Brainstem Auditory Evoked Potentials: BAEP responses elicited with monoaural stimulation delivered to the right and left ears were within normal limits for waves III, IV and V bilaterally. Wave II was slightly delayed for the right and left ears. Wave I was slightly delayed for the left ear. Interpeak latency

between waves III and IV were within normal limits on the right, however, on the left the latency was short. The interpeak latency between waves I-V on the right was 3.7 and on the left was 3.6. These values were within normal limits.

Right Ear								
Wave	I	II	III	IV	V	Interpeak I-III	Interpeak III-V	V*
Latency	1.8	3.0	3.8	4.9	5.5	2	1.7	5.4
Left Ear								
Wave	I	II	III	IV	V	Interpeak I-III	Interpeak III-V	V*
Latency	1.9	3.0	3.9	4.6	5.5	2	0.7	5.7

Baseline EEG: Baseline EEG taken on 09/07/2012 prior to commencing polysomnographic recording for a period of 24Hrs. The ongoing EEG showed mainly diffuse polymorphic delta activity at 2.0-2.5Hz, at 20-30µV. Small amounts of beta activity at 16-18Hz was seen superimposed diffusely, with patchy distribution. Throughout the baseline recording, focal slow activity was seen over the left parietal region.

10.2 Patient-2

Clinical Background: Patient-2 is a 58 year old man who suffered from anoxic brain injury following severe asthma attack in 2008. Following the brain injury, he was also diagnosed with supra-ventricular tachycardia, congestive cardiac failure, tonic-clonic seizure activity and myoclonic jerks. He received initial rehabilitation at the Brain Injury Services of the Royal Hospital for Neuro-disability and underwent SMART and WHIM assessments in 2009. Assessments were completed at 8 months post brain injury which both indicated diagnosis of vegetative state and patient was closely monitored for further 4 months. No significant changes were observed, and patient was transferred to a long term care ward at RHN for provision of specialist nursing home care.

Brain Imaging: CT Brain, 2008- widespread cortical ischaemia and widespread loss of gray-white matter differentiation both in the cortex and basal ganglia.

Medications at the time of study: Amiodarone, Clonazepam, Diltiazem, Ramipril, Frusemide, Levetiracetam, Sodium Valproate, Levothyroxine, Domperidone, Laxatives. Lansoprazole.

Feeding Regime: Daytime via PEG.

Brainstem Auditory Evoked Potentials: Brainstem auditory evoked responses were obtained using monoaural stimulation of the right and left ears. Responses from the right ear were absent. Responses from the left ear were present. Waves I to V were seen however the latencies of waves I to IV were delayed, with wave V arising at the upper limit of the reference range. Interpeak latencies were within normal limits.

Right Ear								
Wave	I	II	III	IV	V	Inter-peak I-III	Inter-peak III-V	V*
Latency	-	-	-	-	-	********	********	-
Left Ear								
Wave	I	II	III	IV	V	Inter-peak I-III	Inter-peak III-V	V*
Latency	1.9	3.5	4.4	5.6	6.0	2.5	1.2	*****

Baseline EEG: Baseline EEG taken prior to commencing polysomnographic recording for a period of 24Hrs. The ongoing EEG showed diffuse polymorphic delta activity at 1.0-2.5Hz between 10-20µV (low amplitude record). Beta components at 18-20Hz was seen diffusely at <15µV. The ongoing recording

was occasionally disrupted by single generalised sharp waves or spike discharges.

10.3 Patient-3

Clinical Background: Patient-3 is a 36 year old man who suffered from anoxic brain injury following cardiac arrest in 2012. He received initial rehabilitation at the Brain Injury Services of the Royal Hospital for Neuro-disability and underwent SMART and WHIM assessments which completed 6 months post brain injury. Both assessments indicated diagnosis of vegetative state. Patient was transferred to a long term care ward at RHN for provision of specialist nursing home care and closely monitored. No changes to consciousness levels were observed.

Brain Imaging: CT Brain, 2012- abnormalities consistent with hypoxic brain injury.

Medications at the time of study: Hyoscine Patch, Lansoprazole, Metformin, Aspirin, Simvastatin, Bisoprolol, Clonazepam, Dantrolene.

Feeding Regime: Daytime via PEG.

Brainstem Auditory Evoked Potentials: Right Ear-Wave I, III, IV, V latencies were within normal limits. Interpeak latency between waves I to III, between waves III to V, between waves I to V were within normal limits. Wave II latency was delayed.
Left Ear-Wave I, IV, V latencies were within normal limits. Interpeak latency between waves I to III are within normal limits. Interpeak latency between waves III to V, between waves I to V were within normal limits. Wave II latency was delayed.

Right Ear									
Wave	I	II	III	IV	V	Interpeak I-III	Interpeak III-V	V*	Interpeak I-V
Latency	1.9	3.2	4.0	5.1	6.2	2.1	2.2	4.3	6.2
Left Ear									
Wave	I	II	III	IV	V	Interpeak I-III	Interpeak III-V	V*	Interpeak I-V
Latency	1.8	3.1	4.3	5.4	6.1	2.5	1.1	4.3	6.1

Baseline EEG: The patient was unresponsive. The EEG was taken whilst the patient was in his wheelchair as an initial baseline preceding a 24-hour polysomnography. The ongoing EEG comprised frequent spike wave discharges which were separated by intervals of low amplitude featureless background. These intervals tended to be short (1-2seconds).

10.4 Patient-4

Clinical Background: Patient-4 is a 51 year old man who suffered from intracerebral and subdural haemorrhage following craniotomy and embolization for right temporal arteriovenous malformation in 2009. He received initial rehabilitation at the Brain Injury Services of the Royal Hospital for Neuro-disability and underwent SMART and WHIM assessments in 2010. Both assessments elicited tracking responses to visual stimuli and were suggestive of diagnosis of Minimally Conscious State. These were completed at 18 months post brain injury. The patient was transferred to a long term care ward at RHN for provision of specialist nursing home care.

Brain Imaging: Right temporal AVM, intracerebral and subdural haemorrhage

Medications at the time of study: Baclofen, Dantrolene, Gabapentin, Lansoprazole, Sodium Valproate

Feeding Regime: Between 16:00 and 04:00 via PEG.

Brainstem Auditory Evoked Potentials: Right Ear: Stimulation of the right ear did not elicit any reproducible BAEP responses. Left Ear: Responses were elicited on stimulation of the left ear. Waves I, II, III were present and latencies were within normal limits. Wave IV was absent. Wave V was delayed.

Right Ear								
Wave	I	II	III	IV	V	Interpeak I-III	Interpeak III-V	V*
Latency	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT			ABSENT
Left Ear								
Wave	I	II	III	IV	V	Interpeak I-III	Interpeak III-V	V*
Latency	1.5	2.7	3.9	ABSENT	7.1	2.4	ABSENT	7.0

Baseline EEG: The ongoing EEG was asymmetric. Right Hemisphere: Over the right hemisphere EMG artefact obscured the frontal derivations. The quiescent channels were of impoverished amplitude devoid of distinguishing characteristics over the central to anterior parietal region. Low amplitude 6Hz theta components seen occipitally.

Left hemisphere: was of higher amplitude, the dominant rhythm was theta activity seen centrally to posteriorly at 6-7Hz. Admixture of delta activity at 1.5-

2.5Hz was seen over the posterior quadrant. Negligible amounts of beta activity were seen anteriorly at 18-22Hz.

10.5 Patient-5

Clinical Background: Patient-5 is a 30 year old woman who suffered from diffuse axonal injury secondary to road traffic accident in 2006. She was treated in the UK following her brain injury and transferred to a nursing home abroad. She was admitted to the Brain Injury Services of the Royal Hospital for Neuro-disability for further rehabilitation and assessment in 2011. The assessments at the RHN indicated that she was in Minimally Conscious State. The patient was transferred to a long term care ward at RHN for provision of specialist nursing home care where she remained in same clinical condition.

Brain Imaging: CT Brain, 2006 - multiple widespread petechial haemorrhage.

Medications at the time of study: Baclofen, Citalopram, Gabapentin

Feeding Regime: Between 16:00 and 02:00 via PEG.

Brainstem Auditory Evoked Potentials: Right and Left Ear-Wave I, II, III, IV and V latencies were within normal limits. Interpeak latency between waves I to III, between waves III to V and between waves I to V are within normal limits.

Right Ear									
Wave	I	II	III	IV	V	Interpeak I-III	Interpeak III-V	Interpeak I-V	V*
Latency	1.4	2.4	3.5	4.6	5.2	2.1	1.7	3.8	5.3
Left Ear									
Wave	I	II	III	IV	V	Interpeak I-III	Interpeak III-V	Interpeak I-V	V*
Latency	1.9	2.7	3.7	4.7	5.3	1.8	1.6	3.4	6.2

Baseline EEG: The ongoing EEG was dominated by delta activity at 1.5-2.0Hz, up to 50μV seen diffusely. Occasional ripples of theta activity at 6-7Hz were seen superimposed over the posterior regions with subtle predilection towards the left side. Beta activity at 15-17Hz was seen over the frontal and central regions bilaterally.

10.6 Patient-6

Clinical Background: Patient-6 is a 43 year old woman who had a history of glioma which was removed at the age of 9 years but complicated with hydrocephalus requiring bilateral ventriculo-peritoneal shunt insertion. She further suffered from severe left temporo-parietal haemorrhage in 2009 which cause worsening of her hydrocephalus and necessitated shunt revision surgery in 2010. She was admitted to the Brain Injury Services of the Royal Hospital for Neuro-disability for further rehabilitation and assessment in 2010. The assessments at the RHN indicated that she was in Vegetative State. The patient was transferred to a long term care ward at RHN for provision of specialist nursing home care where she remained in same clinical condition.

Brain Imaging: CT Brain, 2009- haemorrhage occupying the left temporo-parietal region with surrounding oedema and associated midline shift with extension into the posterior horn of the left lateral ventricle. In addition to this there was high attenuation to the right of the third ventricle which represents further haemorrhage. There was evidence of bilateral shunts and the third ventricle appeared narrow when compared to the previous CT.

Medications at the time of study: Amlodipine, Doxazocin, Levetiracetam, Oxybutinine, Simvastatin

Feeding Regime: 18:00 to 02:00 via PEG

Brainstem Auditory Evoked Potentials: Brain stem responses were poorly formed bilaterally, but the waveforms were still discernible.
Right ear: Wave I, II, IV, V latencies are delayed. Increased interpeak latencies between waves I and III, and also waves III and V were seen.
Left ear: Wave I, II, IV latencies are within normal limits. Interpeak latency between waves III to V are within normal limits. Interpeak latency between waves I to V are within normal limits. Wave V latency is delayed. Delayed wave V with normal latencies for waves I and III indicates possible lesion affecting auditory pathways above the caudal pons, considered as an extreme prolongation of the III-V interval.
Summary- prolongation of the I-III Interpeak latencies bilaterally indicate defect of conduction the pathways within the caudal pons.

Right Ear									
Wave	I	II	III	IV	V	IPL I-III	IPL III-V	IPL IV	V*
Latency	2.0	3.2	5.5	7.1	7.8	3.2	2.6	5.8	absent
Left Ear									
Wave	I	II	III	IV	V	IPL I-III	IPL III-V	IPL I-V	V*
Latency	1.6	2.8	4.8	5.7	6.3	3.2	1.5	4.7	6.4

Baseline EEG: Asymmetric EEG, dominant δ and θ activity on the right.

10.7 Patient-7

Clinical Background: Patient-7 is a 73 year old man who suffered from bilateral ganglia infarcts, and infarction in the left occipital lobe following elective cardiac surgery in 2004. He was admitted to the Brain Injury Services of the Royal Hospital for Neuro-disability for further rehabilitation and assessment in 2005. The SMART and WHIM assessments at the RHN indicated that he was in Minimally Conscious State. The patient was transferred to a long term care ward at RHN for provision of specialist nursing home care where he remained in same clinical condition.

Brain Imaging: Brain CT in 2004- bilateral ganglia infarcts, and infarction in the left occipital lobe, adjacent to the falx. Calcification of both internal carotid arteries and the right vertebral artery was also noted.

Medications at the time of study: Bisoprolol, clonazepam, gabapentin, warfarin

Feeding Regime: 16:00 to 02:00 via PEG

Brainstem Auditory Evoked Potentials: Intermittently agitated during testing, mainly head movements which effected the technical quality of the recorded signals.

Right Ear									
Wave	I	II	III	IV	V	IPL I-III	IPL III-V	IPL I-V	V*
Latency	2.0	0	0	5.2	5.9				5.9
Left Ear									
Wave	I	II	III	IV	V	IPL I-III	IPL III-V	IPL I-V	V*
Latency	1.7	0.0	4.0	4.9	5.2				6.2

Baseline EEG: The ongoing EEG during quiescence was dominated by theta activity at 6-7Hz seen over precentral to posterior regions bilaterally. Occasional delta components at 2-3Hz were intermingled across post central areas. Beta activity at 18-20Hz was seen over the frontal regions bilaterally. Brief episodes of somnolence were demarcated by the appearance of slow lateralised eye movements and quiescent EEG recording.

10.8 Patient-8

Clinical Background: Patient-8 is a 39 year old woman who suffered severe traumatic brain injury (diffuse axonal injury) following a fall from height in 2010. The SMART and WHIM assessments at the RHN in late 2010 indicated that she was in Vegetative State. The patient was transferred to a long term care

ward at RHN for provision of specialist nursing home care where she remained in same clinical condition.

Brain Imaging: Brain CT in 2010- multiple widespread, predominantly frontal, petechial haemorrhages; blood in the ventricles, in the interpeduncular cistern and subarachnoid space.

Medications at the time of study: Baclofen, hyoscine patches, laxatives, lansoprazole

Feeding Regime: 10:00 to 20:00 via PEG

Brainstem Auditory Evoked Potentials:

Right Ear									
Wave	I	II	III	IV	V	IPL I-III	IPL III-V	IPL I-V	V*
Latency	2.4	3.2	4.0	4.6	5.6				7.7
Left Ear									
Wave	I	II	III	IV	V	IPL I-III	IPL III-V	IPL I-V	V*
Latency	2.1	3.3	4.7	6.0	6.8				7.5

Baseline EEG: The ongoing EEG was dominated by delta activity at 1.5-2.0Hz, up to 50µV seen diffusely. Occasional ripples of theta activity at 6-7Hz were

seen superimposed over the posterior regions with subtle predilection towards the left side. Beta activity at 15-17Hz was seen over the frontal and central regions bilaterally.

10.9 Patient-9

Clinical Background: Patient-9 is a 58 year old man who was diagnosed with toxic epidermal necrolysis/ Steven-Johnson Syndrome which led to severe inflammatory brain disease in 2011. The SMART and WHIM assessments at the RHN in late 2012 indicated that he was in Vegetative State. The patient was transferred to a long term care ward at RHN for provision of specialist nursing home care where he remained in same clinical condition.

Evoked potentials investigation has been performed at St Georges Hospital on 9.2.2012. Visual flash evoked potentials showed: no ERG response, fairly formed occipital cortex evoked responses with normal latencies which indicates presence of an intact visual pathway. Auditory evoked potentials were suggestive of mild dysfunction of auditory pathway.

Brain Imaging: MRI scan 1- showed diffuse symmetrical white matter changes suggestive of cytotoxic oedema. Repeat MRI scan showed progression of widespread necrotising process- necrotising leukoencephalopathy.

Medications at the time of study: Bisoprolol, pregabalin

Feeding Regime: 16:00 to 04:00 via PEG

Brainstem Auditory Evoked Potentials: Initial Auditory evoked potentials were suggestive of mild dysfunction of auditory pathway. Repeat BAEP showed:
The right ear -No reproducible responses were obtained on stimulation of the right ear.
The left ear -Wave I latency is within normal limits. Wave IV latency is within normal limits. Interpeak latency between waves I to III are within normal limits. Interpeak latency between waves III to V are within normal limits. Interpeak

latency between waves I to V are within normal limits. Wave II latency is delayed. Wave V latency is delayed. Delayed wave V with normal latencies for waves I and III indicates possible lesion affecting auditory pathways above the caudal pons. This can be considered as prolongation of the III-V interval.

Right Ear									
Wave	I	II	III	IV	V	IPL I-III	IPL III-V	IPL I-V	V*
Latency	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT
Left Ear									
Wave	I	II	III	IV	V	IPL I-III	IPL III-V	IPL I-V	V*
Latency	1.9	3.4	4.7	5.6	6.3				ABSENT

Baseline EEG: Diffuse δ 1-2,5Hz, occasional θ

10.10 Patient-10

Clinical Background: Patient-10 is a 66 year old woman who suffered from anoxic brain injury due to complications following an elective surgery for ovarian cyst removal in 2004. Following extensive neurological investigations and rehabilitation she was admitted to RHN in 2007 for further rehabilitation and specialist assessments. The SMART and WHIM assessments at the RHN in 2007 indicated that she was in Vegetative State. The patient was transferred to a long term care ward at RHN for provision of specialist nursing home care where she remained in same clinical condition.

