



HAL
open science

Is circadian rhythmicity a prerequisite to coma recovery? Circadian recovery concomitant to cognitive improvement in two comatose patients

Florent Gobert, Jacques Luauté, Véronique Raverot, François Cotton,
Frédéric Dailier, Bruno Claustrat, Fabien Perrin, Claude Gronfier

► To cite this version:

Florent Gobert, Jacques Luauté, Véronique Raverot, François Cotton, Frédéric Dailier, et al.. Is circadian rhythmicity a prerequisite to coma recovery? Circadian recovery concomitant to cognitive improvement in two comatose patients. *Journal of Pineal Research*, 2019, 66 (3), pp.e12555. 10.1111/jpi.12555 . hal-04703823

HAL Id: hal-04703823

<https://hal.science/hal-04703823v1>

Submitted on 21 Sep 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Is circadian rhythmicity a prerequisite to coma recovery?

Circadian recovery concomitant to cognitive improvement in two comatose patients

Running title: Circadian recovery and clinical improvement

Florent Gobert, M.D.^{1,2,3}, Jacques Luauté, M.D., Ph.D.^{2,4}, Véronique Raverot, Pharm.D.⁵, François Cotton, M.D., Ph.D.^{6,7}, Frédéric Dailier, M.D.¹, Bruno Claustrat, Ph.D.⁵, Fabien Perrin, Ph.D.*³ and Claude Gronfier, Ph.D.*⁸

¹ Neuro-Intensive care unit, Hospices Civils de Lyon, Neurological hospital Pierre-Wertheimer, 59 Boulevard Pinel, BRON 69677, France

² ImpAct Team (Integrative, Multisensory, Perception, Action & Cognition), Lyon Neuroscience Research Centre (Université Claude Bernard Lyon 1, INSERM U1028, CNRS UMR5292), Bâtiment Inserm 16 avenue Doyen Lépine BRON 69676, France

³ CAP Team (Cognition Auditive et Psychoacoustique), Lyon Neuroscience Research Center (Université Claude Bernard Lyon 1, INSERM U1028, CNRS UMR5292), 50 avenue Tony Garnier, LYON 69366, France

⁴ Neuro-rehabilitation unit, Hospices Civils de Lyon, Neurological hospital Pierre-Wertheimer, 59 Boulevard Pinel, BRON 69677, France

⁵ Hormone Laboratory, Hospices Civils de Lyon, Neurological hospital Pierre-Wertheimer, 59 Boulevard Pinel, BRON 69677, France

⁶ Radiology unit, Lyon-Sud hospital, Hospices Civils de Lyon, 165 Chemin du Grand Revoyet, PIERRE-BENITE 69310, France

⁷ CREATIS-LRMN (CNRS UMR 5220 - INSERM U630), 7 Avenue Jean Capelle O, VILLEURBANNE 69100, France

⁸ Univ Lyon, Universite Claude Bernard Lyon 1, Inserm, Stem Cell and Brain Research Institute
U1208, 69500 Bron, France

Additional Authorship Options:

Fabien Perrin and Claude Gronfier should be considered joint senior author.

Corresponding Author and Lead contact

Florent Gobert

Neuro-Intensive care unit, Hospices Civils de Lyon, Neurological hospital Pierre-Wertheimer,
Lyon, France 1

59, Boulevard Pinel, 69003, Lyon FRANCE

+33 (0)6 78 64 90 66

florent.gobert01@chu-lyon.fr

Title: 121 characters including spaces

Abstract: 260 words

Text: 4293 words for the text + 666 for Figures Titles and Legends

44 references

3 figures

Abstract

Circadian rhythmicity (CR) is involved in the regulation of all integrated functions, from sleep-wake cycle regulation to metabolic function, mood and cognition. However, the interdependence of CR, cognition and consciousness has been poorly addressed.

To clarify the state of CR in coma and to determine the chronological relationship between its recovery and consciousness after brain lesions, we conducted a longitudinal observational study investigating how the state of CR was chronologically related with the recovery of behavioural wakefulness, cognition and/or awareness. Among 16 acute comatose patients, we recruited two 37-year-old patients with a persistent disorder of consciousness, presenting diencephalic lesions caused by severe traumatic brain injuries. Two biological urinary markers of CR were explored every 2-hours during 24h (6-sulfatoxymelatonin, free-cortisol) with a dedicated methodology to extract the endogenous component of rhythmicity (environmental light recording, near-constant-routine protocol, control of beta-blockers). They presented an initial absence of rhythmic secretions, and a recovered CR 7 to 8 months later. This recovery was not associated with the restoration of behavioural wakefulness, but with an improvement of cognition and awareness (up to the minimally conscious state). MRI showed a lesion pattern compatible with the interruption of either the main hypothalamo-sympathic pathway or the accessory habenular pathway.

These results suggest that CR may be a prerequisite for coma recovery with a potential but still unproven favourable effect on brain function of the resorted circadian melatonin secretion and/or the functional recovery of the suprachiasmatic nucleus (SCN). Assessing circadian functions by urinary melatonin should be further explored as a biomarker of cognition reappearance and investigated to prognosticate functional recovery.

