Optimizing Circadian Rhythm and Characterizing Brain Function in Prolonged Disorders of Consciousness

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Presentation outline

1. Brief introduction to circadian rhythms and sleep in healthy subjects
2. Why study and optimize sleep in PDOC? Aim of our research.
3. Investigation of sleep in PDOC: Baseline Studies
4. Results of the interventional study

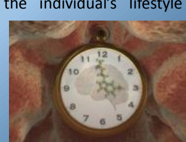
Circadian Rhythm and Sleep in Healthy

What is circadian rhythm?

- One of the most characteristic features of the nature is its rhythmicity
- Rhythms are either externally imposed, internally generated, or more frequently a combination of these two factors
- In Latin "Circa" means about and "dies" means day.
- Circadian= about a day
- The most prominent example of human circadian rhythm: sleep/ wake cycles

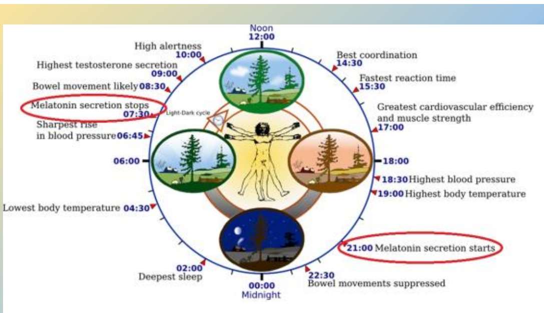
How is sleep regulated?

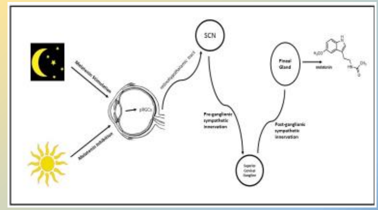
- Human circadian rhythm has two control mechanisms: internal and external.
- It is mainly regulated by the internal clock in the suprachiasmatic nucleus (SCN).
- Light-dark cycle is the major synchronizer of human circadian rhythms.
- Exogenous component is driven by the individual's lifestyle and environmental factors.

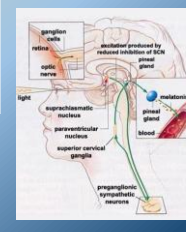


How can we assess the human circadian rhythm?

1. Studying the endocrine cycles that are regulated by the internal clock at SCN
2. Studying one of the most important end product: sleep

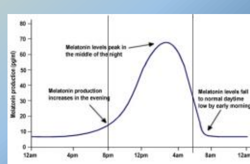






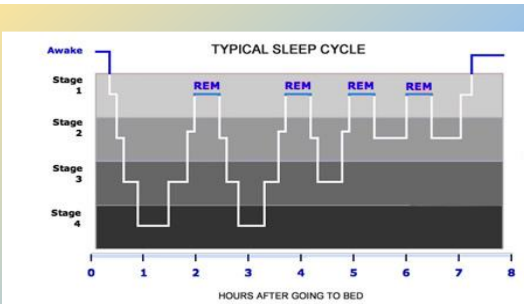
Melatonin: A Phase Marker of Circadian Rhythm

- Melatonin is used as reliable phase-marker of human circadian rhythm as it is secreted 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.

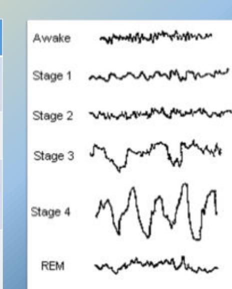


How do we sleep and what is a normal sleep?

- Even within a night's sleep we have rhythms.
- During sleep, we usually pass through five phases of sleep: stages 1, 2, 3 &4, and *REM* (rapid eye movement) sleep. These stages progress in a cycle from stage 1 to REM sleep, then the cycle starts over again with stage 1.
- The first REM sleep period usually occurs about 70 to 90 minutes after we fall asleep. A complete sleep cycle takes 90 to 110 minutes on average.
- We spend almost 50% of our total sleep time in stage 2 sleep, about 20% in REM sleep, and the remaining 30% in the other stages.



Sleep Stages	EEG Characteristics in Normal individuals
Stage 1	Beginning of sleep, slow eye movements and overall muscle relaxation
Stage 2	Presence of spindles (12-15 Hz, 10-50 μV, 0.5-2 s) and κ-complexes
Stage 3/4	High amplitude (>75 μV) and slow frequency (0.5-2 Hz) activity dominating ≥20% of the 30-s epoch
REM	Muscular atonia, lack of spindles and slow wave sleep, presence of rapid eye movements, predominant theta rhythm



What happens in different stages?