Brain Imaging: MRI scan 2004- extensive diffuse signal change in the left temporal lobe, the left occipital and parietal cortices consistent with ischaemic damage.

Medications at the time of study: Clobazam, diazepam levetiracetam, mebeverine, mirtazapine, omeprazole, paracetamol, ramipril, zopiclone, amlodipine, atorvastatin

Feeding Regime: three milkshakes during the day PO, water at night via PEG

Brainstem Auditory Evoked Potentials:
Right and Left Ear-Wave I, II, III, IV, V latencies are within normal limits. Interpeak latency between waves I to III, between waves III to V, between waves I to V are within normal limits.

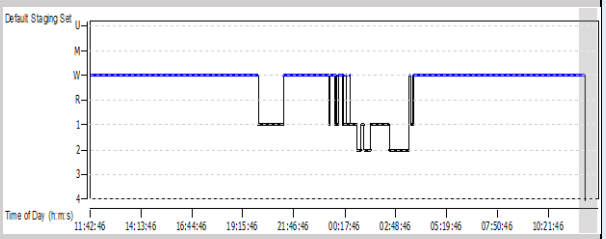
Right Ear									
Wave	I	II	III	IV	V	IPL I-III	IPL III-V	IPL I-V	V*
Latency	1.7	2.6	3.8	5.1	5.6	2.1	1.8	3.9	5.6
Left Ear									
Wave	I	II	III	IV	V	IPL I-III	IPL III-V	IPL I-V	V*
Latency	1.5	2.5	3.9	5.3	?5.8	2.4	1.9	4.3	5.8

Baseline EEG: The wake EEG showed copious amounts of beta activity seen at 15-17Hz.

APPENDIX-2: INTERVENTIONAL STUDY SLEEP DATA AND HYPNOGRAPHIES

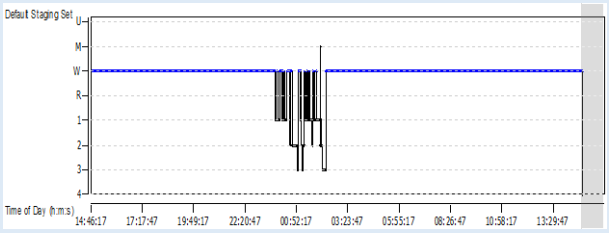
Patient-1

Baseline-1



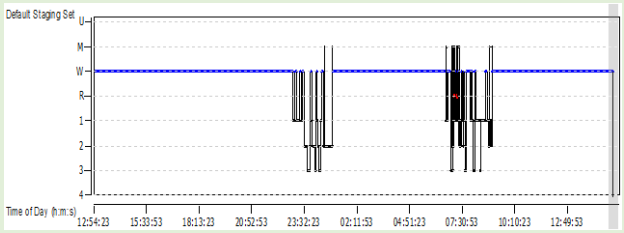
Stages	Time	% Sleep Time	% Time in Bed
WAKE	1163	-	80.40%
Stage 1	192.5	67.80%	13.30%
Stage 2	91.5	32.20%	6.30%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	0	0.00%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	284	100.00%	19.60%

Baseline-2



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1349	-	93.30%
Stage 1	51.5	53.40%	3.60%
Stage 2	31.5	32.60%	2.20%
Stage 3	13	13.50%	0.90%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	0.5	0.50%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	96	99.50%	6.60%

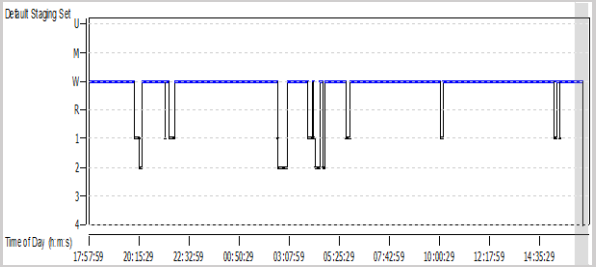
Post-Intervention



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1370	-	88.00%
Stage 1	83.5	44.90%	5.40%
Stage 2	71.5	38.40%	4.60%
Stage 3	25	13.40%	1.60%
Stage 4	0	0.00%	0.00%
REM	1	0.50%	0.10%
MT	5	2.70%	0.30%
UNS	0	-	0.00%
NREM (1+2+3+4)	180	96.80%	11.60%

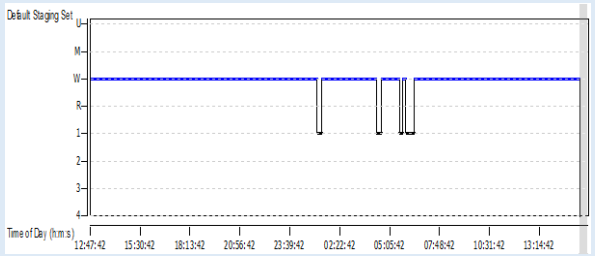
Patient-2

Baseline-1



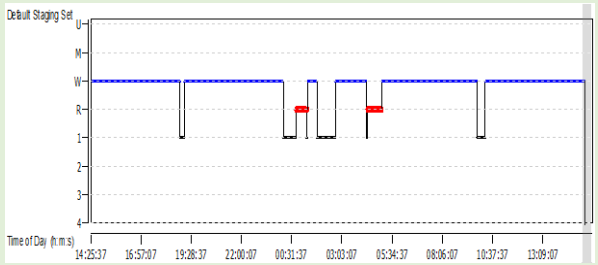
Stages	Time	% Sleep Time	% Time in Bed
WAKE	1206	-	90.40%
Stage 1	78	60.70%	5.80%
Stage 2	50.5	39.30%	3.80%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	0	0.00%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	128.5	100.00%	9.60%

Baseline-2



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1521.5	-	95.50%
Stage 1	72	100.00%	4.50%
Stage 2	0	0.00%	0.00%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	0	0.00%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	72	100.00%	4.50%

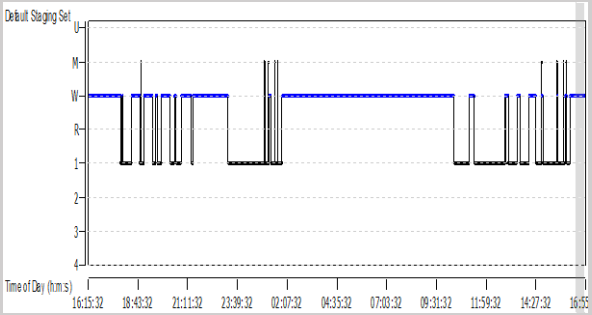
Post-Intervention



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1270	-	85.80%
Stage 1	132	62.70%	8.90%
Stage 2	0	0.00%	0.00%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	78.5	37.30%	5.30%
MT	0	0.00%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	132	62.70%	8.90%

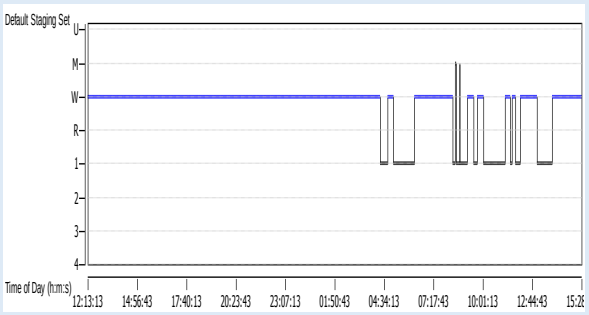
Patient-3

Baseline-1



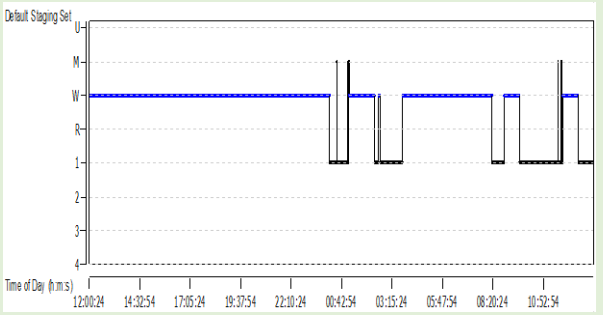
Stages	Time	% Sleep Time	% Time in Bed
WAKE	919.5	-	63.30%
Stage 1	527	99.00%	36.30%
Stage 2			
Stage 3			
Stage 4			
REM			
MT	5.5	1.00%	0.40%
UNS		-	
NREM (1+2+3+4)	527	99.00%	36.30%

Baseline-2



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1343.5	-	82.20%
Stage 1	288	98.60%	17.60%
Stage 2			
Stage 3			
Stage 4			
REM			
MT	4	1.40%	0.20%
UNS		-	
NREM (1+2+3+4)	288	98.60%	17.60%

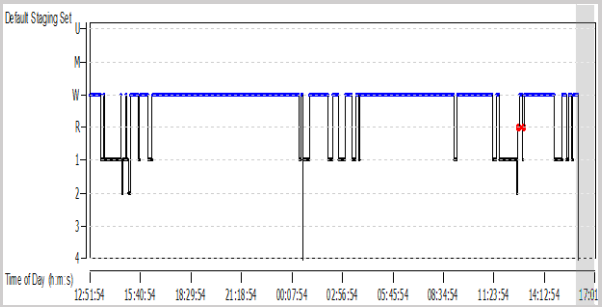
Post-Intervention



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1173.5	-	77.00%
Stage 1	346.5	98.90%	22.70%
Stage 2			
Stage 3			
Stage 4			
REM			
MT	4	1.10%	0.30%
UNS		-	
NREM (1+2+3+4)	346.5	98.90%	22.70%

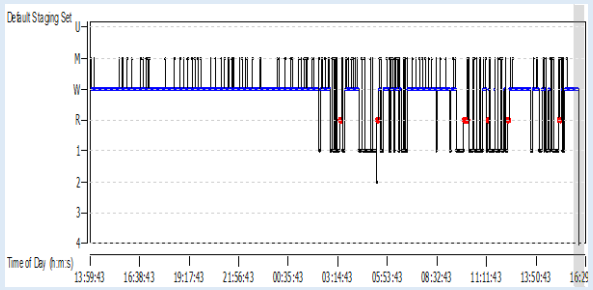
Patient-4

Baseline-1



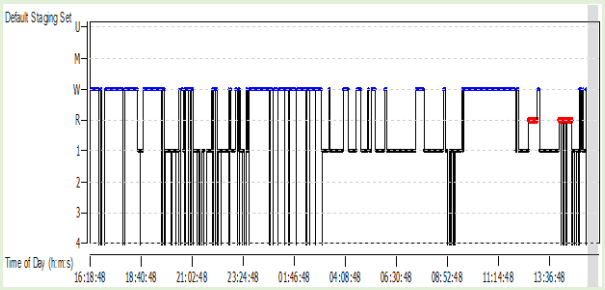
Stages	Time	% Sleep Time	% Time in Bed
WAKE	1309.5	-	80.50%
Stage 1	300	94.80%	18.40%
Stage 2	9	2.80%	0.60%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	7.5	2.40%	0.50%
MT	0	0.00%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	309	97.60%	19.00%

Baseline-2



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1115	-	71.80%
Stage 1	302	68.90%	19.40%
Stage 2	1.5	0.30%	0.10%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	43	9.80%	2.80%
MT	92	21.00%	5.90%
UNS	0	-	0.00%
NREM (1+2+3+4)	303.5	69.20%	19.50%

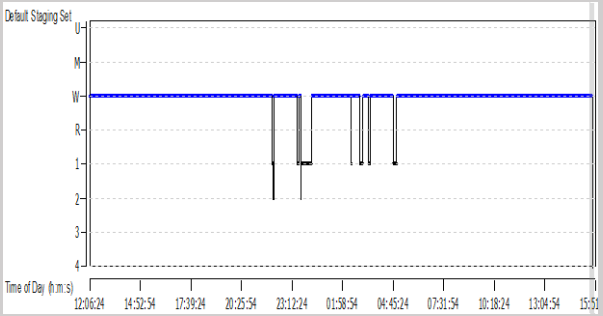
Post-Intervention



Stages	Time	% Sleep Time	% Time in Bed
WAKE	682.5	-	49.40%
Stage 1	547.5	90.90%	39.60%
Stage 2	0	0.00%	0.00%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	54.5	9.10%	3.90%
MT	0	0.00%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	547.5	90.90%	39.60%

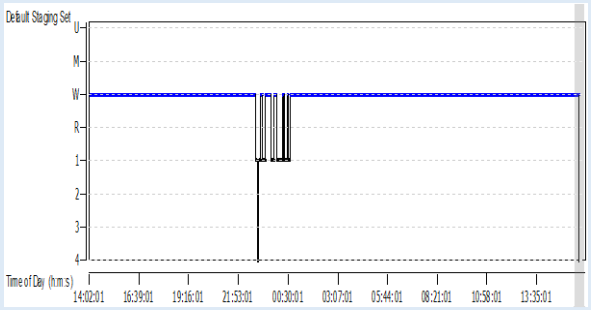
Patient-5

Baseline-1



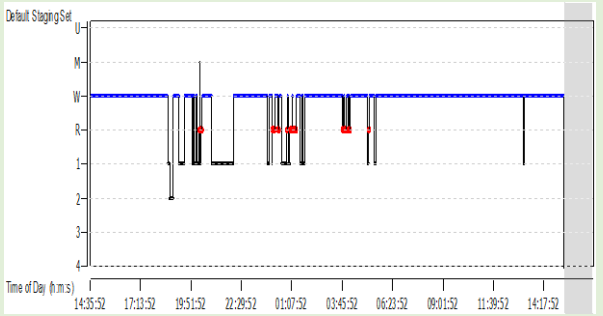
Stages	Time	% Sleep Time	% Time in Bed
WAKE	1576	-	95.80%
Stage 1	67	96.40%	4.10%
Stage 2	2.5	3.60%	0.20%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	0	0.00%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	69.5	100.00%	4.20%

Baseline-2



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1455	-	95.10%
Stage 1	70.5	100.00%	4.60%
Stage 2	0	0.00%	0.00%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	0	0.00%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	70.5	100.00%	4.60%

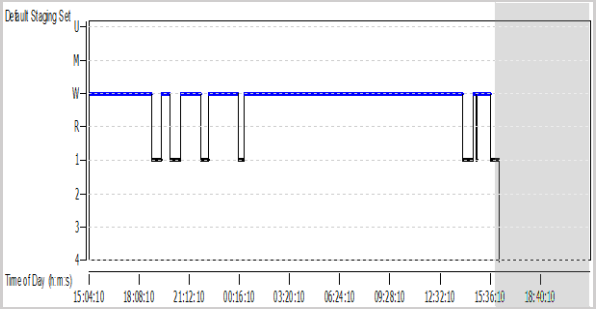
Post-Intervention



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1262	-	84.90%
Stage 1	156.5	69.70%	10.50%
Stage 2	11	4.90%	0.70%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	56.5	25.20%	3.80%
MT	0.5	0.20%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	167.5	74.60%	11.30%

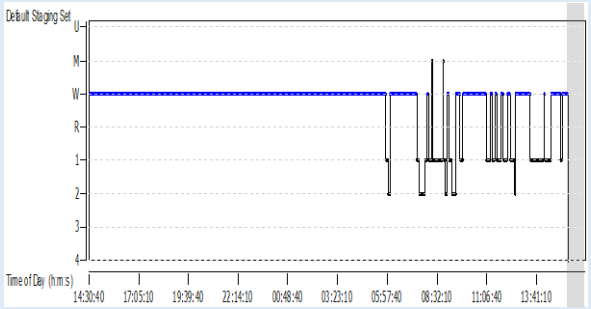
Patient-6

Baseline-1



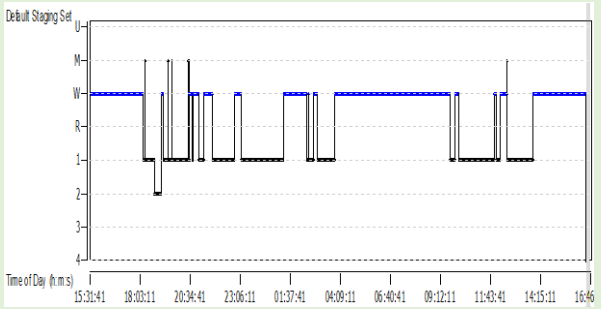
Stages	Time	% Sleep Time	% Time in Bed
WAKE	1308.5	-	87.90%
Stage 1	180.5	100.00%	12.10%
Stage 2	0	0.00%	0.00%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	0	0.00%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	180.5	100.00%	12.10%