Keywords: Circadian Rhythm, Melatonin, Hypothalamus, Coma, Cognition, Biomarkers, Traumatic Brain Injury

Authors' contributions

FG, JL, FP and CG have designed the study.

FG has acquired the data.

FG, JL, VR, FC, FP and CG have analysed and interpreted the data.

JL, FP and CG have supervised the study.

JL, FD, BC, FP and CG have provided critical revision of the manuscript for important intellectual content.

Declaration of Interests

The authors have declared that no conflict of interest exists.

Is circadian rhythmicity a prerequisite to coma recovery?

Circadian recovery concomitant to cognitive improvement in two comatose patients

Abstract

Circadian rhythmicity (CR) is involved in the regulation of all integrated functions, from sleep-wake cycle regulation to metabolic function, mood and cognition. However, the interdependence of CR, cognition and consciousness has been poorly addressed.

To clarify the state of CR in coma and to determine the chronological relationship between its recovery and consciousness after brain lesions, we conducted a longitudinal observational study investigating how the state of CR was chronologically related with the recovery of behavioural wakefulness, cognition and/or awareness. Among 16 acute comatose patients, we recruited two 37-year-old patients with a persistent disorder of consciousness, presenting diencephalic lesions caused by severe traumatic brain injuries. Two biological urinary markers of CR were explored every 2-hours during 24h (6-sulfatoxymelatonin, free-cortisol) with a dedicated methodology to extract the endogenous component of rhythmicity (environmental light recording, near-constant-routine protocol, control of beta-blockers). They presented an initial absence of rhythmic secretions, and a recovered CR 7 to 8 months later. This recovery was not associated with the restoration of behavioural wakefulness, but with an improvement of cognition and awareness (up to the minimally conscious state). MRI showed a lesion pattern compatible with the interruption of either the main hypothalamo-sympathic pathway or the accessory habenular pathway.

These results suggest that CR may be a prerequisite for coma recovery with a potential but still unproven favourable effect on brain function of the resorted circadian melatonin secretion and/or the functional recovery of the suprachiasmatic nucleus (SCN). Assessing circadian

functions by urinary melatonin should be further explored as a biomarker of cognition reappearance and investigated to prognosticate functional recovery.

Keywords: Circadian Rhythm, Melatonin, Hypothalamus, Coma, Cognition, Biomarkers, Traumatic Brain Injury

Abbreviations:

CR: Circadian rhythmicity

SCN: Suprachiasmatic nucleus

TBI: Traumatic Brain Injury

CRS-R: Coma Recovery Scale - Revised

UFC: Urinary Free Cortisol

AMT6S: 6 sulfatoxymelatonin

AUC: Area Under the Curve

Introduction

The central role of circadian rhythmicity (CR) in human physiology and pathology has been a subject of growing interest over the past years, and the attribution of the Nobel Prize in Physiology to circadian biologists in 2017 recently shed light on this field. Circadian rhythmicity is driven by the suprachiasmatic nucleus (SCN)¹ of the hypothalamus. This structure displays a rhythmic activity, conveyed to a large number of brain and extra-cerebral structures through neural and humoral messages². The endogenous, genetically based near-24h rhythmicity of this clock requires daily resetting, in order to maintain synchrony between external and circadian time³, and expression of physiological rhythms at appropriate times.

The pleiotropic clinical consequences of circadian disruption are experienced in social circumstances (jet lag, shift work) or in experimental conditions³⁻⁵. Night work is considered as a risk factor for sleep, cardiovascular, psychiatric diseases and cancer⁶. The precise mechanisms involved are thought to involve circadian disruption, leading to alterations of the gene expression, cell division, hormone secretion and immunity, but also of integrated function such as the sleep/wake cycle, mood, cardiovascular and gastro-intestinal functions^{4,6}. Variations in cognitive performance are controlled by CR, and they are high during daytime (before melatonin onset) and low during nighttime (nadir after the melatonin peak)⁵. Circadian regulation of cognition is thought to counteract the significant loss of performance associated with the increasing sleep pressure occurring during daytime⁷, in order to maintain a stable and high cognitive throughput during wakefulness.

The multifunctional consequences of circadian disruption might also interfere with consciousness, as an overall expression of brain functions. Lesional coma and awakening from coma offer the opportunity to investigate the link between CR and consciousness. Wakefulness, cognition and awareness are supposed to be transiently lost during coma⁸. The restored eyes-opening/eyes-closed alternations defines practically the border⁹ between coma and vegetative

state (VS¹⁰ or unresponsive wakefulness syndrome¹¹). After VS, a cognitive improvement can occur toward the minimally conscious state (MCS)⁸ or Cortically Mediated State¹² an entity characterized by a partial/unstable awareness and cognition, without any functional communication or ability for object use.