- REM sleep: synaptic re-modeling essential to learning and cognition can occur
- NREM Stage 2: synaptic plasticity/ targeted bidirectional plasticity in the neocortex.
- NREM Stage 3 &4: slow wave-dependent activity would serve to strengthen memory circuits- consolidation of memories
- Motor skills learning in NREM, Visual learning in SWS and REM

How much sleep do we need?

- Adults aged 18 to 60 years should sleep 7 or more hours per night on a regular basis to promote optimal health and reduce the risk of adverse health outcomes.
- Sleeping more than 9 hours per night on a regular basis may be appropriate for young adults, individuals recovering from sleep debt, and individuals with illnesses. For others, it is uncertain whether sleeping more than 9 hours per night is associated with health risk.

American Academy of Sleep Medicine and Sleep Research Society.

Circadian Rhythm and Sleep in PDOC

The Problem in Context

- Limited research on circadian rhythm and sleep patterns of patients with PDOC. However, a handful of studies, which investigated sleep in PDOC, indicated that these patients may have abnormal sleep patterns as well as circadian rhythms.

SLEEP AND CIRCADIAN RHYTHM IN PDOC

- **Diagnostic Value: O**bservation of sleep spindles and REM sleep was highly correlated with clinical scores (de Biase et al. Sleep Medicine, 2014)
- **Prognostic value:** More structured sleep was associated with positive clinical outcomes in sub-acute DOC. (Arnaldi et al. Clinical Neurophysiology, 2015)
- **Circadian System in PDOC:** VS patients present alteration in night melatonin secretion and reduced light-induced melatonin suppression suggesting disruption of circadian system in DOC. (Guaraldi et al. Chronobiol Int. 2014)

To our knowledge, there are no previous studies performed which examined the influence of circadian rhythm optimization on brain functions of patients with PDOC.

Aim of the Research Study

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 light, melatonin and caffeine?

3) Could these interventions lead to improvement of consciousness/ brain functions measured by using either clinical assessment tools or neurophysiological investigations?

Setting and Participants

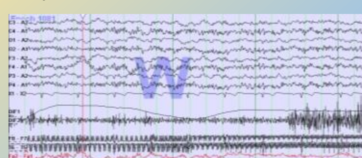
- 14 patients (7 female, aged 30-73) with PDOC were included in the baseline studies which aimed to investigate the melatonin circadian rhythm and sleep pattern/ structures.
- 10 of the patients were then included in the interventional study. These 10 patients were residents in the specialist nursing home section of RHN.
- Time since the brain injury varied between 1 and 9 years
- At the time of study all patients were medically stable and had established medication regimes.
- 6/14 fed during the day, 8/14 fed during evening/night hours, all turned/ repositioned every 4-6 hours

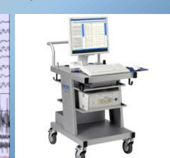
Methods used to answer Q1

- Brainstem auditory evoked potentials
- Coma Recovery Scale-revised (CRS-R) assessments
- 30-minute resting EEGs
- 24-hour polysomnographies (PSG)
- 4-hourly saliva melatonin measurements for 48 hours

Resting EEG

- **All patients had abnormal baseline EEG with low amplitude record.**



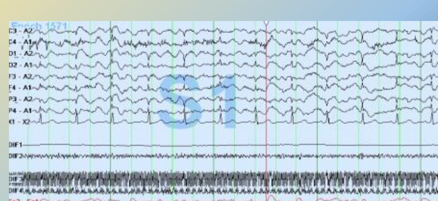


Typical resting state EEG of patients with PDOC: Characterized by diffuse polymorphic delta activity at 1.0-2.5Hz between 10-20μV (low amplitude record).

Polysomnography (PSG)

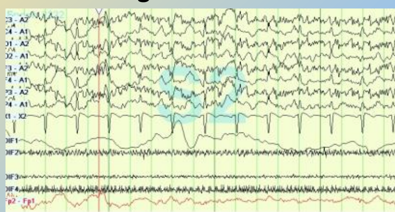
- 24hour PSG was performed using a Video-EEG monitoring system and Trex ambulatory EEG headbox which incorporated polygraphic inputs for the measurement of additional physiologic parameters.
- Sleep staging was done using visual inspection method adopted from AASM 2007.

PSG in PDOC- Stage 1



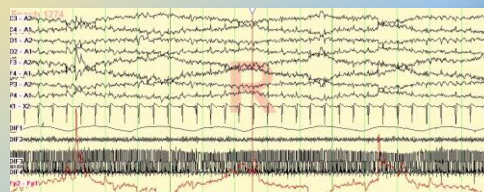
Stage 1 sleep: Characterized by absence of eye blinks as well as markers of other sleep stages

PSG in PDOC- Stage 2



Stage 2 sleep: Identified by presence of spindles which is usually in the 6-9 Hz frequency range

PSG in PDOC- REM



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

Sleep/ Wake Patterns

- All patients had abnormal sleep patterns with one of the following conditions:

a) increased sleep activity during daytime with wakefulness at night (6/14),
b) increased sleep activity in both day and night time (1/14),
c) reduced sleep activity in both day and night time (5/14)
d) short sleep mostly at night (2/14).