Baseline-2



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1215	-	81.80%
Stage 1	224	82.70%	15.10%
Stage 2	46	17.00%	3.10%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	1	0.40%	0.10%
UNS	0	-	0.00%
NREM (1+2+3+4)	270	99.60%	18.20%

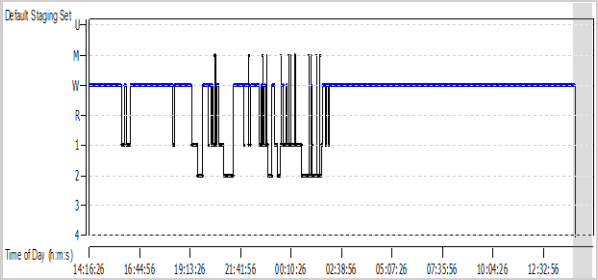
Post-Intervention



Stages	Time	% Sleep Time	% Time in Bed
WAKE	869.5	-	58.00%
Stage 1	603	95.90%	40.30%
Stage 2	22	3.50%	1.50%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	3.5	0.60%	0.20%
UNS	0	-	0.00%
NREM (1+2+3+4)	625	99.40%	41.70%

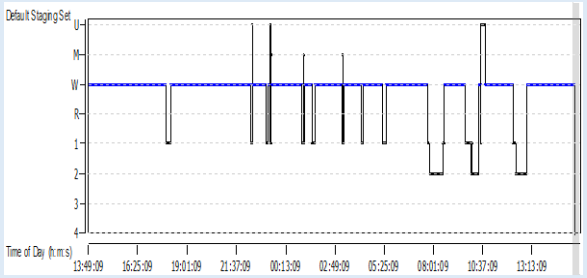
Patient-7

Baseline-1



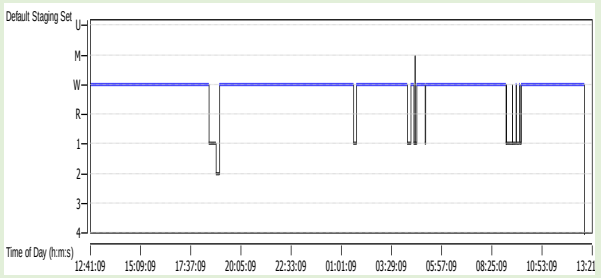
Stages	Time	% Sleep Time	% Time in Bed
WAKE	1123.5	-	79.10%
Stage 1	173	58.30%	12.20%
Stage 2	116.5	39.30%	8.20%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	7	2.40%	0.50%
UNS	0	-	0.00%
NREM (1+2+3+4)	289.5	97.60%	20.40%

Baseline-2



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1319	-	86.10%
Stage 1	100.5	50.20%	6.60%
Stage 2	98	49.00%	6.40%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	1.5	0.80%	0.10%
UNS	13	-	0.80%
NREM (1+2+3+4)	198.5	99.20%	13.00%

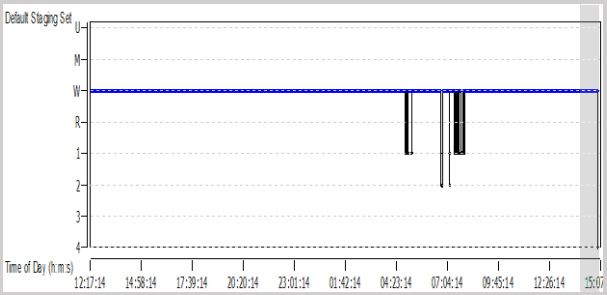
Post-Intervention



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1359.5	-	91.80%
Stage 1	87	88.30%	5.90%
Stage 2	11	11.20%	0.70%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	0.5	0.50%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	98	99.50%	6.60%

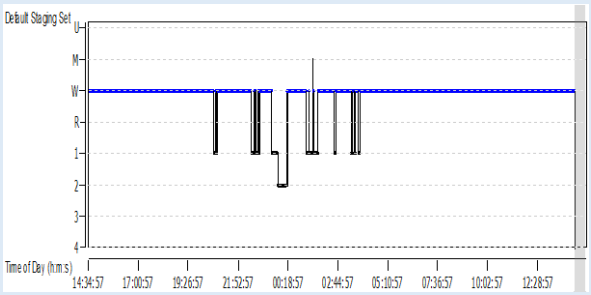
Patient-8

Baseline-1



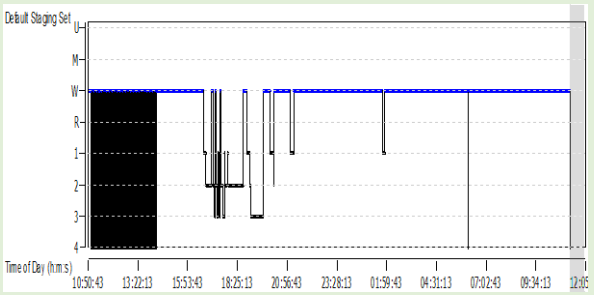
Stages	Time	% Sleep Time	% Time in Bed
WAKE	1508.5	-	97.60%
Stage 1	31	82.70%	2.00%
Stage 2	6.5	17.30%	0.40%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	0	0.00%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	37.5	100.00%	2.40%

Baseline-2



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1308	-	92.20%
Stage 1	83.5	75.90%	5.90%
Stage 2	26	23.60%	1.80%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	0.5	0.50%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	109.5	99.50%	7.70%

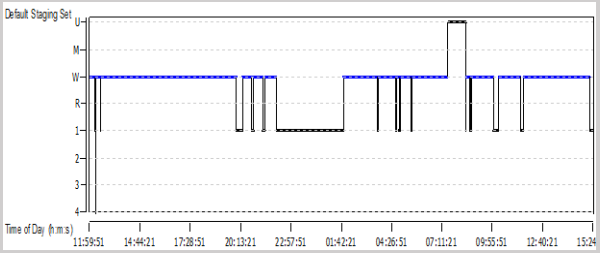
Post-Intervention



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1174.5	-	80.10%
Stage 1	49.5	25.20%	3.40%
Stage 2	93.5	47.60%	6.40%
Stage 3	53.5	27.20%	3.60%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	0	0.00%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	196.5	100.00%	13.40%

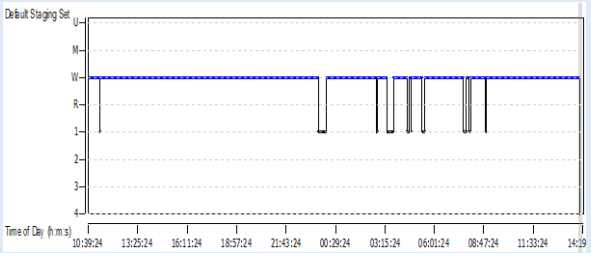
Patient-9

Baseline-1



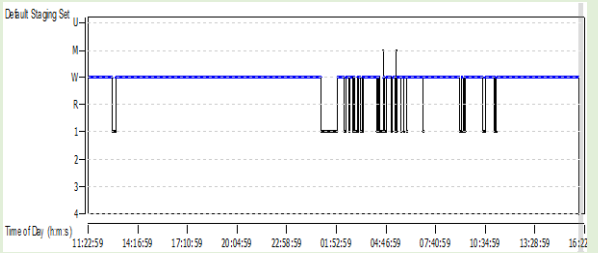
Stages	Time	% Sleep Time	% Time in Bed
WAKE	1278.5	-	77.70%
Stage 1	306	100.00%	18.60%
Stage 2	0	0.00%	0.00%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	0	0.00%	0.00%
UNS	59.5	-	3.60%
NREM (1+2+3+4)	306	100.00%	18.60%

Baseline-2



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1558	-	95.00%
Stage 1	82.5	100.00%	5.00%
Stage 2	0	0.00%	0.00%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	0	0.00%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	82.5	100.00%	5.00%

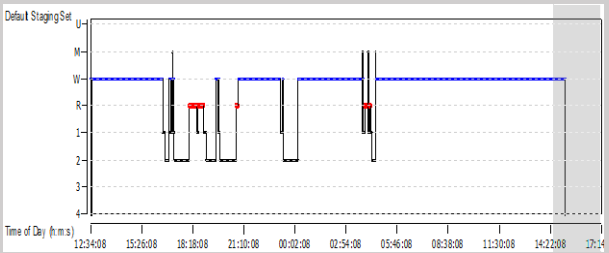
Post-Intervention



Stages	Time	% Sleep Time	% Time in Bed
WAKE	1553.5	-	90.70%
Stage 1	159	99.40%	9.30%
Stage 2	0	0.00%	0.00%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	1	0.60%	0.10%
UNS	0	-	0.00%
NREM (1+2+3+4)	159	99.40%	9.30%

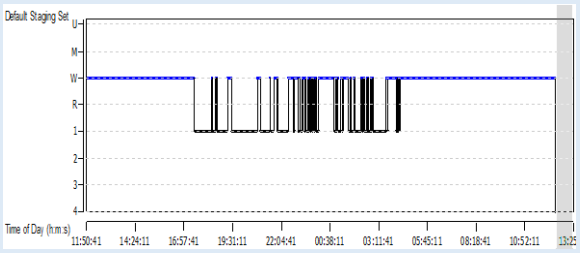
Patient-10

Baseline-1



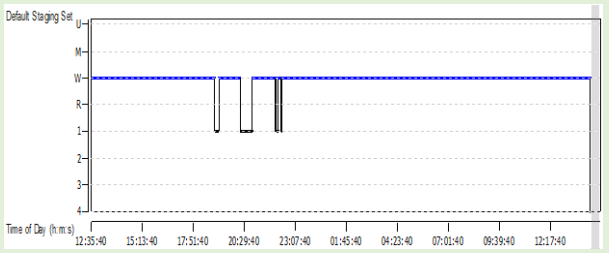
Stages	Time	% Sleep Time	% Time in Bed
WAKE	1222.5	-	78.40%
Stage 1	49.5	14.90%	3.20%
Stage 2	210.5	63.50%	13.50%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	68	20.50%	4.40%
MT	3.5	1.10%	0.20%
UNS	0	-	0.00%
NREM (1+2+3+4)	260	78.40%	16.70%

Baseline-2



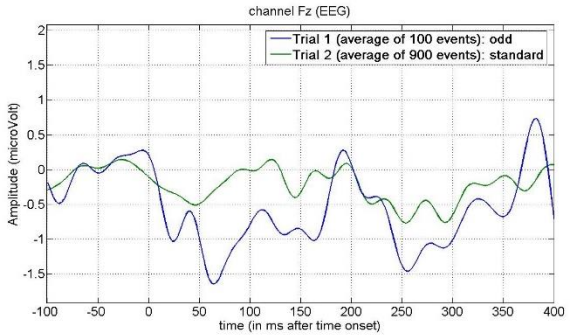
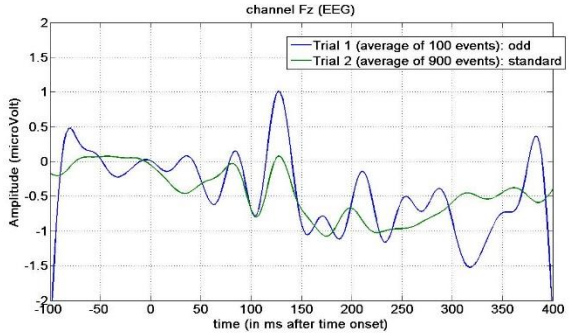
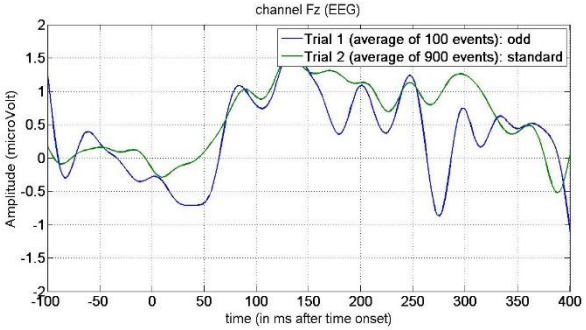
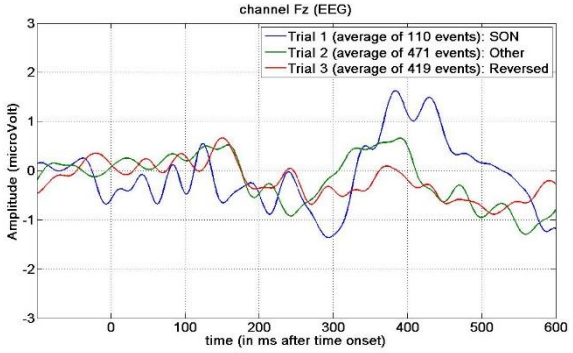
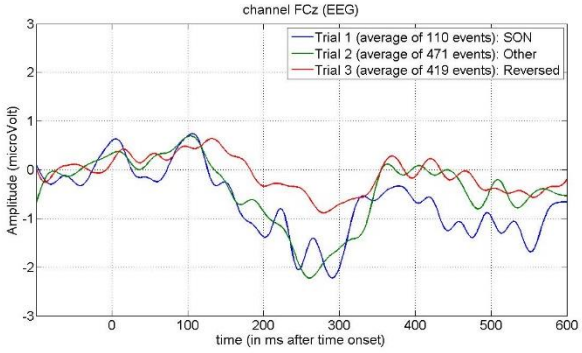
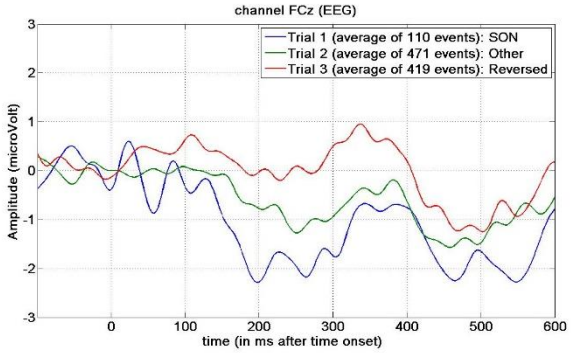
Stages	Time	% Sleep Time	% Time in Bed
WAKE	1054.5	-	71.30%
Stage 1	423	100.00%	28.60%
Stage 2	0	0.00%	0.00%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	0	0.00%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	423	100.00%	28.60%

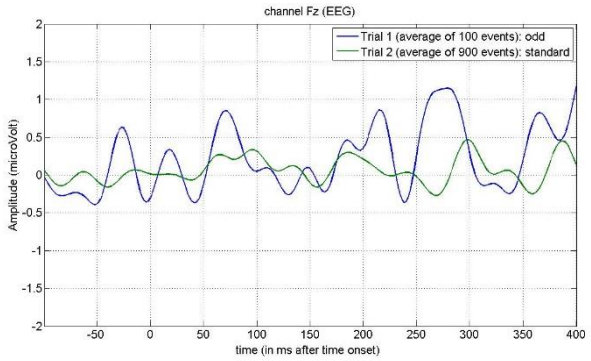
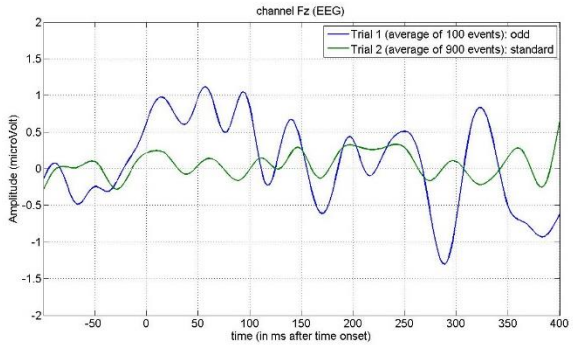
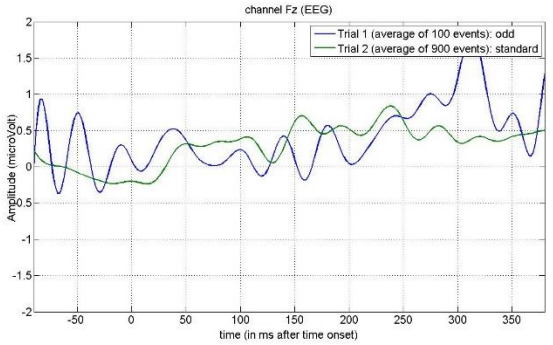
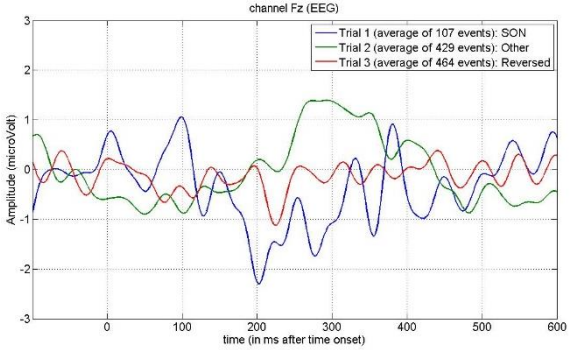
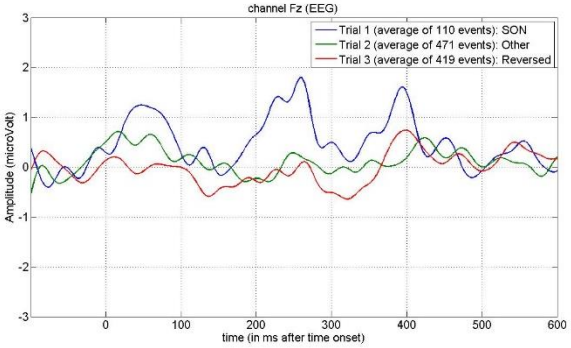
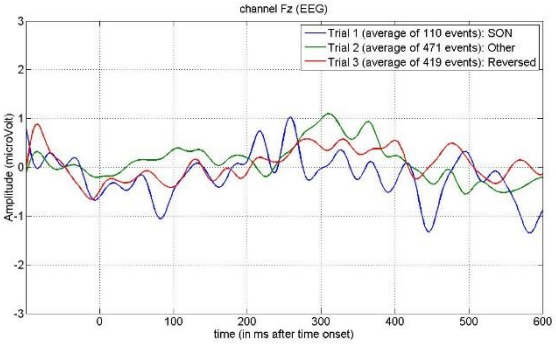
Post-Intervention