The literature is inconclusive as to whether CR exists in coma, and how it evolves in post-comatose patients (VS/MCS). Circadian rhythms in coma have been inconstantly reported¹³. Four-days actimetric recordings have been performed in 55 chronic post-comatose patients¹⁴. The absence of motor cyclicality was significantly more frequently observed for VS than MCS. However, actimetry is a poor indicator of the circadian system after traumatic brain injury as several cofounding factors can appear. In particular, the motor tonus modified by spasticity could be more severely impaired in the VS group. Body temperature rhythmicity was reported in VS and MCS patients, with a link between circadian period (closeness to 24h) and arousal¹⁵. Melatonin were reported to remain low at night during VS¹⁶, however, the study was too short (a single night) to conclude on CR.

In order to clarify the state of circadian rhythmicity in coma and investigate how CR relates with recovery of behavioural wakefulness, cognition and/or awareness in post-comatose patients, we explored two patients suffering from traumatic diencephalic lesions, longitudinally over 7-8 months, from coma or acute VS to late MCS. Two biological urinary markers of circadian rhythmicity were precisely explored (6-sulfatoxymelatonin and free cortisol, every two hours during 24h) in order to assess the origin of circadian arrhythmicity (a disruption of the central clock would abolish both melatonin and cortisol rhythms, whereas the existence of one hormonal rhythm would indicate a functional central clock but a dysfunctional efference to the hormonal pathway showing abolished rhythmicity). Potential biases were excluded or limited thanks to a dedicated methodology to collect data and extract the endogenous

component of rhythmicity (environmental light recording, near-constant routine protocol, continuous management of coma, control of beta-blockers' use).

Methods

Patients

The selection criteria for this study were: i) patient between 18 and 80 years-old; ii) MRI performed during the first month after coma; iii) confirmation of delayed coma recovery > 7 days after sedation (first assessment); iv) persistent disorder of consciousness 6 months after the brain injury (second and/or third assessment).

Biology

The levels of urinary free cortisol (UFC, CMIA technology, Abbott Diagnostics, Illinois, USA) and urinary 6 sulfatoxymelatonin (AMT6S, the main hepatic melatonin metabolite, assessed by RIA¹⁷) were measured over two to three 24h segments over 7-8 months. Samples were taken every 2 hours during each 24h assessment (12 samples), starting between 6:00 PM and 9:00 PM. We used a bladder probe and a per-hour urine quantification, excepted for one assessment (Patient 1, assessment 2: in the absence of a bladder probe, the samples were collected after each spontaneous urination). Results are given in ng/h for AMT6S and nmol/h for UFC.

AMT6S assays were run in duplicate samples by batch. Samples from the same assessment were run all together in a batch, but samples from different observations were run in different batches. The intra-assay CVs were 3.8% and 4.3% at 550 and 3300 ng/L respectively. The inter-assay CVs were 19% and 12% at 2500 and 70000 ng/L respectively. The limit of quantification was found at 125 ng/L.

Assays of UFC were run in single samples on a i2000 instrument (Abbott diagnostics). The intra-assay CVs were 5.7% and 2% at 62.8 and 345 nmol/L respectively. The inter-assay CVs were 4.8%, 3.7% and 3.7% at 125, 451 and 977 nmol/L respectively. Limit of quantification was found at 15 nmol/L.

Urinary instead of plasmatic markers have been previously used as an appropriate markers of CR to diagnose Circadian Rhythm Sleep Wake Disorders¹⁸, to study the CR of blind subjects¹⁹ and to analyse UFC and aMT6S oscillations in elderly subjects with a close 90-min sampling rate²⁰. The common relationship between these markers have been recently modelled and seemed well predictable²¹.

Environment

As it was not possible for ethical reason to hold patients in a dim-light protocol (explaining the term “near-constant routine protocol”), environmental light intensity was recorded for each assessment, i.e. during 24h in each patient’s room (CHY630, IDDM+®) excepted for the third assessment of Patient 1 for technical reasons. The Area Under the Curve (AUC) for day- and night- light exposure was computed using a Python script. The limits for night and days were based on official hours of solar day/night for each recording day. The day/night light ratio of ambient light was compared between assessments and patients. Considering every day and night AUC values together, we tested the hypothesis of a persistent environmental variability in this setting.

Imagery

MRI was systematically performed within the first month after post-traumatic brain injury (TBI) and concomitantly with the following biological assessment. Structural sequences

included systematically a T2-sequence to screen Diffuse Axonal Injury (FLAIR or axial SET2) and a T2-Gradient Echo sequence to look for microbleeds. Sagittal T1 sequences focused on brainstem and hypothalamic lesions were also provided for their anatomical precision.

Statistical analysis

Circadian rhythmicity of melatonin and cortisol concentrations was assessed using a least-square single-harmonic regression model²² (SigmaPlot12, Systat Software Inc) on log-transformed values. In our model, the circadian period was constrained between 23h30 and 24h30. Regressions were considered statistically significant with p-values <0.05.

The overall dynamic of metabolite excretion (AMT6S and UFC) absolute values was compared between each consecutive assessment using one-sided paired t-test. A Bonferroni correction for multiple comparisons was provided, namely $p = 0.05/3$ for Patient 1 (with 3 curves per hormones inducing three possible comparisons) and $p = 0.05/1$ for Patient 2 (with 2 curves per hormones inducing a single comparison). Day/night AUC values were statistically compared using one-tailed t-test.