- Only 4 of the 14 patients had REM sleep and 9 of the 14 patients had stage-2 sleep during time spent asleep. None of the patients showed presence of deep sleep (stage 3&4).

Melatonin

- Saliva melatonin samples were collected every 4 hours using oral swabs (Salimetrics, USA) over 48 hours.
- Melatonin levels were measured using radioimmuno assay analysis.
- Raw saliva melatonin results were plotted as a function of time

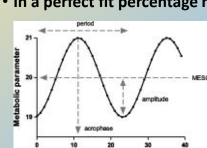
  - to explore whether an obvious rhythmicity could be recognized or not;
  - to make macroscopic/ qualitative analysis of noise;
  - to see if there is cycle-to-cycle variability or not

Is there a melatonin peak?

- **Review of the saliva melatonin results showed that 12/14 patients had peak melatonin levels reached in daytime in either 24 hour period.**
- **Only 3 patients (2 MCS, 1 VS) had delayed peak melatonin levels between 4am and 6am in both days suggesting degree of preserved rhythmicity.**

Cosinor analysis

- **Cosinor analysis investigates if the data are better described by a cosine curve than straight line.**
- **Data was analysed with cosinor analysis for the detection of rhythmicity.**
- **In a perfect fit percentage rhythm is 100%.**



Hung Hsuchou et al. J Appl Physiol 2013;115:995-1003

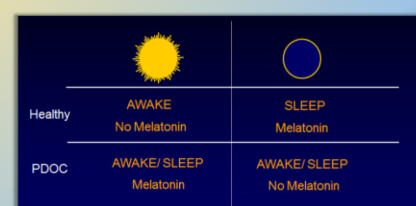
Cosinor Analysis of Melatonin Results

- **Cosinor analysis of saliva melatonin results revealed that the mean % rhythmicity was only 29.5 in PDOC patients.**
- **Range 10% - 66.4%, SD: 16.6**

Saliva Melatonin % Rhythm Results

Age/Gender	Brain Injury Aetiology	Time Since Brain Injury	Diagnosis	Feeding Regime	Melatonin % Rhythm
73/ Male	Stroke	8 years	MCS	16:00-02:00	66.4
66/ Female	Anoxic	6 years	Low MCS	Daytime	51.9
40/ Female	TBI	3 years	MCS	Daytime	46.5
55/Male	Stroke	9 months	MCS	16:00-04:00	40.5
71/ Female	Stroke	5 years	MCS	Daytime	34.6
59/ Female	Anoxic BI	8 years	VS	Daytime	32.9
36/ Male	Anoxic BI	2 years	VS	Daytime	28.2
68/ Male	Stevens- Johnson S.	2 years	VS	16:00-04:00	23.5
53/Male	TBI	7 months	MCS	16:00-04:00	19
52/ Male	SAH	4 years	MCS	16:00-04:00	16.8
43/Female	Stroke	3 years	MCS	18:00-02:00	16.5
58/Male	Anoxic BI	5 years	Low MCS	Daytime	13.5
30/ Female	TBI	5 years	MCS	16:00-02:00	12.9
38/Male	Anoxic BI	4 years	MCS	16:00-02:00	10

Melatonin and Sleep in PDOC



Baseline Study Results

- Our findings suggest that circadian rhythm of patients with PDOC is impaired significantly -as indicated by the low melatonin circadian rhythmicity.
- Both sleep/ wake patterns and sleep structure are abnormal in PDOC. Very few patients had REM sleep and none had Stage 3/4 deep sleep.
- Feeding time appear to have strong influence on maintaining circadian rhythm.

Interventional study participants

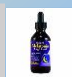
Patient	Age/ Gender	Cause of BI	Diagnosis
Patient-1	71/ Female	ICH	MCS
Patient-2	58/Male	Anoxic BI	low MCS
Patient-3	36/ Male	Anoxic BI	VS
Patient-4	52/ Male	SAH	MCS
Patient-5	30/ Female	TBI	MCS
Patient-6	43/Female	Stroke	MCS
Patient-7	73/ Male	Stroke	MCS
Patient-8	40/ Female	TBI	MCS
Patient-9	68/ Male	CNS Inflammation	VS
Patient-10	66/ Female	Anoxic	MCS

13 weeks for each patient

Week 1	Week 5	Week 6-9	Week 10	Week 11	Week 13
BAEP, EEG Baseline-1 investigations	Baseline-2 Investigations	Intervention	Intervention Post-Intervention Investigations	24 Hour saliva collection for melatonin	24 Hour saliva collection for Melatonin

FIVE WEEKS INTERVENTION

MELATONIN TREATMENT
3 mg Melatonin (1mg/ml) was given via percutaneous enteral gastrostomy (PEG) tube at 6pm.