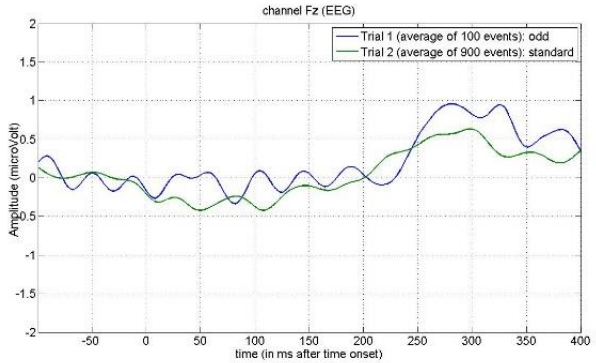
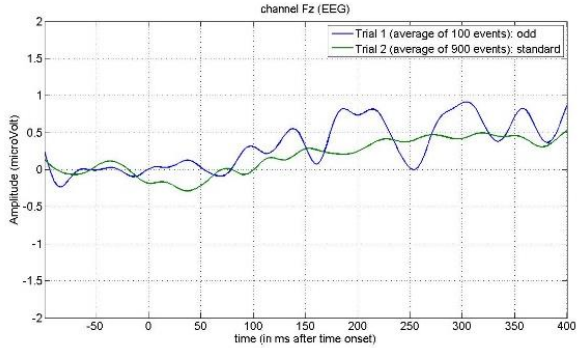
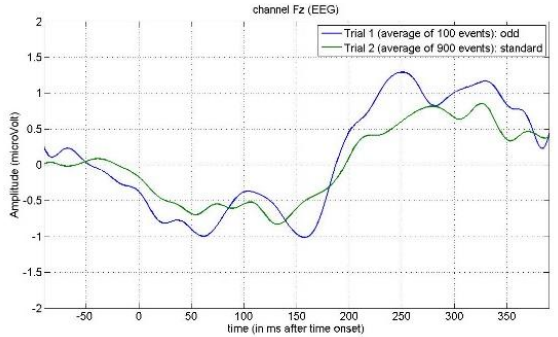
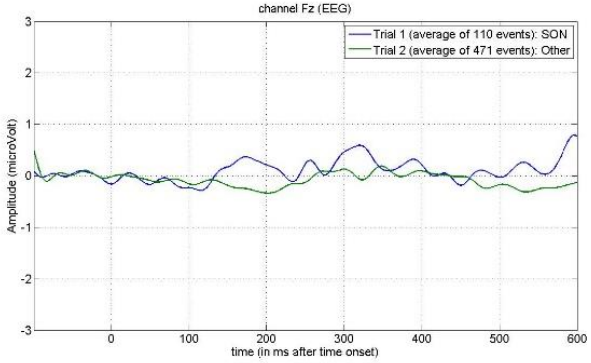
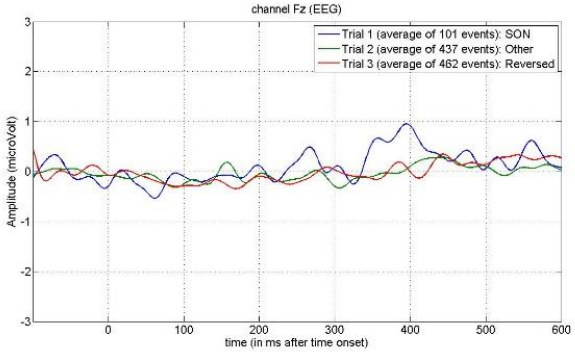
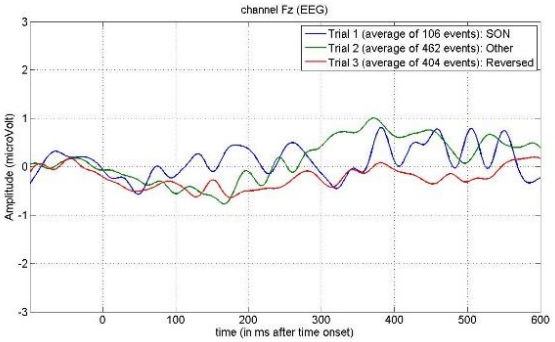


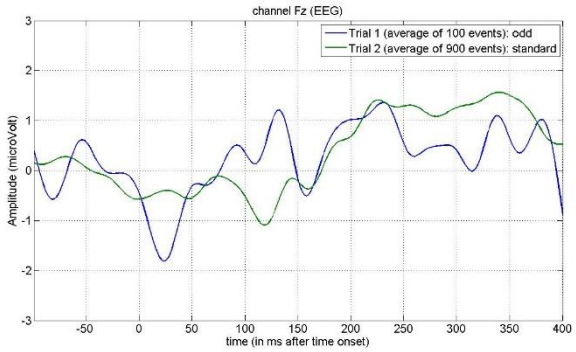
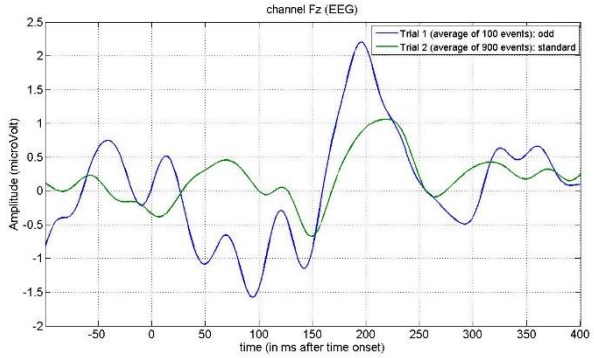
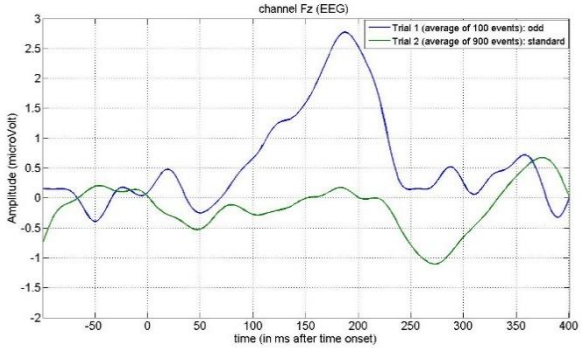
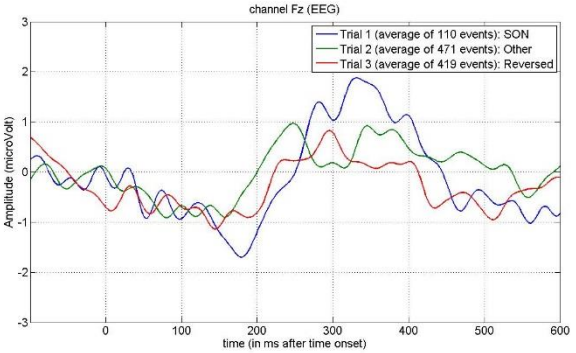
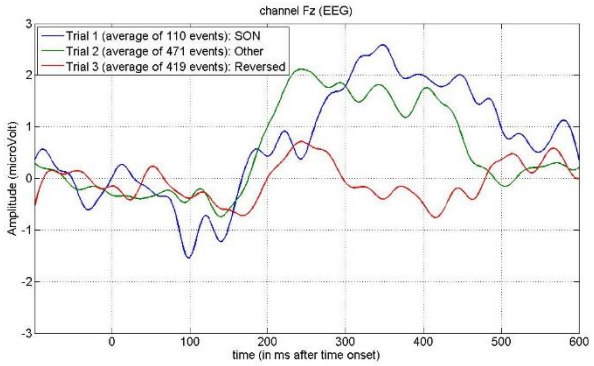
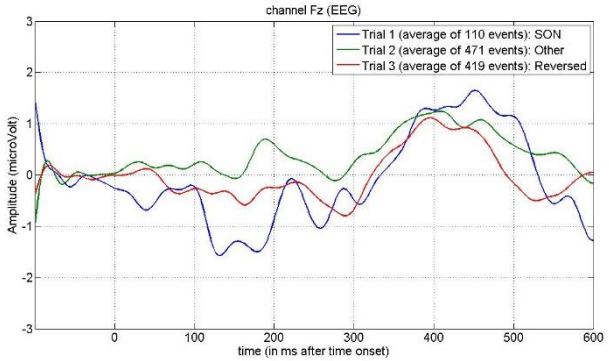
Stages	Time	% Sleep Time	% Time in Bed
WAKE	1483.5	-	96.00%
Stage 1	62	100.00%	4.00%
Stage 2	0	0.00%	0.00%
Stage 3	0	0.00%	0.00%
Stage 4	0	0.00%	0.00%
REM	0	0.00%	0.00%
MT	0	0.00%	0.00%
UNS	0	-	0.00%
NREM (1+2+3+4)	62	100.00%	4.00%

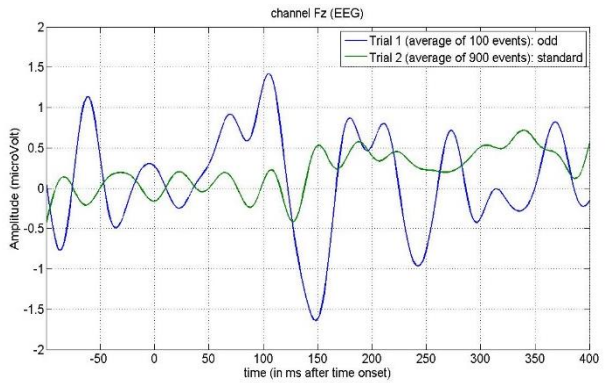
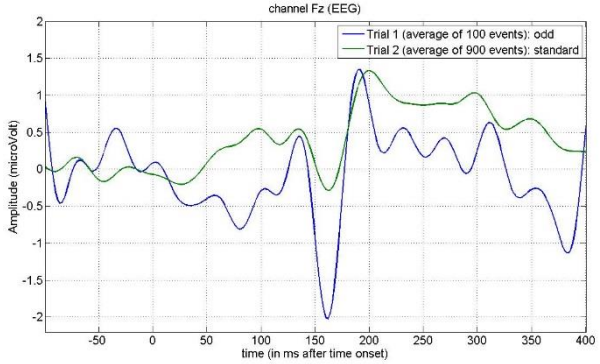
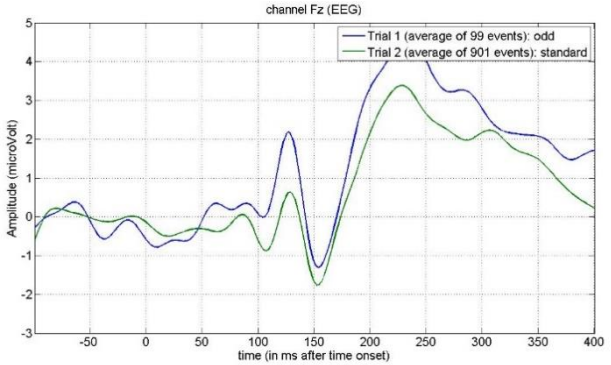
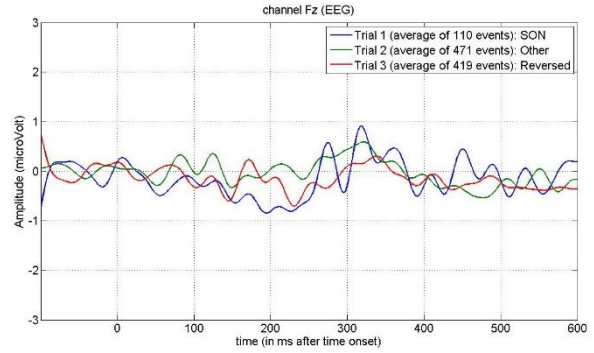
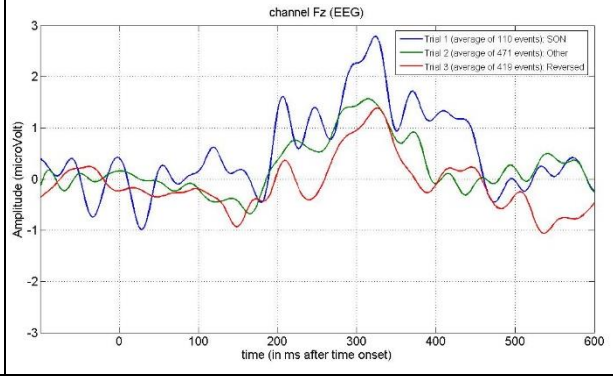
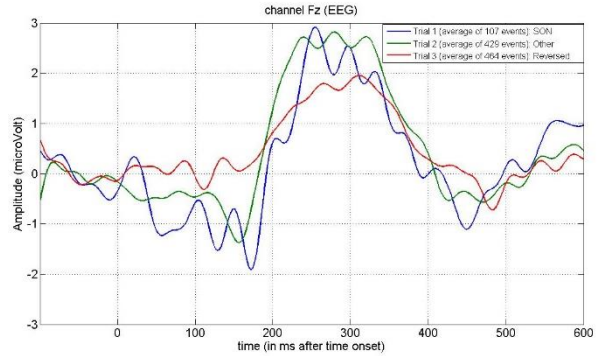
APPENDIX 3: EVENT RELATED POTENTIALS