Study approval

The experiment was conducted in agreement with the guidelines of the Declaration of Helsinki and approved by the local Ethic Committee (CPP Sud-Est II, NCT02742506), including a written informed consent from the patients' relatives.

Results

Population description

Among sixteen comatose patients recruited in ICU and biologically evaluated between January 2014 and October 2017, two patients (37-year-old males) were unable to follow simple

commands 6 months after the brain injury and were therefore eligible for inclusion to analyse longitudinally their circadian rhythmicity. They presented a severe traumatic brain injury (TBI) and an initial Glasgow coma score (GCS) of 3 and 4 respectively and were recorded between April 2014 and April 2015 with a clinical follow-up for two years. The clinical evolution was assessed by the Coma Recovery Scale–Revised (CRS-R)¹², the behavioural gold standard to classify recovery after coma.

Patient 1 was comatose at his first assessment on day 29 post-TBI (19 days after sedation withdrawal) with a GCS = 6 (E1V1M4), no eye-opening period and a CRS-R = 2 (with auditory function: 0, visual function: 0, motor function: 2, verbal function: 0, communication: 0, wakefulness: 0). Two follow-up assessments were carried out at 6 and 8 months post-TBI. Clinical status was then respectively compatible with VS (GCS = 9 – E4V1M4, eye-opening periods, CRS-R = 5) then improved moderately to MCS (GCS = 9 – E4V1M4, CRS-R = 10 with auditory function: 1, visual function: 3*, motor function: 2, verbal function: 2, communication: 0, wakefulness: 2).

Patient 2 was already in VS at his first assessment at day 20 post-TBI (18 days post-sedation) with a GCS = 9 (E4V1M4), eye-opening periods and a CRS-R = 4 (with auditory function: 0, visual function: 0, motor function: 2, verbal function: 0, communication: 0, wakefulness: 2). A single follow-up assessment was performed at 7 months post-TBI, while clinical status was compatible with MCS (GCS = 11 – E4V1M6, CRS-R = 16 with auditory function: 3*, visual function: 4*, motor function: 5*, verbal function: 1, communication: 0, wakefulness: 3).

Concerning the potential pharmacological biases, a first dose of propranolol was administered to Patient 1 in the middle of the first assessment (100 mg at 8:00 AM). Patient 2 was under propranolol during both recordings (first assessment: 40 mg at 6:00 AM; second assessment: 40 mg at 8:00 AM and 6:00 PM).

Concerning the potential metabolic biases, these patients did not display significant variations of biological hepatic and renal parameters across the study.

Concerning the clinical assessments of core body temperature, data were not collected with a high and stable sampling rate across longitudinal assessments. However, based on the analysis of the consecutive 3 days (before, during and after the 5 circadian biological assessments), it appeared that fever was observed for both patients for the ICU assessments during the first month post-TBI (maximal temperature at 38,9°C for Patient 1 and 38,2°C for Patient 2).

Biological description of melatonin-cortisol circadian rhythms (Fig1 and Fig2)

Circadian rhythmicity was not statistically significant in comatose state (Patient 1, assessment 1 in Fig1.A-B) nor in vegetative state (Patient 1, assessment 2 in Fig1.C-D; Patient 2, assessment 1 in Fig2.A-B), for both AMT6S and UFC profiles.

When Patient 1 (Fig1.E-F) and Patient 2 (Fig2.C-D) evolved to MCS, a significant circadian rhythm was observed for AMT6S (sinusoidal regression, $p=0.008$ for Patient 1 and $p=0.001$ for Patient 2) and UFC profiles (sinusoidal regression, $p=0.04$ for Patient 1 and $p=0.005$ for Patient 2).

Absolute values were higher in every initial assessments than the normative values for AMT6S and UFC excretion in young people obtained from the data published by Kripke et al., namely at 728 ± 688 ng/h for aMT6S and 3.3 ± 2.1 nmol/h for UFC²⁰.

For Patient 1, the mean levels of AMT6S decreased significantly between assessments 1 and 2 (2153.75 ± 679.86 vs 796.7 ± 280.88 ng/h, $p<0.0001$) and between assessments 2 and 3 (796.7 ± 280.88 vs 201.88 ± 102.02 ng/h, $p<0.0001$). A non-significant decreasing trend was observed for UFC between assessments 1 and 2 (18.3 ± 7.7 vs 10.1 ± 4.3 , $p = 0.032$). The

difference between assessments 2 and 3 (10.1 ± 4.3 vs 8.2 ± 5.2 nmol/h, $p = 0.011$) and between assessments 1 and 3 were significant ($p = 0.0000113$).

For Patient 2, the mean values of AMT6S was decreasing between assessment 1 and 2 (1186 ± 702 vs 680 ± 633 ng/h) but the difference was not significant ($p = 0.079$). The UFC decrease over time was significant (21.2 ± 12 vs 8.7 ± 13.7 nmol/h, $p = 0.017$).