LIGHT TREATMENT:
2500 lux blue light given between 8am and 9am.

CAFFEINE TREATMENT
Caffeine 100 mg twice a day (at 08:00 and 12:00 hours)

Light

- **Visual and non-visual effects on human physiology.**
  - **resets the timing of circadian pacemaker**
  - **acutely improves subjective and objective measures of alertness**
  - **suppresses pineal melatonin production**
- **Light intensity, duration of exposure to light, wavelength and timing of exposure can have an effect on circadian rhythms.**
- **Blue light in the range of 440-480 nm is highly effective in phase-shifting the human circadian clock.**

Light Treatment

- **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.**

Melatonin Treatment

- **Used in treatment of sleep disorders associated with circadian rhythm disruption.**
- **Melatonin is usually given 1-2 hours before usual bedtime around between 8 and 10pm.**
- **The patients with PDOC 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.**

Melatonin Treatment

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

Caffeine

- **Caffeine is a weak psycho-stimulant**
- **It has circadian effects**
- **It can inhibit melatonin production due to its adenosine receptor antagonist properties**
- **Beneficial in restoring low levels of wakefulness and to counteract deteriorations in task performance related to sleep deprivation**
- **Re-synchronization of circadian rhythm can be achieved quicker when given alongside of melatonin**

Caffeine Treatment

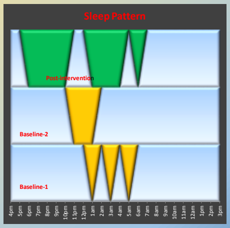
- We aimed to administer caffeine 100 mg twice a day (at 08:00 and 12:00 hours) as part of our intervention due to its stimulant and circadian rhythm effects.
- Caffeine was introduced in gradual manner as PDOC patients are not given any caffeine containing feed or fluids normally.
- Tablets were crushed and dissolved according to usual nursing practice of giving tablets via feeding tube.

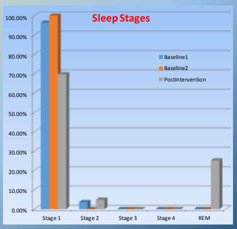
INTERVENTIONAL STUDY RESULTS

- With intervention, improvement of sleep stages and/or sleep-wake patterns were detected in 8/10 patients.
- Increase in %Melatonin Rhythm following intervention was statistically significant (p=0.012).
- There was statistically significant improvement of CRS-R scores with intervention (p=0.034).

Example: Patient-5 (30yo, Female, TBI, MCS)

CRS_Ave_pre:13 CRS_post:18



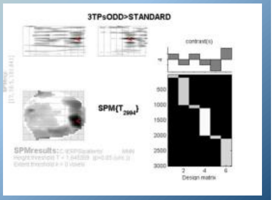


Event Related Potential (ERP) Studies

- ERPs reflect post-synaptic potentials which last up to hundreds of milliseconds and provide covert and continuous measure of neural processing.
- For example; Mismatch negativity (MMN) is regarded as an indicator of pre-attentive sensory memory process. In the recent years several studies examining MMN in PDOC uniformly suggested that MMN seems to have potential value in predicting clinical improvement in patients with chronic PDOC.
- We measured ERP responses using frequency oddball paradigm, auditory roving oddball paradigm and subject's own name paradigm

Some Promising Results on ERPs…

- Data is currently being analysed using Statistical Parametric Mapping (SPM) techniques
- On this example the increase in brain responses to auditory stimuli following intervention was statistically significant; in the correct part of brain; and at the correct time point.
- Similar responses observed in other patients and further detailed analysis is being performed.
- Results will be published once analysis is complete.



Conclusions

1. **Circadian rhythm and sleep of patients with PDOC are severely impaired**
2. **We were able to improve circadian rhythm and sleep with 5 week long Melatonin, Light and Caffeine treatment on patients with PDOC years after their brain injury.**
3. **More importantly, improvement of circadian rhythm and sleep lead to improvement of consciousness as evidenced improvement of CRS-R scores (and possibly with improvement of ERP responses)**

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Next Steps…

- Multicentre study with increased number of patients in earlier stages of PDOC.
- Inclusion of changing feeding time from night to day time as part of intervention.

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Royal Hospital for Neuro-disability

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THANK YOU!

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