PT-1		
Oddball Baseline-1	**Oddball Baseline-2**	**Oddball Post-intervention**
		
SON Baseline-1	**SON Baseline-2**	**SON Post-intervention**
		

PT-2		
Oddball Baseline-1	**Oddball Baseline-2**	**Oddball Post-intervention**
		
SON Baseline-1	**SON Baseline-2**	**SON Post-intervention**
		

PT-3		
Oddball Baseline-1	**Oddball Baseline-2**	**Oddball Post-intervention**
		
SON Baseline-1	**SON Baseline-2**	**SON Post-intervention**
		

PT-4		
Oddball Baseline-1	**Oddball Baseline-2**	**Oddball Post-intervention**
		
SON Baseline-1	**SON Baseline-2**	**SON Post-intervention**
		

PT-5		
Oddball Baseline-1	**Oddball Baseline-2**	**Oddball Post-intervention**
		
SON Baseline-1	**SON Baseline-2**	**SON Post-intervention**
		

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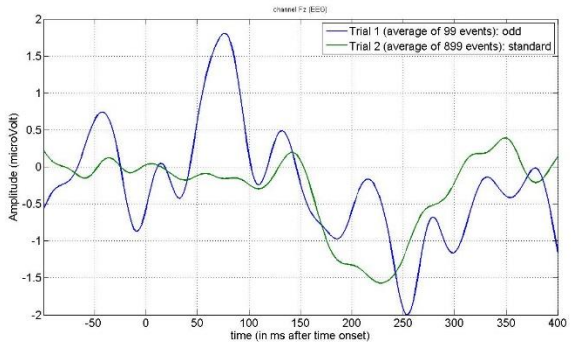	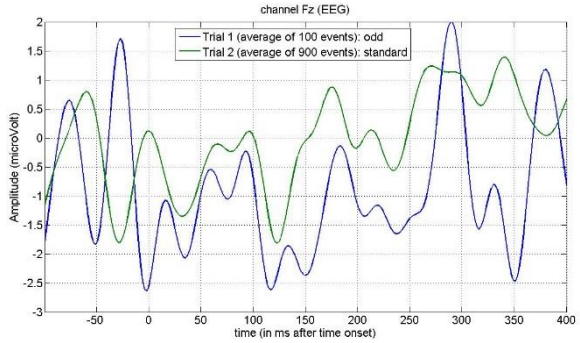	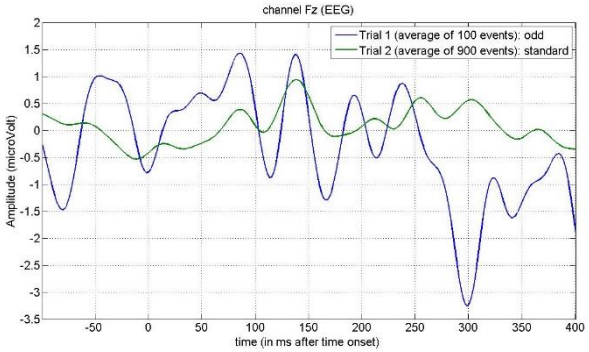
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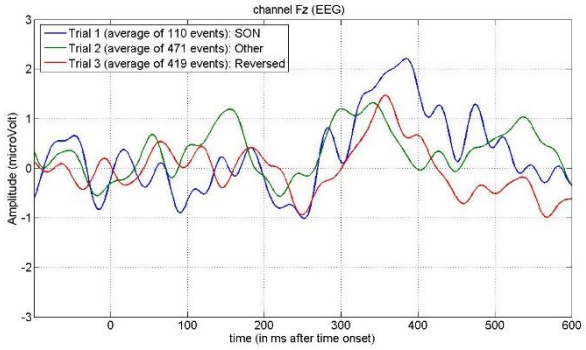	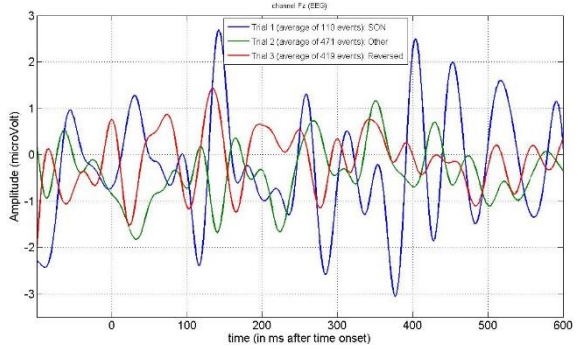	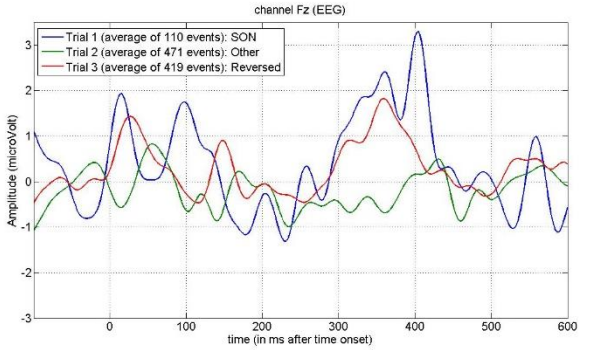

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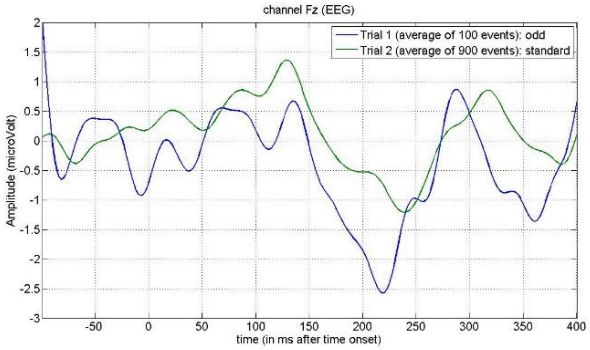	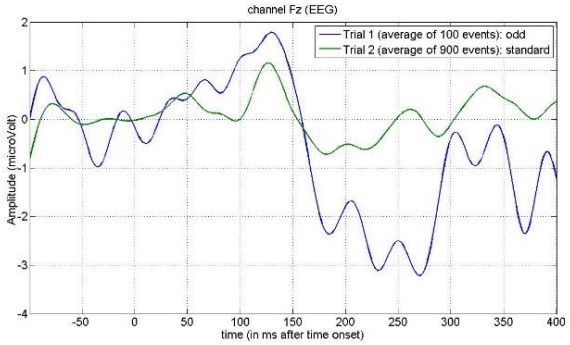	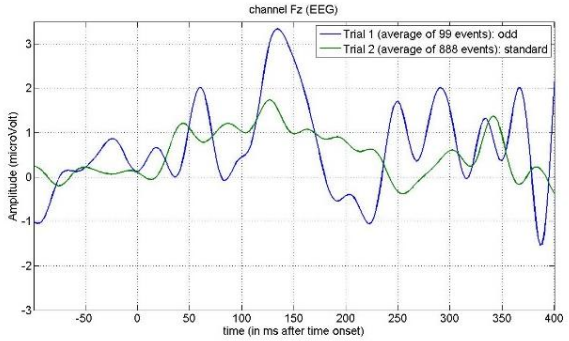
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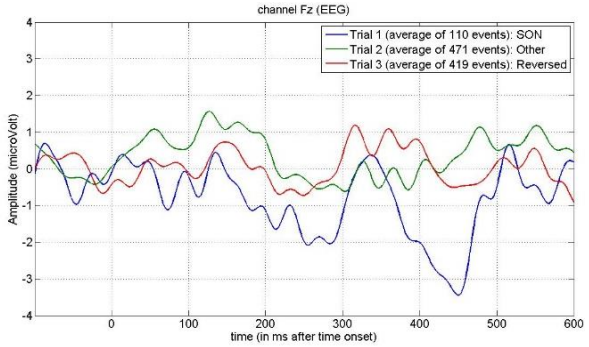	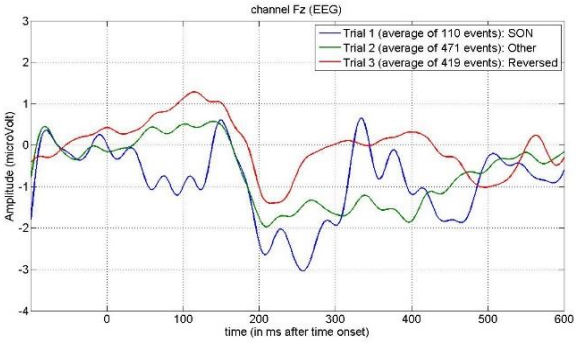	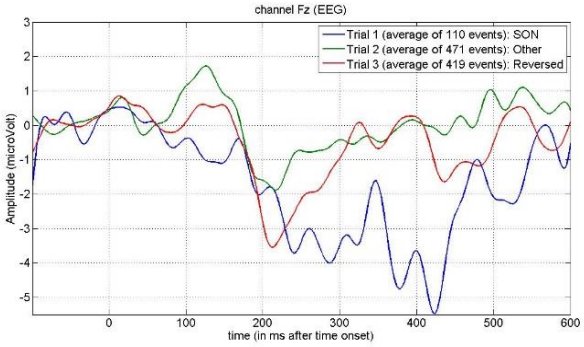

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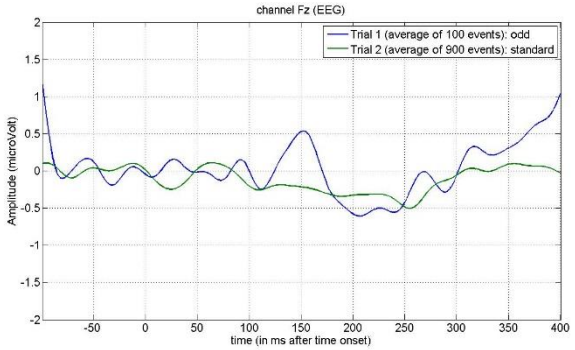		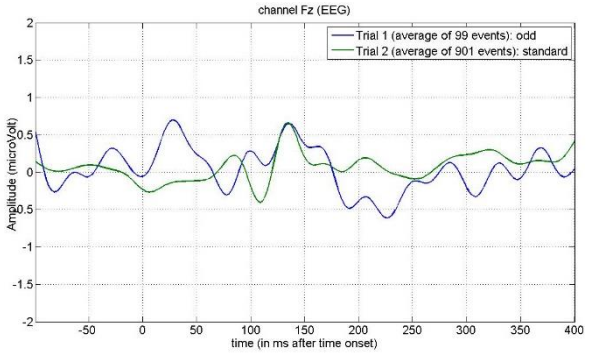
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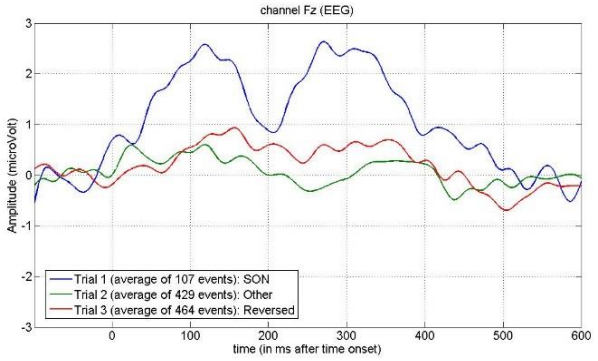	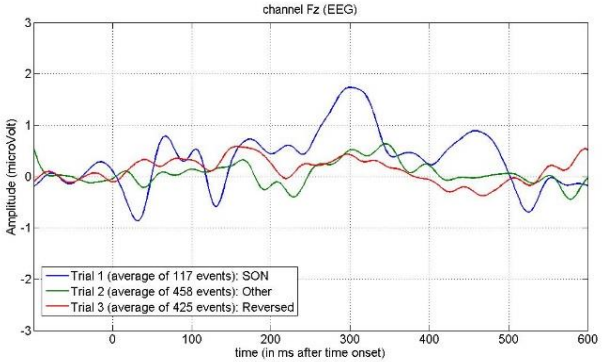	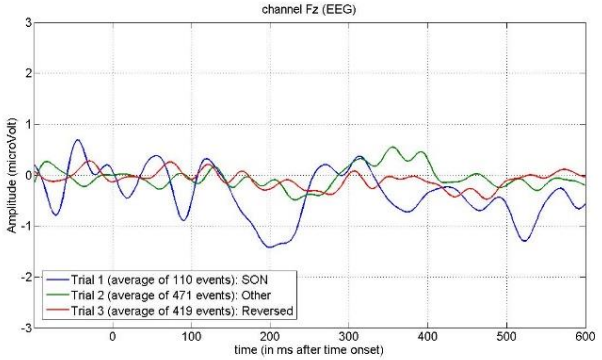

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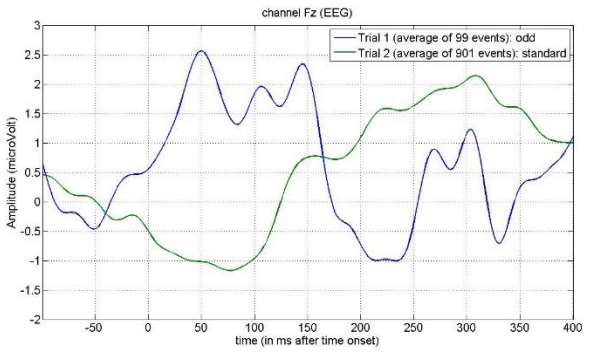	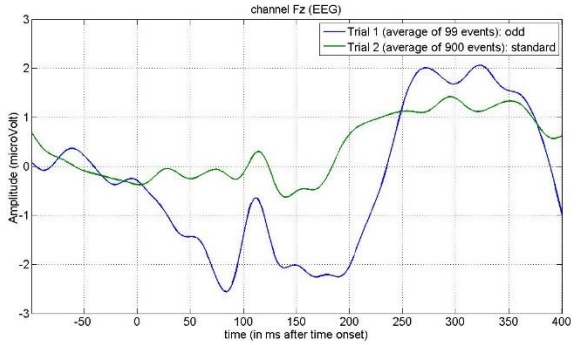	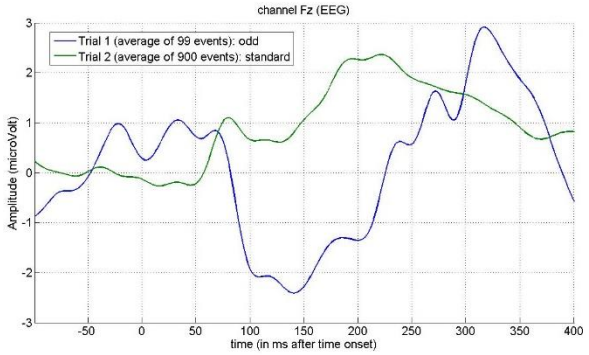
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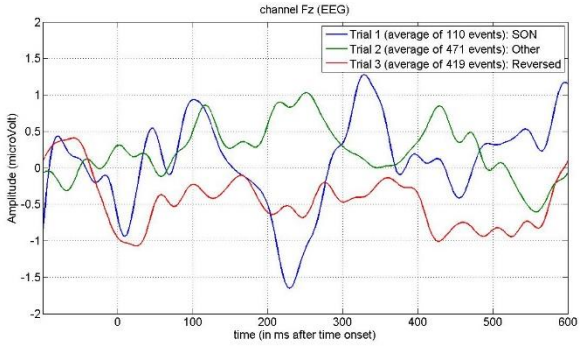	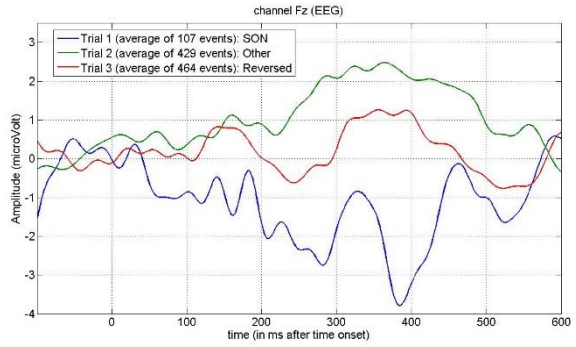	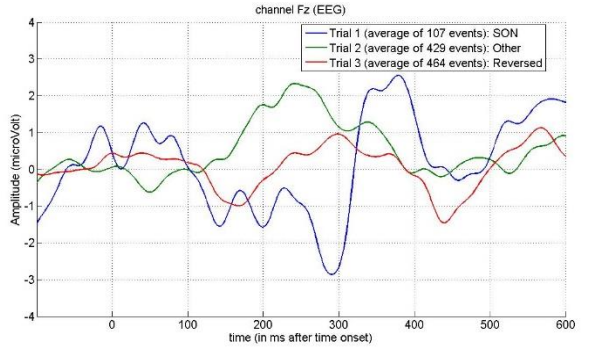