Neuro-anatomical correlates of CR disruption using longitudinal MRI analysis

For Patient 1, a unilateral hypothalamic microbleed (Fig3.A) and a diffuse axonal injury related to periaqueductal vasogenic oedema (Fig3.B) were observed. A follow-up MRI detected a diffuse cortico-sub-cortical atrophy (Fig3.C).

For Patient 2, a severe diencephalic lesion was observed in T2-Gradient Echo, FLAIR and T1 (Fig3.D-F) in the habenular commissure. The follow-up sagittal T1 imaging shows the atrophy severity (Fig3.F-G) while the habenular microbleed decreases (Fig3.D-H).

Environmental recordings

The mean day/night ratio light exposure AUC was 11.53 (SD=7.5) over the recordings of both subjects. A significant difference in light intensity was found between day and night values (one-tailed t-test=0.023). No significant difference in light exposure was found between patients ($p=0.17$) or between assessments ($p=0.41$ and $p=0.4$ for Patient 1 and Patient 2, respectively). Across all assessments, mean illuminance levels (in lux) were assessed at 211.6 and 34.8 for daytime and nighttime respectively. For Patient 1 and 2 respectively, daytime illuminances were 201,6 (SD=20.4) and 221,6 (SD=95.9) and nighttime illuminances were 22.3 (SD=5.3) and 47.4 (SD=44.4).

Correlated color temperatures were not measured in our study, but they are likely to have been around 5500-6500 K during daytime (due to a combination of solar and artificial light), and around 4000 K during nighttime.

Discussion

Two patients with traumatic diencephalic lesions were explored longitudinally. Initially absent, circadian rhythmicity for the excreted urinary metabolites of melatonin and cortisol recovered 7 to 8 months later. Based on this excretion parameters, we may infer a circadian secretion for both hormones, suggesting that CR disruption after brain lesions can recover over time, concomitantly to a partial clinical recovery of consciousness. Both patients were arrhythmic (as observed after SCN lesions²) during coma and VS (i.e. when behavioural wakefulness reappeared). When Patient 1 partially regained a cognitive content (MCS), we observed a concomitant recovery of melatonin and cortisol circadian profiles, with a slight delay in phase compared to values measured in normal young subjects²⁰, but a normal phase angle between the two rhythms. When Patient 2 reached the same state of consciousness, he showed a normal circadian rhythmicity and phase angle between the two hormones. The recovered phase relationship between melatonin and cortisol, and their normal phases within the 24-hours, indicate that not only was the circadian timing system functional, but also that it was appropriately entrained to the environmental light-dark cycle².

The SCN drives melatonin rhythmicity through two pathways²³: i) the main one reaches the central sympathetic system (at the level of the cervical and superior thoracic spinal cord) via the peri-aqueductal gray matter and the peripheral sympathetic system (via the nervi conari) ; ii) an auxiliary one, through a pathway via the habenular commissure directly to the pineal gland, found in rodents²³ but whose pineal regulatory role has not been explored in humans. Focal hypothalamic lesions have been implicated in case reports of pathological CR assessed

by sleep-wake and temperature recordings²⁴. For Patient 1 of the present study, the widespread lesion of the hypothalamic pacemaker and of the brainstem pathway towards the sympathetic tract (innervating the pineal gland) could explain the abolition of melatonin rhythmicity. For Patient 2, the habenular commissure would be the only candidate to explain a disconnection between the central drive (SCN) and its melatonergic output (the pineal gland), but, as mentioned above, the existence of this pathway is unknown in humans. The concomitant absence and subsequent recovery of cortisol and melatonin rhythmicity argue for a more global circadian disruption, at the level of the SCN, rather than a disconnection of the pineal gland and of the adrenal cortex, although no direct lesion of the SCN was observable in structural images.

Methodological considerations

Physiological variations of behavioural wakefulness are determined by the interaction of homeostatic and circadian drives¹. To demonstrate the dynamics of CR recovery, a strict and highly controlled methodology is fundamental. Such conditions are fulfilled in the present study due to clinical, environmental and therapeutic specificities of coma. Indeed, CR in comatose patients with enteral continuous feeding can be rigorously assessed with biological measures, as it is done in the highly controlled “constant routine protocol” (the gold standard to experimentally unmask circadian rhythmicity²⁵). The absence of constant light levels in our study would not qualify our protocol as a gold standard “constant routine” condition, but the low intensities measured are likely to have limited impacts, if any, in our patients whose eyes were closed most of the time. Despite this favourable clinical condition, no homogeneous literature has risen from ICU recordings of coma to reliably prove the absence of CR after specific lesions. Core body temperature rhythmicity has been used as a marker of CR, but temperature can be masked by fever and antipyretic treatment or controlled hypothermia during invasive management²⁶ and as such is not considered as a precise marker of CR in humans

anymore²⁷. Urinary^{18-20,28-30}, plasmatic²⁷ or salivary²⁸ samples of melatonin and cortisol, considered as gold standard markers of CR, should be more precise to explore the pathophysiological processes in particular because melatonin variability (in plasma) was lower than the one of temperature²⁷. Small ICU cohorts using cortisol³¹ or melatonin¹³ have shown that circadian patterns can be observed in a few cases. However, the insufficient sampling rates, the assessment of a single hormone, and the inclusion of non-severe traumatic brain injury under sedation during the first days after insult limit the strength of such conclusions^{13,31}.