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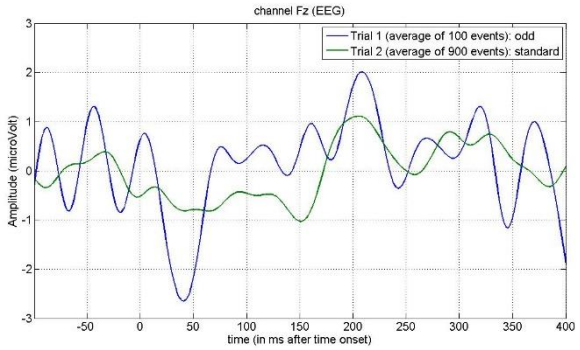	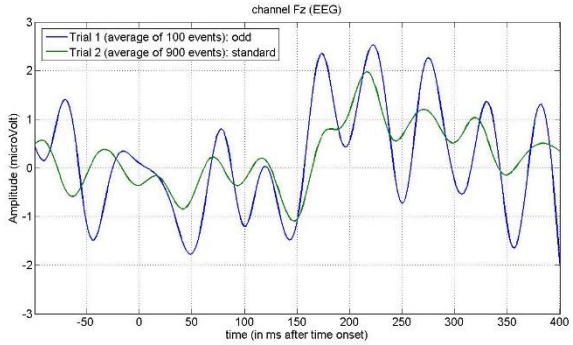	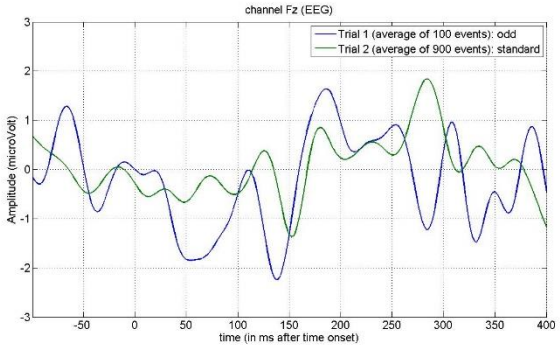
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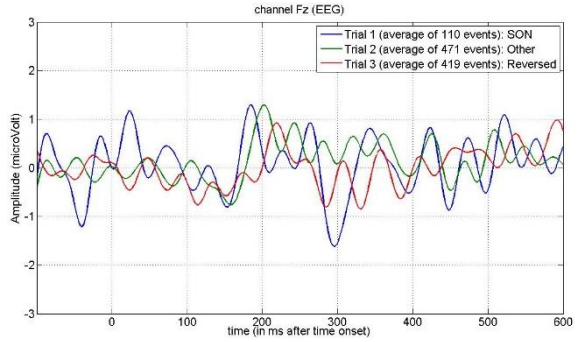	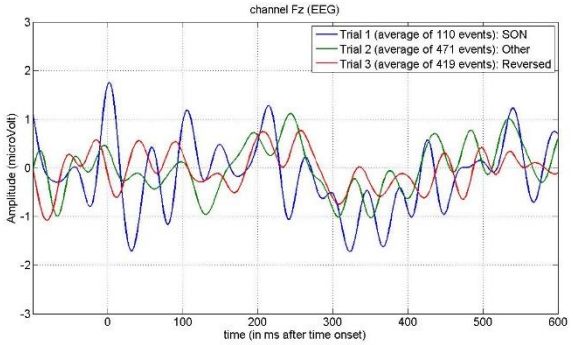	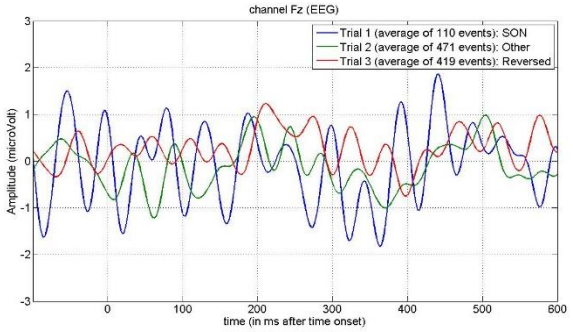

APPENDIX 4: PUBLISHED PAPERS THAT CONTRIBUTE TO THE STUDY DESIGN

A Rehabilitation Unit at Night: Environmental Characteristics of Patient Rooms

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Agnieszka Kempny: Royal Hospital for Neuro-Disability, London, United Kingdom; UCL Institute of Neurology, London, United Kingdom
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Keywords: Light, Noise, Temperature, Humidity, Rehabilitation, Environment, Rehabilitation Unit

Abstract

Purpose: The aim of this study was first of all to investigate the sound, light, temperature and humidity levels in a rehabilitation ward and to establish whether the measured levels were within the recommended levels or not; secondly to explore influence of the type of the patient rooms on obtained measurements.

Methods: Measurements of environmental characteristics were recorded on three non-consecutive nights by data loggers in a 12-bedded residential neurological rehabilitation unit for patients with disorders of consciousness. The obtained measurements were compared with the recommendations of the World Health Organization and the Society of Light and Lighting.

Results: Recordings were within recommended levels for light and humidity overnight. Average noise levels were above the recommended levels. There were abrupt increases of light and noise levels which were high enough to cause sleep fragmentation**.** Mean temperature levels were higher than recommended.

Conclusions: Our results indicated that the patients are at risk of disturbed sleep for the duration of their stay in rehabilitation unit. Exposure to generally high noise levels, as well as the sudden increases of noise and light intensities can prevent reaching restful nighttime sleep and may negatively impact on rehabilitation process due to impaired memory, learning and well-being.

Introduction

Fatigue, lethargy, day time reduced arousal level and low mood are common problems that are encountered in neurological rehabilitation units. These problems are usually attributed to physical, or if present, cognitive impairment of patients. However, very little attention is paid to sleep patterns. It has been previously demonstrated that sleep fragmentation caused by relatively brief interruptions leads to measurable changes in daytime alertness, cognitive functioning including memory and learning, psychological functioning and neuroplasticity.[1-5] Daytime sleepiness and fatigue have been shown to be related to difficulties in sleeping.[6] Sleep disturbance is also a significant risk factor for obesity, diabetes, myocardial infarction, stroke and coronary artery disease.[7] Impaired immune function, decreased inspiratory muscle endurance and delirium have also been reported as adverse consequences of sleep disruption.[8-10]

The commonest preventable causes of such interruptions in ward based settings are background noise and light with sudden interruptions being particularly disruptive.

Noise levels are expressed as in decibel (dB). While the noise level in a quiet bedroom at night is around 30 dB, the noise generated by loud hand clapping at one meter distance is 130 dB. To the human ear, a gain of 10 dB is perceived as twice as loud. Light intensity levels on the other hand, are expressed as in lux. While average luminance in well-lit office is around 500 lux, good main road lighting luminance levels are approximately 100 lux. Environments that are too hot or cold may also be disturbing. Standards for ward based hospital environments to minimise sleep disturbance developed by the World Health Organization (WHO) and The Society of Light and Lighting.

According to the Guidelines for Community Noise[11], the noise levels in hospital rooms should be 35 dB or less. The recommended maximum intensity for individual events at the patient's bedside is 45 dB. Temperature and humidity should be maintained between 20-22 C° and 30-60 % respectively.[12] The Society of Light and Lighting recommends that night and observation illuminance in patient rooms should be below 5 lux and should not exceed 10 lux.[13]

Studies examining the environmental characteristics in intensive care and acute health care settings have demonstrated that the levels of noise and light were above the recommended levels for hospitals and sleep disruption was common.[14, 15]

The duration of stay in a rehabilitation unit varies greatly but in comparison with other types of inpatient admissions is very lengthy. Therefore night time environmental characteristics of patient rooms, namely light, noise, temperature and humidity levels need to be optimised not only to provide comfortable accommodation but to improve sleep quality and consequently to minimize the problems that may be faced due to poor sleep.

This is the first study to examine levels of sound, light, temperature and humidity in a rehabilitation setting. The aim of this study was first of all to investigate the sound, light, temperature and humidity levels in different types of rooms in a rehabilitation ward and to establish whether the measured levels were within the recommended levels or not; secondly to explore influence of the type of the patient rooms on obtained measurements.

Methods

Study Setting

The study setting was a 12-bed inpatient neurological rehabilitation unit part of a large rehabilitation center located in London, United Kingdom. The unit is located on the third floor of the main building which is approximately 20m away from a busy main road. There are one 4-bedded, two 2-bedded and four one-bedded rooms in the unit.

The unit specializes in assessment and rehabilitation of patients with severe acquired brain injuries leading to disorders of consciousness such as vegetative and minimally conscious states.

Patients are usually returned to bed in the late afternoon due to limited sitting tolerance and are repositioned in bed every four hours. Evening medications are given by nursing staff at 18.00, 20.00 and 22.00 hours and morning medications are given at 08.00 hours. Due to their disability patients are not able to control any of the devices that might be a source for light or noise.

Design

Measurements of light, sound, temperature and humidity over a 12 hour period, between 7pm and 7am, were obtained in occupied one bedded, two bedded and four bedded patient rooms. The measurements were repeated on another night within an unoccupied one-bedded room in order to gain better understanding of contributory factors arising from presence of patients such as increased room temperature, and more frequent entry of nursing staff to the rooms. Measurements were repeated on three separate occasions during late autumn/winter months in order to obtain information on use of artificial lighting by staff.

Six two-hourly intervals beginning at 7pm were chosen to capture the variability between high and low activity times. Between 7pm and 9pm nursing staff handover takes place and some patients may have still have visitors on the ward. On the other hand, between 11pm till 5am, only bed positioning and observation of patients are performed. After 5am activity increases due to administration of medications and feed.

The study was approved by the local audit committee. Due to the nature of their illness the patients were not able to consent or communicate. Nevertheless, at the time of data logger placement they were told of the purpose and nature of the study. The night shift ward staff and the patient relatives were informed of the audit study through written information sheets and verbal communication.

Measurement of Environmental Characteristics

SOUND

Sound levels were measured using a Tecpel 331 sound level meter (Tecpelco Ltd, Taiwan). The Tecpel 331 sound level meter has an accuracy of ± 0.5 decibels (dB). The dimensions of the device are 275 x 64 x 30 millimeters. The sound level meter

was programmed to measure sound level ranges of 30 to 130 dB. The Tecpel 331 sound level meter can measure from 25 dB in a quite closed environment. Sound measurements were sampled every 30-seconds and stored in the data logger's internal memory. The data collected by the device was downloaded onto a computer using the SE322 software provided by Tecpelco to be used with the sound data logger. During a 12 hour period 1440 sound measurements were obtained.

LIGHT

Light levels were measured with the Tecpel 536 light meter/ RS232 data logger device (Tecpelco Ltd, Taiwan), and stored in the device's internal memory. The Tecpel light meter sensor measures light intensity with a range of zero to 2000 Lux. This device measures 146 x 70 x 39 millimeters. The light measurements were sampled every 5 seconds. The data collected by the light meter was downloaded onto a computer using RS232 software provided by Tecpelco. Over a 12 hour period 8640 light measurements were recorded.

TEMPERATURE AND HUMIDITY

Temperature and humidity were measured using CEM DT-172 temperature and humidity data logger (CEM, China). This device has measurement range of zero to 100%RH (relative humidity) and -40 to 70 °C with accuracy of ±3%RH and ±1% °C. The dimensions of this device are 94 x 48 x 33 millimeters. Both temperature and humidity levels were sampled every 30 minutes. Twenty-four measurements of temperature and humidity were collected in the 12 hour period.

Data Collection Procedures

At the onset of data collection all data loggers were initialized using their software. All three data loggers were mounted to a hard plastic frame measuring 20cm x 30cm and placed within one meter distance of patient's bedside at the level of the head and at least two meters away from the room door. The location of data loggers did not interfere with nursing care activities. Overnight nursing care of the patients was not altered during the study. The patients were continued to be observed as normally and re-positioned in bed every four hours as part of their postural management program. Despite being in vegetative state some patients are able to make sounds such as groaning or laughing. At the time of the study, none of the patients were known to vocalize, and no monitoring equipment was being used that generated noise or light signals.

Data was collected continuously between 7:00pm and 7:00am on three different nights in one-bedded, two-bedded and four-bedded rooms which were occupied by patients to their maximum capacity i.e., all four beds were occupied in four-bedded room. The measurements were repeated on another night within an unoccupied one-bedded room in order to gain better understanding of contributory factors arising from presence of patients such as increased room temperature, and more frequent entry of nursing staff to the rooms.

At the end of the measurements nursing staff were asked to find out if there were any unusual events during the night, such as a medical emergency in the adjacent ward. Results of the study were presented to nursing staff in order to obtain their feedback and views on nighttime sleep environment of patients.

Data Analysis

All stored data was downloaded from data loggers into a desktop computer for analysis using their own connection USB cables and specific software. Numeric data was then loaded into Excel (Microsoft, USA) and transferred into Origin-Pro version 8.5 (OriginLab Corp., USA) for statistical analysis. Light and noise during each 2-hour interval and temperature and humidity during 12-hour period were calculated using descriptive statistics (mean, SD, SE and range). Examination of the data represented graphically suggested that this was normally distributed. Variability of environmental measures for the three different types of rooms was analyzed with one way independent ANOVA, followed by Tukey's means comparison *post hoc* test.

Results

TEMPERATURE AND HUMIDITY

The mean temperature within the unoccupied one bedded room was 25.28 C° which was higher than in the occupied rooms and well above recommended levels. The average nighttime temperature within the occupied one and two-bedded rooms were 24.21°C (SE= 0.17) and 23.29°C (SE= 0.30) respectively. These were above the recommended upper threshold of 22°C. The average nighttime temperature in the four bedded room was 21.72°C (SE= 0.19), which was within the recommended range of room temperature in patient rooms (figure 1).

The results show that the type of room significantly affected the room temperature levels, $F(2, 213) = 29.24$, $p<0.05$. Tukey's post hoc tests revealed significant differences between all types of rooms at the 0.05 level.

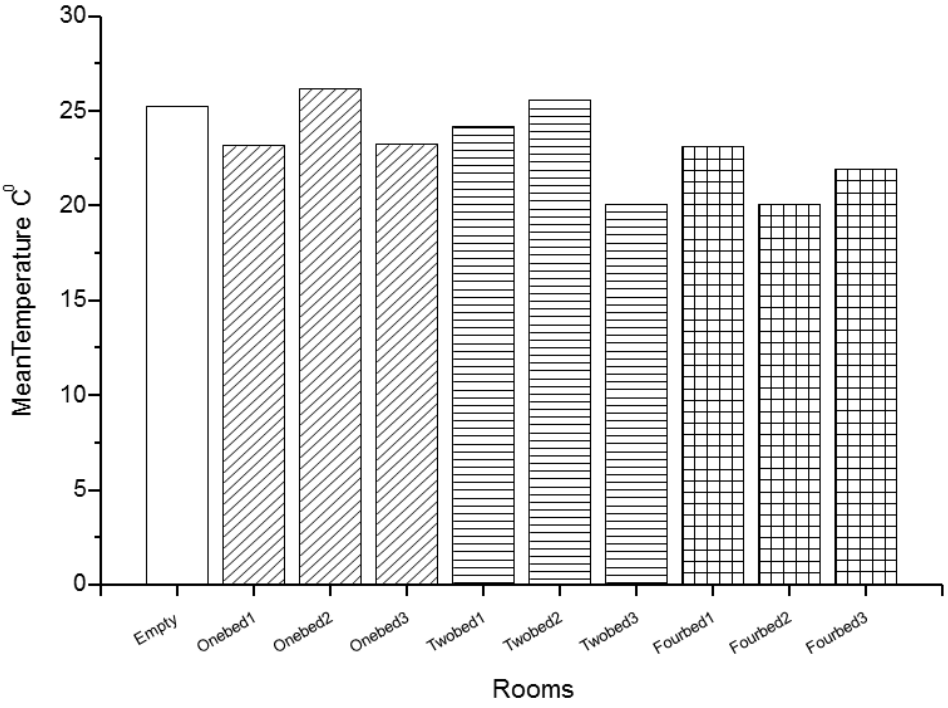

Figure 2: Mean temperature levels in empty and occupied rooms recorded over three nights.

Mean humidity levels in all rooms were between 29 %RH and 41 %RH, hence maintained within the recommended range. Minimal variation in humidity was present throughout each nighttime period (figure 2).

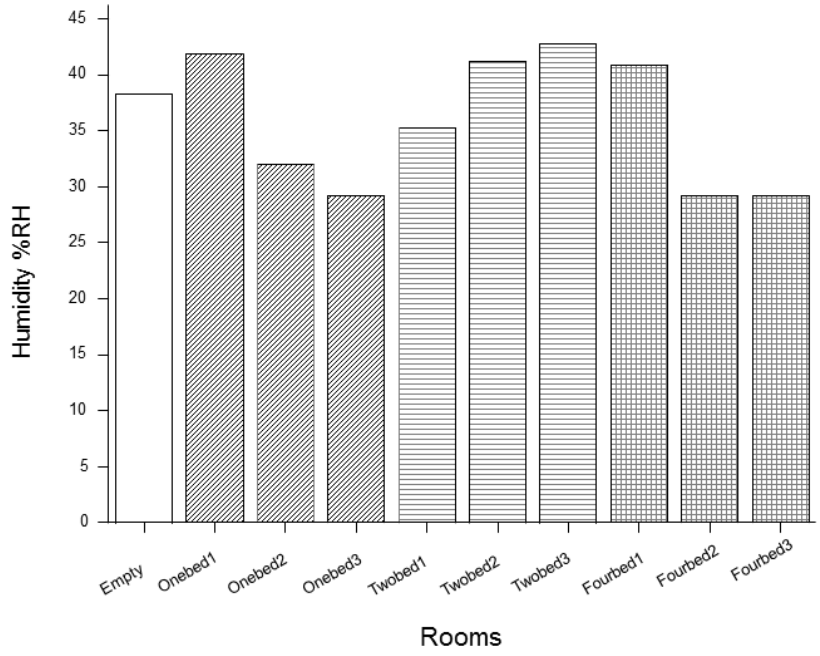

Figure 3: Mean humidity levels in empty and occupied rooms recorded over three nights.

Both temperature and humidity levels were consistent throughout the night without any sudden changes.

NOISE

Recorded noise levels ranged between 28 and 84 dB throughout the study. Mean noise levels of the occupied rooms within the two-hour periods, however, varied between 36 and 52 dB. There were abrupt increases of noise levels in excess of 70 dB in all types of rooms. The mean noise levels in all different types of patients' rooms within the each 2-hour interval are shown on figure 3.

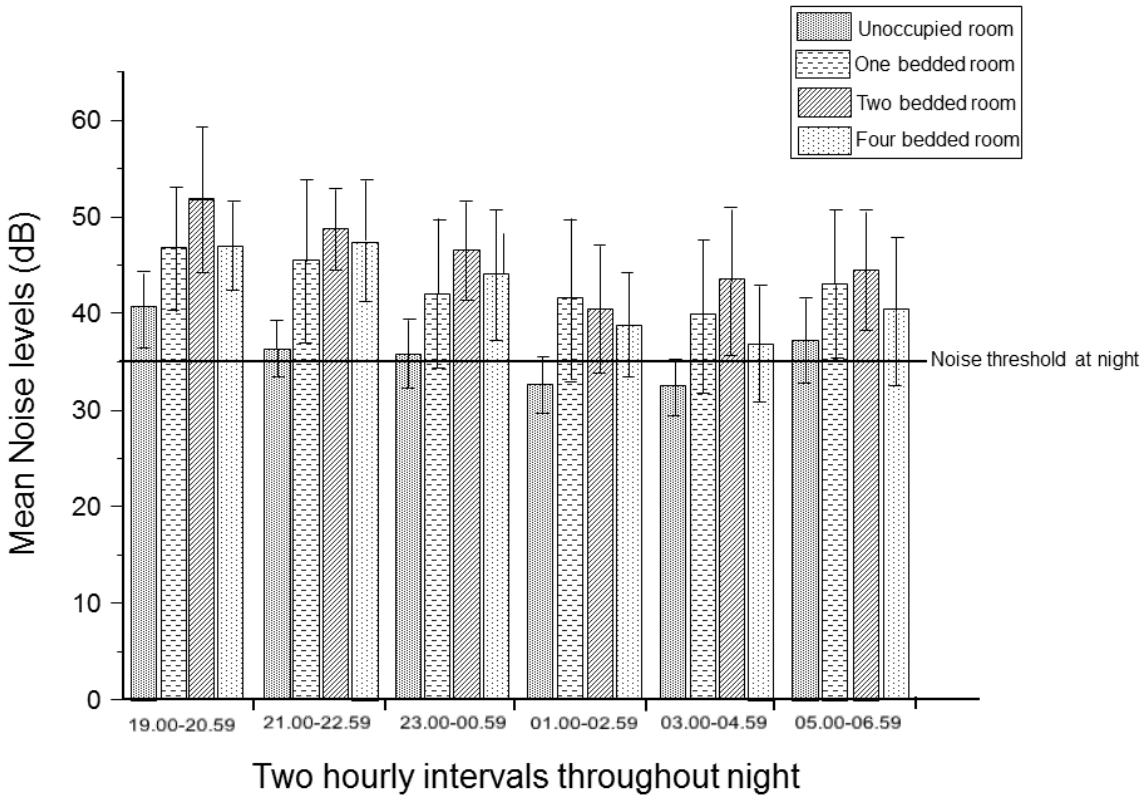

Figure 4: Mean noise levels in each two hourly intervals recorded in different rooms from 7pm to 7am.

Overall one way ANOVA test revealed that at the 0.05 level the means were significantly different for the measurements obtained in two-hour periods ($F(5, 1434) = 1001.57$). Tukey's post hoc test confirmed the significant differences between the all two hourly intervals except, between 1 and 3 am indicating sound intensities were less variable within this time period. The mean noise levels obtained on three different nights were collapsed and one way ANOVA test was performed. This revealed that at the 0.05 levels the mean noise levels were significantly different in different types of rooms ($F(3,2396)=108.47$).

LIGHT

The recorded levels of light in the unit ranged between 0 and 164 lux. Overall light intensity was greatest between 7pm and 9pm, consistent with the visiting and nursing activity times. Mean light levels after 9 pm varied between 0 and 108 lux. There were abrupt increases of light levels in excess of 100 lux in occupied one bedded room on night 1 and night 2 which indicates main lights being turned on. The mean light levels in all different types of patients' rooms within the each 2-hour interval are shown on figure 4.

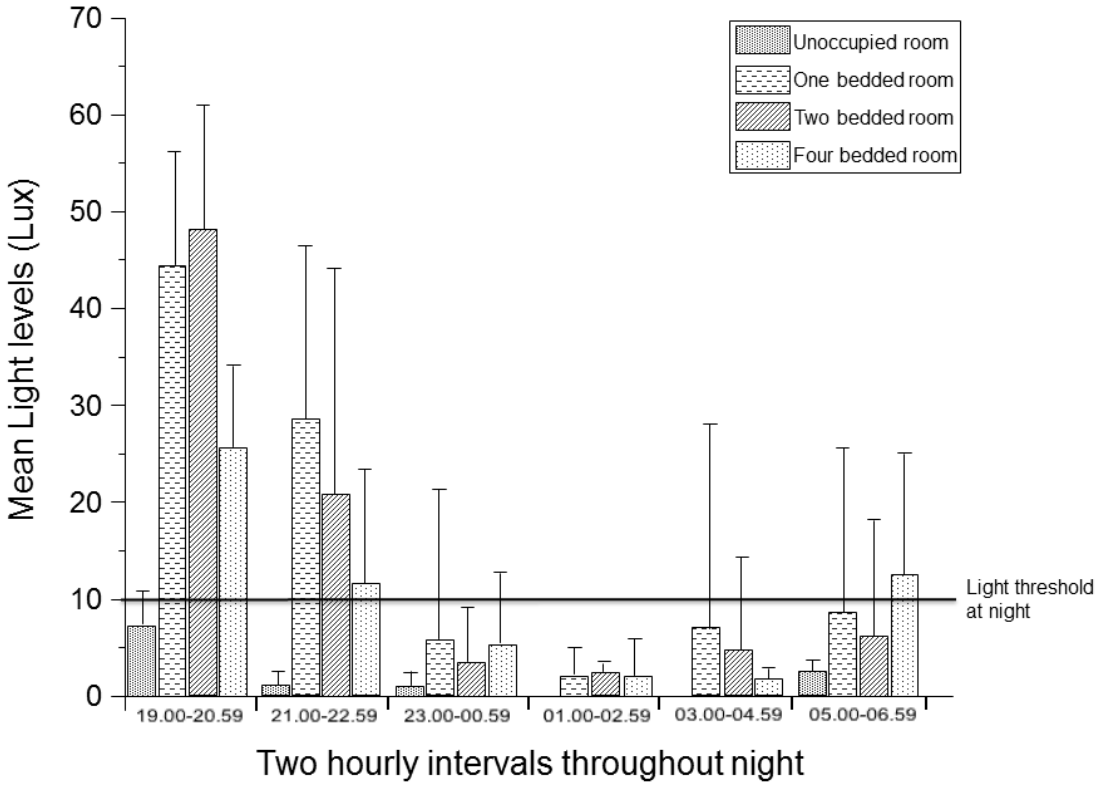

Figure 5: Mean light levels in each two hourly intervals recorded in different rooms from 7pm to 7am.

Overall one way ANOVA test revealed that at the 0.05 level the mean light levels were not significantly different for the measurements obtained in two-hour periods between 11pm and 7am ($F(3, 12) = 2.86$) indicating that rooms were reasonably dark within this time frame and within the recommended light levels. The mean light levels between 11pm and 7 am ranged from 1.61 to 7.42. Tukey's post hoc test confirmed was not significant at 0.05 level.

Further statistical tests were performed to reveal any differences arising from the number of patients occupying the rooms however no significant difference was found. ($F (3, 20)= 1.40$).

STAFF FEEDBACK

Following the presentation of the study findings to staff positive and constructive feedback were received and following points were noted: Door fittings and bins may be the source of noise, night staff may need training on which light sources to use for nighttime observations and patients with severe brain injuries may not be able to regulate their body temperatures.

Discussion

This study showed that the environmental characteristics are not at optimum levels during night time in the patient rooms. Interestingly the only parameter which was consistently within ideal levels was humidity levels which we had the least control over.

Temperature measurements showed that, although throughout the night stable, the levels were above the recommended levels in one bedded and two bedded rooms. Temperature in the four bedded room was maintained within the recommended range.

On the other hand, humidity levels within the rooms were stable and within the recommended range for healthcare settings in all rooms throughout the night.

The findings of this study identified that measurements of sound levels in our rehabilitation ward for patients with profound brain injuries were not always within the recommended levels. Mean sound levels in all occupied rooms were consistently in excess of the 35 dB and therefore, not within the recommendations made by the World Health Organization for healthy sleep environment in healthcare settings. Moreover, there were abrupt increases of sound levels as high as 84 dB which is equivalent to sound of an alarm clock at two feet distance. Hence, the 45 dB threshold of recommended maximum intensity for individual events at the patient's bedside was exceeded in all three types of patient rooms. Interestingly, in the unoccupied one bedded room, mean sound levels varied between 32 and 41 dB throughout the night. This indicates that entries to the rooms by night staff significantly contributed to the increased noise levels in patient rooms as it is unlikely that the patients with disorders of consciousness would be the source of noise.

In our rehabilitation ward, light intensity levels were reasonably maintained within the recommended levels by the Society of Light and Lighting between 11pm and 7am. The differences amongst the different types of rooms, including the unoccupied one bedded room, were not statistically significant. This suggests that nursing staff were able to perform their clinical care activities using the night observation lights. However, there was also evidence of use of main lights, on occasions, in one bedded occupied room.

The major limitation of this study was that all measurements were performed within the same season- late autumn/winter. This study was focusing on the bedside care

environment of patients with disorders of consciousness; therefore, findings may not necessarily be generalized to other rehabilitation settings where patients can potentially influence the environmental factors more than in our unit. On the other hand, our study provides better understanding of nighttime environmental factors mainly influenced by nursing staff activity and buildings rather than factors depend on patients. This study did not aim at identifying sources of increased noise or light intensity. Nevertheless, it is also possible that night staff may have altered their behavior as they were aware of the study.

The findings of this study indicate that there is a need to explore sources of excessive nighttime noise in order to create a healthier sleep environment for patients. These may include check and maintenance of fixture and fittings present in the rooms, such as doors and rubbish bins. More importantly, education and training may be needed to increase awareness on importance of sleep and environmental factors. Although, the temperature levels in the rooms were above the recommended levels, it was noted that nursing staff in our rehabilitation unit believed that patients with profound brain injuries were not able to regulate their own body temperatures and therefore were in need of warmer than recommended bedside environment. We were not able to find any studies done on group of patients with disorders of consciousness that can support this theory.

Nighttime patient care i.e. changing feed, turning the patients will always need to be performed by nursing staff nevertheless, implications of this study for clinical practice include assessment of current practice, education and training of night staff to raise awareness on importance of sleep and environmental factors and development of strategies to reduce noise levels at night with the objective of providing better sleep

environment and reduce possible complications of interrupted sleep. During their stay patients undergo a series of clinical assessments looking for the evidence of consciousness and awareness. Therefore, it is imperative that the patients maintain good arousal levels during the assessments.

The results of this study suggest that there is a need for further studies exploring the bedside care environment in other rehabilitation settings. Studies that are designed to identify the sources of excessive sound and light intensity such as observational studies on night time care are also needed. Once the sources of excessive sound and light identified, interventional studies aiming to optimize bedside environment in rehabilitation settings are warranted. Further research on body temperature regulation of patients with profound brain injuries is considered necessary with the intention of investigating whether recommended levels of room temperatures are applicable to this patient population.

Acknowledgements

We thank patients, their families and ward nursing staff for allowing us to carry out measurements in the rooms. We especially thank Luke Price from Laser and Optical Radiation Dosimetry Group, Health Protection Agency for providing us with very valuable information of environmental light measurement techniques. This research/study/project was funded by the Neurodisability Research Trust and the Royal Hospital for Neuro-disability and supported by researchers at the National Institute for Health Research University College London Hospitals Biomedical Research Centre.

Declaration of Interest

The authors report no declarations of interest.

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LATE RECOVERY OF AWARENESS IN PROLONGED DISORDERS OF CONSCIOUSNESS- A cross-sectional cohort study

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Keywords: Vegetative state, Minimally conscious state, Unresponsive Wakefulness Syndrome

Abstract

Purpose: To detect any improvement of awareness in prolonged disorders of consciousness in the long term.

Methods: 34 patients with Prolonged Disorders of Consciousness (27 vegetative state and 7 minimally conscious state; 16 male; aged 21 to 73) were included in the study.

All patients were initially diagnosed with vegetative/ minimally conscious state on admission to our specialist neurological rehabilitation unit. Re-assessment was performed 2 to 16 years later using Coma Recovery Scale-Revised.

Results: Although remaining severely disabled, 32% of the patients showed late improvement of awareness evidenced with development of non-reflexive responses such as reproducible command following and localization behaviours. Most of the late recoveries occurred in patients with subarachnoid haemorrhage (5/11, 45.5%). The ages of patients within the late recovery group (Mean=45, SD=11.4) and non-recovery group (Mean=43, SD=15.5) were not statistically different (p=0.76).

Conclusions: This study shows that late improvements in awareness are not exceptional in non-traumatic Prolonged Disorders of Consciousness cases. It highlights the importance of long term follow up of patients with Prolonged Disorders of Consciousness, regardless of the aetiology, age and time passed since the brain injury. Long term follow up will help clinicians to identify patients who may benefit from further assessment and rehabilitation. Although only one patient achieved recovery of function, recovery of awareness may have important ethical implications especially where withdrawal of artificial nutrition and hydration is considered.

Keywords: Disorders of Consciousness, Vegetative State, Minimally Conscious State, Recovery, Consciousness, Unresponsive Wakefulness Syndrome

INTRODUCTION

Severe disorders of consciousness (DOC) which include vegetative state (VS) and minimally conscious state (MCS) are known to have a very poor clinical outcome and despite extensive research are still poorly understood.[1-4] VS is characterized by complete lack of awareness of the self and the environment, accompanied by sleep-wake cycles with either complete or partial preservation of hypothalamic and brain stem autonomic functions[5]. The diagnosis of MCS is based on the presence of minimal but definite behavioural evidence of self or environmental awareness on clinical assessment[6]. In MCS behavioural responses are characteristically inconsistent and often subtle; hence patients require repeated assessments by experienced clinicians to differentiate MCS from VS. When DOC lasts more than one month it is defined as Prolonged Disorders of Consciousness (PDOC).

Several research studies have shown that it is possible to detect the presence of covert awareness/ consciousness in such patients, by utilizing advanced electrophysiological methods and/or advanced functional neuroimaging techniques in cases where clinical assessments are unable to detect any behavioural sign of awareness. [7-11] Despite these promising developments within severe DOC brain research, currently the diagnosis of VS and MCS is made on clinical grounds.

Although limited and inconsistent, patients with MCS may demonstrate agency and, on rare occasions, may be able to communicate their choices and opinions with respect to their basic treatment and care. Therefore, the distinction between VS and MCS has important ethical implications for the patient, their family and carers, medical, nursing and therapy staff and for wider society especially where withdrawal of clinically assisted nutrition and hydration is considered.

National Clinical Guidelines on Prolonged Disorders of Consciousness[2] state that vegetative state may be classified as a 'permanent VS' if it has persisted for more than six months following non-traumatic brain injury and more than one year following traumatic brain injury as after these time points recovery is deemed 'highly improbable'.

Our current knowledge of long-term outcome in severe DOC is incomplete largely because once a diagnosis is made, patients are discharged to diverse care settings and their follow up rarely extends beyond 12 months after brain injury.[1,12,13] A recent study examined the long-term prognosis (for a mean of 25.7 months from onset of brain injury) in 50 patients with VS. This study reported that late recovery was detected in 25% of the patients; suggesting that late recovery of responsiveness may occur

more frequently than previously appreciated.[14] The study also demonstrated a higher chance of recovery in the post-anoxic brain injury sub-group (21.4%) than in earlier published studies which were in the form of case reports.[15-21] Luauté et al. showed that a third of patients in MCS with mixed aetiologies improved more than 1 year post ictus.[22] In this study however, Glasgow Outcome Scale was used as main outcome measure with no specific attention to improvement of awareness/ responses to given stimuli . We wished to add to the small but growing body of knowledge on the long-term prognosis of PDOC. We specifically wanted to focus on detecting changes in in awareness which can be detected only with structured and detailed assessments and otherwise may be unnoticed. The clinical setting of our unit gave us the unique opportunity to investigate outcomes in PDOC patients many years and even decades after the original ictus.