There are limitations in our study. Our sample size is limited to two patients. The existence of pharmacological treatments could interfere with CR. However, the one-shot dose of beta-blocker did not create a spurious rhythm for Patient 1 in comatose state. In addition, CR recovered in spite of an on-going beta-blocker treatment for Patient 2. The rhythmic evolution of melatonin therefore excludes potential beta-blocking effects, as they should have prevented the recovery of melatonin rhythmicity. The absence of constant light levels could also be a limitation of our study, as light levels at night could inhibit nocturnal melatonin secretion¹ and therefore mask its circadian rhythmicity. Moreover, the possibility that comatose patients could present no eye-opening periods during all the nycthemeral recording brings no guarantee of light deprivation as it has been reported previously that light effect can persist through closed eyelids³². However, the protocol describing this effect was far different from physiological conditions and alternative light stimulations, closer to the conditions of the present study, might not systematically induce such an effect³³.

By scrutinizing the evolution of absolute values of AMT6S and UFC across assessments, it appeared that, despite light levels at night could inhibit nocturnal melatonin secretion³⁴ and therefore mask its circadian rhythmicity, AMT6S excretion was not abolished during the initial assessments in ICU. As this excretion was higher (but not rhythmic) during the first assessments for both patients, we exclude that the existence of a light-dark cycle could explain our results,

because of light conditions. Indeed, the stability of light conditions (based on AUC comparisons) did not mask circadian rhythmicity by suppressing AMT6S excretion during coma and VS state and it did not avoid rhythmicity to emerge at follow-up.

Altogether, we believe that CR reappearance associated with the progressive decrease of melatonin secretion (presenting an initial inappropriate increase of its absolute value) indicate a restored central control of the circadian timing system on the pineal gland, and is not related to medication nor light exposure.

Finally, it should be notified that the clinical status was assessed by a single CRS-R score, despite the proposal to run several assessments to avoid misclassification due to clinical fluctuations³⁵. In this previous paper (published after the beginning of the present study), CRS-R results were higher in the morning, but the authors included only chronic post-comatose patients after 2 years of evolution.

Despite these limitations, we propose that CR reappearance indicate a restored central control of the circadian timing system on melatonin and cortisol secretion and is not related to medication nor light conditions.

Circadian mechanisms of recovery

The absence of circadian rhythmicity in coma and VS states could be explained by an alteration of the circadian system *per se*, or by an alteration of the functional connectivity between the circadian clock and its effectors. Studies in mice have shown that circadian rhythmicity can be abolished under constant light exposure. In humans, studies have shown that abnormal light dark cycles (space class missions for astronauts) or bright light exposures at singular times³⁶ can also abolish circadian rhythmicity. In all these cases, circadian rhythmicity normally recovers to normal levels after a while. Although loss of circadian rhythms was related

to brain injury in our patients, it is conceivable that progressive circadian recovery might relate to similar mechanisms.

The progressive decrease of melatonin level is coherent with a recovered inhibition of photic information via the SCN on melatonin secretion during the day²³. However, rodent models of SCN-pineal pathway by selective lesions have suggested that influences of SCN on hormonal secretion were not straightforward³⁷. It could be both excitatory (explaining the increased peak melatonin when SCN-pineal pathway is functional) and inhibitory (explaining the higher mean melatonin level after SCN lesion than the basal daytime secretion, normally reduced by a physiological SCN control). The interdependency of cyclicity restoration and absolute level reduction in these clinical cases could be explained by a dissociated recovery of these divergent pineal control systems.

There could be reciprocal interactions between circadian recovery and cognition/awareness, as SCN inputs and outputs show a complex reciprocal influence on numerous brain areas, including an anatomical connection through the sub-para-ventricular zone to the hypothalamic dorso-medial nucleus. Three SCN projections¹ are important for our purpose: i) to the ventro-lateral pre-optic nucleus to promote sleep; ii) to the orexin secreting neurons of the lateral hypothalamic area to increase wakefulness stability; iii) to the periventricular nucleus to control hormonal release of melatonin and cortisol.

Synaptic plasticity modification by CR and the possible anti-oxidant effect of a rhythmic melatonin have been proposed to explain the negative effects of circadian disruption in pathology. The severity of ischemia in a mice model of cerebral infraction was related to circadian disruption, emphasizing a neuroprotective effect of normal CR during stroke in humans³⁸. Imbalanced inflammatory response was proposed as a possible mechanistic link between altered CR and health² through molecular properties of melatonin (and subsequent cellular messengers) and its rhythmic secretion. Thus, recovery of CR could be indirectly

involved in brain repair, explaining some of the positive effects usually attributed to sleep. The circadian control of cognition could also be related to a long-term potentiation rhythmicity³⁹ (on the cAMP–MAPK–CREB signal transduction pathway) or to the effect of synchronized peripheral clocks in the brain.