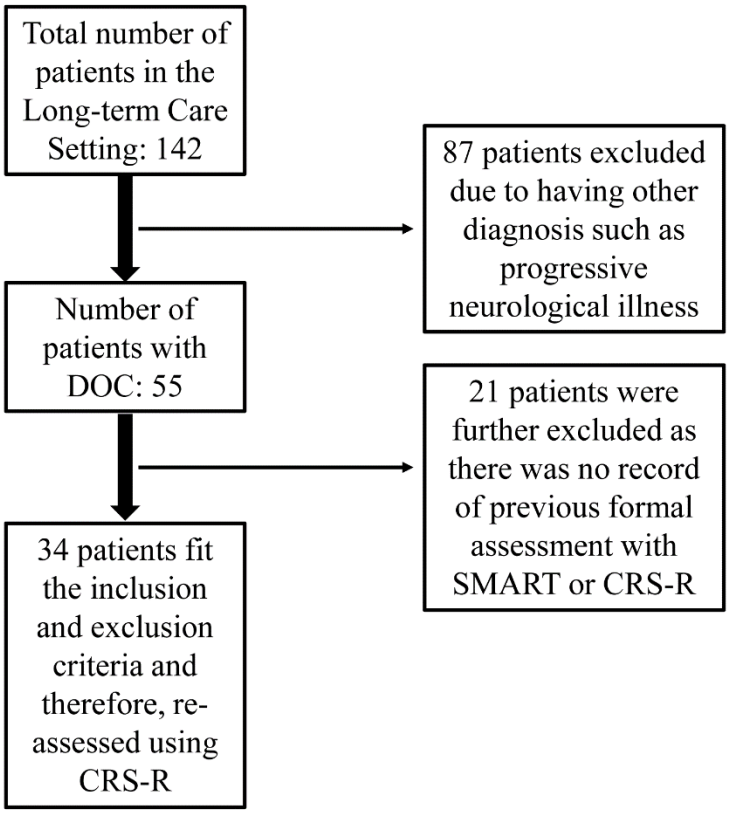
METHODS

The study was conducted in a long term care setting specializing in management and care of patients with profound brain injuries. The patients were given regular sensory stimulation provided in sensory rooms and were exposed to art and music therapy sessions as well as to regular social events. The number of residents in the long term care setting is around 140 and 55 of these had diagnosis of DOC and 34 of these patients received their initial assessments and rehabilitation at our rehabilitation unit where Sensory Modality Assessment Rehabilitation Technique (SMART) assessment is most commonly used to diagnose DOC. SMART [23] is a valid and robust tool used in the assessment of DOC. It is recognised by the Royal College of Physicians guidelines and in the English high courts as the tool of choice to detect awareness and identify potential in patients with DOC.

Although recommended as a good practice, regular and formal re-assessment of PDOC patients is not routinely and widely carried out in the United Kingdom. Following discharge to long term care setting, our patients were monitored closely by clinicians who are experienced in care of people with disorders of consciousness and no apparent recovery of awareness was reported. Nevertheless, due to lack of regular and formal re-assessments subtle changes or improvements masked by aphasia and/or severe motor weakness may have gone unnoticed.

The patients were in a stable medical condition. Case notes of all the patients with a disorder of consciousness were screened and the following features were considered as exclusion criteria: disorders of consciousness secondary to neurodegenerative illnesses; patients who did not have an initial formal assessment of consciousness using validated assessment techniques (SMART± Coma Recovery Scale-Revised (CRS-R) or Wessex Head Injury Matrix) and, patients with severe pathologies independent from the brain injury such as advanced cancer.

The eligibility criteria for this cohort study were that patients: had a diagnosis of VS or MCS established by using SMART assessment, as it was the validated assessment tool most often used in our cohort ± another validated assessment tool; had a brain injury secondary to acquired and non-progressive neurological illness; and, were medically stable at the time of re-assessment. The flow chart in Figure 1 shows the selection criteria for the follow up study.



We documented a range of demographic variables (age and sex), cause of brain injury (e.g. traumatic, anoxic, subarachnoid haemorrhage, ischaemic stroke), time from brain injury to initial SMART assessment, the initial SMART assessment outcome, time from brain injury to follow up assessment, time between first SMART assessment and the follow up assessment and the outcome of the follow up assessment.

All the patients were re-assessed 2 to 16 years after the initial DOC diagnosis by two clinicians who were experienced in clinical assessments of patients with disorders of consciousness. The assessments were undertaken in a quiet, well lit room while patients were in the sitting position. The main outcome measure was recovery of awareness/ responsiveness according to the clinical criteria for MCS and for emergence from MCS, assessed with CRS-R.[24] The SMART and CRS-R apply similar stimuli and specify the method of application, to exclude extraneous variables. CRS-

R was chosen as the method of re-assessment as it is a quick and reliable assessment tool for screening purposes.[25] CRS-R includes all the modalities of the SMART assessment with the exclusion of the gustatory and olfactory sensory stimulation techniques. Another difference between SMART and CRS-R is that, CRS-R uses a mirror to assess visual tracking, whereas SMART uses a moving person and a picture of a baby. On the other hand, both tools assess visual tracking in both horizontal and vertical planes. As it was shown that assessment of visual pursuit with mirror is superior to with moving person or object[26,27]; SMART assessment proformas were examined in detail and all additional behaviours which suggested MCS were recorded.

Two clinicians were present during CRS-R assessments. One of the investigators (SD or AK) was not involved in the initial review of case notes and was blinded to the initial diagnosis of the patients. Due to resource constraints, we were not able to have two blinded examiners at the same time however, CRS-R scoring sheets were only completed upon consensus of both clinicians on patients' responses during the assessment. In a few occasions where there was disagreement between the assessors on the responses elicited, the CRS-R scores were recorded for the lower assessment. For example, if one of the clinicians did not agree on the presence of a consistent movement to command, this was scored as "not present".

Data were analysed with SPSS 21 (IBM SPSS, Chicago, IL, USA). Permission for the study was obtained from the Royal Hospital for Neuro-disability audit committee.

RESULTS

34 patients (16 male) met the inclusion and exclusion criterion. The mean age at the time of brain injury was 43 (Range 17-70 years, SD 13 years). The mean age of the

study population at the time of re-assessment was 49 (Range 21-73 years, SD 12 years).

The causes of brain injury were: anoxic brain injury in 15 patients; cerebrovascular accident in 13 patients (subarachnoid haemorrhage in 11 patients and massive ischaemic stroke in two patients); traumatic brain injury (TBI) in six patients. 27 (79%) of the patients had a diagnosis of VS on the initial SMART assessment, with seven (21%) diagnosed as being in the MCS. Time from brain injury to initial formal DOC diagnosis with SMART assessment varied between five and 38 months (Mean=10.9, SD=7.1 months). The diagnosis remained same at the time from completion of SMART assessment and admission to long term care facility as patients were under constant review and continued to receive monitoring assessments during period of rehabilitation.

The time from brain injury to re-assessment was between 2 and 16 years. Mean duration of follow-up from brain injury was 6 years. (SD 3 years). Mean time from the initial SMART assessment to re-assessment was 5 years (SD 3.17 years). The time interval between the brain injury and re-assessment was 5.9 (SD 3.4 years, range 2-11 years). (See table 1)

Table 2: Clinical features of patients

		TBI (*n*=6)	Anoxic (*n*=15)	Subarachnoid haemorrhage (*n*=11)	Ischaemic Stroke (*n*=2)	Total Sample (*n*=34)
Mean Age(SD)		38.5(10.9)	49.2(12.5)	53.6(9.2)	58(21.2)	49.2(12.4)
Mean Months BI to SMART (SD)		10.2(2.8)	12.9(10.1)	9.19(3.3)	8.5(2.1)	10.9(7.2)
Mean Years BI to CRS-R (SD)		4.9(2.1)	6.9(3.9)	4.9(3.0)	7.0(1.4)	5.9(3.4)
SMART Outcome	VS	4	15	8	0	27
	MCS	2	0	3	2	7
	Exit MCS	0	0	0	0	0
CRS-R Outcome	VS	2	12	2	1	17
	MCS	4	3	8	1	16
	Exit MCS	0	0	1	0	1
Improvement		33% (2/6)	20% (3/15)	46% (6/13)		32% (11/34)

TBI= Traumatic Brain Injury, VS= Vegetative State, MCS, Minimally Conscious State, CRS-R= Coma Recovery Scale- Revised, SMART= Sensory Modality Assessment Rehabilitation Technique, SD= Standard Deviation

The results of the re-assessment using CRS-R showed that all patients remained severely disabled. However, 32% of the patients showed improvement of awareness with development of more complex responses than they had during initial assessment. The CRS-R scores and responses of the patients who showed improvement in their awareness state are shown in table 2 where we show the patient outcome data categorized by aetiology and on a scale of VS, MCS and exit MCS. Most of the late recoveries occurred in patients with cerebrovascular accidents (6/13, 46%). The ages

of patients within the late recovery group (Mean=45, SD=11.4) and non-recovery group (Mean+43.5, SD=15.5) were not statistically different (p=0.76).

Only one patient, who suffered from severe subarachnoid haemorrhage and was previously in minimally conscious state, progressed to the level of functional verbal communication and object use which is the criteria for exit MCS/ emergence from DOC. Eight patients had changes of diagnosis (VS to MCS) between initial assessment and re-assessment (table 2).

Patient ID Sex/Age/Aetiology	Time BI to SMART (months)	Time SMART to CRS-R (years)	SMART Findings	SMART Diagnosis	CRS-R Findings						
					Auditory function	Visual function	Motor function	Oromotor/ Verbal function	Communication	Arousal	CRS-R Total Score (Diagnosis)
Patient 1 M/ 65/SAH	7	9	Visual startle only Motor withdrawal No vocalization	VS	Reproducible movement to command	Visual startle	Object manipulation	Vocalization/ Oral movement	Non-functional: intentional	Eye opening without stimulation	13 (MCS)
Patient 2 F/ 44/ SAH	8	2	Reflexive responses in tactile, visual (pupil constriction only) and auditory	VS	Consistent movement to command	Visual pursuit	Flexion withdrawal	Oral reflexive movement	None	Eye opening without stimulation	12 (MCS)
Patient 3 F/ 21/ TBI	15	3	Visual tracking No command following	MCS	Reproducible movement to command	Visual pursuit	Flexion withdrawal	Oral reflexive movement	None	Attention	12(MCS)
Patient 4 F/ 39/ TBI	12	2	No visual fixation/ tracking Auditory startle only	VS	Localization to sound	Visual pursuit	Abnormal posturing	Vocalization/oral movement	None	Attention	11 (MCS)
Patient 5 M/ 38/ Anoxia	12	2	Inconsistent focusing on a familiar face, eye opening to auditory stimulus	VS	Reproducible movement to command	Object localization/ reaching	Object manipulation	Oral reflexive movement	None	Attention	15 (MCS)
Patient 6 F/ 45/ SAH	9	10	Startle and withdrawal responses to visual and auditory stimuli, no localization	VS	Localization to sound	Visual pursuit	Localization to noxious stimulation	Vocalization/oral movement	None	Attention	13 (MCS)

Patient 7 F/ 65/ Anoxia	38	4	No visual responses, localization of sound	VS	Reproducible movement to command	None	Flexion withdrawal	Vocalization/ oral movement	None	Attention	10 (MCS)
Patient 8 M/ 43/ Infarct	7	5	Localizing responses at visual, auditory and motor domains.	MCS	Consistent movement to command	Object recognition	Functional object use	Intelligible verbalization	Functional: accurate	Attention	23 (exit-MCS)
Patient 9 M/ 57/ Anoxia	10	2	Visual startle but no visual fixation	VS	Auditory startle	Fixation	Flexion withdrawal	Oral reflexive movement	None	Eye opening w/o stimulation	8 (VS/ MCS minus)
Patient 10 F/ 70/ SAH	12	2	No localization of sound, no visual tracking	VS	Reproducible movement to command	Fixation	Object manipulation	Intelligible verbalization (lip read)	Non-functional: intentional	Attention	16 (MCS)
Patient 11 F/ 43/ SAH	10	2	Flexion withdrawal only, visual startle	VS	Reproducible movement to command	Object recognition	Object manipulation	Vocalization/ oral movement	None	Attention	17 (MCS)

Table 3: CRS-R Scores of patients with improvement of awareness

TBI= Traumatic Brain Injury, VS= Vegetative State, MCS, Minimally Conscious State, SAH=Subarachnoid Haemorrhage, CRS-R= Coma Recovery Scale- Revised, SMART= Sensory Modality Assessment Rehabilitation Technique, F=Female, M=Male

DISCUSSION

This study shows that late improvements in awareness are not exceptional in non-traumatic VS and MCS patients, regardless of age. Previous studies have reported better outcomes in traumatic VS patients than in our study. However, our study included only four traumatic VS patients and two traumatic MCS patients. The improvement rate was 33% within this subgroup but it is not possible to comment further on how aetiology differentially affects outcome due to the small subgroup sample size. The most significant finding of the present study is that approximately a third of patients in late phase of recovery from severe brain injury showed measurable improvements in their level of awareness. These changes were found in both VS and MCS patients including patients who suffered from non-traumatic brain injuries.

The main methodological difference between this study and previously published work is that all patients included in our study were initially diagnosed at the attached specialist brain injury rehabilitation unit by highly experienced clinical staff using at least two different validated assessment techniques (SMART, WHIM [28]). In our study, the timeline between the brain injury and re-assessment using CRS-R varied between 2 and 16 years. In comparison to previous studies this is an unusually long time window between validated assessments. During this time, very slow processes of neural recovery may have taken place including re-establishment of disrupted brain networks essential for consciousness. The care pathway within the Royal Hospital for Neuro-disability is that patients are initially assessed and treated within a specialist brain injury rehabilitation unit; typically for a period of 4 months. If

the patient is stable yet showing no consistent improvement in their DOC they are then transferred to a specialist nursing home environment where they continue to have maintenance level therapy input along with interventions to prevent complications such as pressure sores and contractures. They also are involved with group activities including music therapy. The current study did not aim to investigate the influence of access to specialist rehabilitation or the specialist nursing home settings; however, the rehabilitation and the care received by this cohort of patients may have some bearing on unexpectedly high percentages of improvement of awareness.

In our study only one patient emerged from disorders of consciousness and 8 out of 11 patients with improvement of awareness had a diagnosis change of VS to MCS. This may have significant ethical and legal implications. For example, in the United Kingdom, for the purposes of the law and withdrawal of treatment decisions, the distinction between VS and MCS is important. For people in VS, when considering applications for declaratory relief for withdrawal of clinically assisted nutrition and hydration, the English Courts work on the principal assumption of life-sustaining treatment is not in their best interests and favour withdrawal of life-sustaining treatment. Whereas, for the people in MCS, the decisions are made using a balance sheet approach where perceived benefits from continuation of treatment will be weighed against countervailing disadvantages.[29] As the differentiation between VS and MCS is the cornerstone of decision-making in English courts; regular assessments with validated assessment tools such as SMART, Wessex Head Injury Matrix, CRS-R is necessary and will inform clinicians and families of the patients when considering best interests of the patients with disorders of consciousness.

Our study has some limitations. Firstly, this is a cross-sectional cohort study with 34 patients in one particular long term care setting; hence the results may not be generalizable to the whole PDOC patient population. Second, this was not designed as a prospective follow up study, therefore, fails to provide evidence for possible influential factors for recovery of consciousness as well as how fast and when recovery occur. Another methodological limitation of this study was use of different assessment tools at the time of initial diagnosis and re-assessment (SMART and CRS-R retrospectively). However, both assessment tools are validated diagnostic tools for DOC and both use clear and stringent techniques to assess same modalities. Finally, this study has not captured data on the patients who died while resident in the long term care setting.

Conclusions

This study highlights the importance of long term follow up of patients with disorders of consciousness, regardless of the aetiology, age of the patient and time passed since the brain injury. Recovery of awareness in a third of patients over a long period of time, albeit with a poor functional outcome, supports the findings of recent studies showing that late recovery is possible[14] and it provides behavioural support for the concept that there may be long term axonal regrowth and neural plasticity in disorders of consciousness. [30,31] Our results further increase the ethical dilemmas faced by staff involved in making treatment decisions in this vulnerable patient group. The phenomenon of very late recovery of awareness has an important bearing on questions of withdrawal of artificial nutrition and hydration. Our study raises the question as

to whether the word ‘permanent’ is being used appropriately in the diagnostic term “Permanent Vegetative State” as reported in the recent Royal College of Physicians Guidelines. Prospective multi-centre studies that involve a variety of rehabilitation and long term care settings are now needed in order to comprehend long term prognostic outcomes and mechanisms of recovery in severe PDOC states.

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