Several candidate mechanisms could explain a favourable effect of circadian recovery on clinical improvement, both indirectly thanks to a concomitant sleep recovery^{1,40} and directly by an hormonal effect³ and by the neurophysiological consequences of a resorted SCN function as well. Thus, CR and sleep could have a synergistic effect on brain functions based on an endogenous anti-inflammatory mechanism^{39,41} related to the neuroprotective property of melatonin in particular after neurological insults such as ischemic strokes in human (based on its anti-oxidant properties)^{38,42} or rats models of subarachnoid hemorrhage⁴³, or their combined role on synaptic plasticity and cortical excitability³⁹. Whatever the cause of CR disruption, an improvement of the brain damages might contribute to a virtuous cycle for several physiological brain functions. Thus, such as reticular and thalamus integrity, recovery of circadian functions could be part of the “background conditions for full consciousness”⁴⁴.

Perspectives

A more precise understanding of the underlying mechanisms of CR disruption could be of utmost importance, and lead to modify the ICU environment so as to favour clinical recovery with an adapted level of light, an appropriate light/dark cycle, or with a pharmacological intervention on the suprachiasmatic nucleus (i.e. daily melatonin administration), all aimed at restoring circadian rhythmicity or enhancing its weakened amplitude. First of all, it could help us to precise a major blind spot of the present observational study by precisising whether coma recovery implies or is implied by circadian recovery. It could also help us determine in an original way patients’ sensitivity to their environment, while reactivity is usually only assessed by direct sensorial stimulations. To increase the reliability of this approach, further

observational studies including systematic and repeated CR assessments during coma in ICU and follow-up assessments after discharge are needed to observe the circadian restoration pace and confirm its value as biomarker of clinical recovery. Urinary melatonin recordings could be regarded as an appropriate candidate as it is functionally and anatomically closely related to the circadian pacemaker, and embeds less confounding factors than temperature and cortisol do in ICU settings. We should in particular assess the external validity of this relation between melatonin rhythmicity and clinical improvement for patients presenting no lesions on this specific pathway, but a functional alteration using a hybrid (morphological/functional) imaging. In a prognostic approach, long term follow-up could analyse whether patients recovering CR, either early or lately, or not, have a different functional outcome beyond awareness recovery. Then, the efficacy of enhanced day-night gradient in ICU ambience or other therapeutic approaches at different steps of coma management might be investigated.

Systematic CR assessment may describe another natural history of coma by giving new insights in the biological patterns associated to coma frontiers. In this novel pathophysiological approach, CR could contribute to a positive dynamic on brain function recovery after coma, including its cognitive dimension, and might thus be a prerequisite to coma recovery. A direct positive effect of melatonin and in particular of its restored circadian secretion should be explored specifically in further studies. After having observed the chronological correlation between cognitive evolution and CR recovery, further interventional studies should be designed to look for the direction of causality, if any, between the normalization of circadian functions and awareness.

References

1. Saper CB, Scammell TE, Lu J. Hypothalamic regulation of sleep and circadian rhythms. *Nature*. 2005;437(7063):1257-1263.
2. Roenneberg T, Merrow M. The Circadian Clock and Human Health. *Curr Biol*. 2016;26(10):R432-443.
3. Czeisler CA, Gooley JJ. Sleep and circadian rhythms in humans. *Cold Spring Harb Symp Quant Biol*. 2007;72:579-597.
4. Foster RG, Wulff K. The rhythm of rest and excess. *Nat Rev Neurosci*. 2005;6(5):407-414.
5. Wyatt JK, Ritz-De Cecco A, Czeisler CA, Dijk DJ. Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day. *Am J Physiol*. 1999;277(4 Pt 2):R1152-1163.
6. ANSES Collective expert report: Assessment of the health risks for professionals exposed to atypical working hours, especially night work. 14 rue Pierre et Marie Curie, 94701 Maisons-Alfort Cedex2016.
7. Dijk DJ. Circadian regulation of sleep propensity, sleep structure and alertness: A symphony of paradoxes. *Acta Neuropsychiatr*. 1995;7(2):24-26.
8. Giacino JT, Ashwal S, Childs N, et al. The minimally conscious state: definition and diagnostic criteria. *Neurology*. 2002;58(3):349-353.
9. Landsness E, Bruno MA, Noirhomme Q, et al. Electrophysiological correlates of behavioural changes in vigilance in vegetative state and minimally conscious state. *Brain : a journal of neurology*. 2011;134(Pt 8):2222-2232.
10. PVS TM-STFo. Medical aspects of the persistent vegetative state (1). *The New England journal of medicine*. 1994;330(21):1499-1508.
11. Laureys S, Celesia GG, Cohadon F, et al. Unresponsive wakefulness syndrome: a new name for the vegetative state or apallic syndrome. *BMC Med*. 2010;8:68.
12. Naccache L. Minimally conscious state or cortically mediated state? *Brain*. 2017.
13. Paparrigopoulos T, Melissaki A, Tsekou H, et al. Melatonin secretion after head injury: a pilot study. *Brain Inj*. 2006;20(8):873-878.
14. Cruse D, Thibaut A, Demertzi A, et al. Actigraphy assessments of circadian sleep-wake cycles in the Vegetative and Minimally Conscious States. *BMC Med*. 2013;11:18.
15. Blume C, Lechinger J, Santhi N, et al. Significance of circadian rhythms in severely brain-injured patients: A clue to consciousness? *Neurology*. 2017;88(20):1933-1941.
16. Guaraldi P, Sancisi E, La Morgia C, et al. Nocturnal melatonin regulation in post-traumatic vegetative state: a possible role for melatonin supplementation? *Chronobiol Int*. 2014;31(5):741-745.
17. Harthe C, Claustrat B, Brun J, Chazot G. Direct radioimmunoassay of 6-sulfatoxymelatonin in plasma with use of an iodinated tracer. *Clin Chem*. 1991;37(4):536-539.
18. Auger RR, Burgess HJ, Emens JS, Deriy LV, Thomas SM, Sharkey KM. Clinical Practice Guideline for the Treatment of Intrinsic Circadian Rhythm Sleep-Wake Disorders: Advanced Sleep-Wake Phase Disorder (ASWPD), Delayed Sleep-Wake Phase Disorder (DSWPD), Non-24-Hour Sleep-Wake Rhythm Disorder (N24SWD), and Irregular Sleep-Wake Rhythm Disorder (ISWRD). An Update for 2015: An American Academy of Sleep Medicine Clinical Practice Guideline. *J Clin Sleep Med*. 2015;11(10):1199-1236.
19. Skene DJ, Lockley SW, James K, Arendt J. Correlation between urinary cortisol and 6-sulphatoxymelatonin rhythms in field studies of blind subjects. *Clin Endocrinol (Oxf)*. 1999;50(6):715-719.
20. Kripke DF, Youngstedt SD, Elliott JA, et al. Circadian phase in adults of contrasting ages. *Chronobiol Int*. 2005;22(4):695-709.
21. Abeysuriya RG, Lockley SW, Robinson PA, Postnova S. A unified model of melatonin, 6-sulfatoxymelatonin, and sleep dynamics. *J Pineal Res*. 2018;64(4):e12474.
22. Gronfier C, Wright KP, Jr., Kronauer RE, Czeisler CA. Entrainment of the human circadian pacemaker to longer-than-24-h days. *Proc Natl Acad Sci U S A*. 2007;104(21):9081-9086.
23. Moller M, Baeres FM. The anatomy and innervation of the mammalian pineal gland. *Cell Tissue Res*. 2002;309(1):139-150.

24. Cohen RA, Albers HE. Disruption of human circadian and cognitive regulation following a discrete hypothalamic lesion: a case study. *Neurology*. 1991;41(5):726-729.
25. Duffy JF, Dijk DJ. Getting through to circadian oscillators: why use constant routines? *J Biol Rhythms*. 2002;17(1):4-13.
26. Dauch WA, Bauer S. Circadian rhythms in the body temperatures of intensive care patients with brain lesions. *J Neurol Neurosurg Psychiatry*. 1990;53(4):345-347.
27. Klerman EB, Gershengorn HB, Duffy JF, Kronauer RE. Comparisons of the variability of three markers of the human circadian pacemaker. *J Biol Rhythms*. 2002;17(2):181-193.
28. Deacon SJ, Arendt J. Phase-shifts in melatonin, 6-sulphatoxymelatonin and alertness rhythms after treatment with moderately bright light at night. *Clin Endocrinol (Oxf)*. 1994;40(3):413-420.
29. Lockley SW, Skene DJ, James K, Thapan K, Wright J, Arendt J. Melatonin administration can entrain the free-running circadian system of blind subjects. *J Endocrinol*. 2000;164(1):R1-6.
30. Lockley SW, Dijk DJ, Kostı O, Skene DJ, Arendt J. Alertness, mood and performance rhythm disturbances associated with circadian sleep disorders in the blind. *J Sleep Res*. 2008;17(2):207-216.
31. Savaridas T, Andrews PJ, Harris B. Cortisol dynamics following acute severe brain injury. *Intensive Care Med*. 2004;30(7):1479-1483.
32. Figueiro MG, Rea MS. Preliminary evidence that light through the eyelids can suppress melatonin and phase shift dim light melatonin onset. *BMC Res Notes*. 2012;5:221.
33. Hatonen T, Alila-Johansson A, Mustanoja S, Laakso ML. Suppression of melatonin by 2000-lux light in humans with closed eyelids. *Biol Psychiatry*. 1999;46(6):827-831.
34. Perrin F, Peigneux P, Fuchs S, et al. Nonvisual responses to light exposure in the human brain during the circadian night. *Current biology : CB*. 2004;14(20):1842-1846.
35. Cortese MD, Riganello F, Arcuri F, et al. Coma recovery scale-r: variability in the disorder of consciousness. *BMC Neurol*. 2015;15:186.
36. Jewett ME, Kronauer RE, Czeisler CA. Light-induced suppression of endogenous circadian amplitude in humans. *Nature*. 1991;350(6313):59-62.
37. Papp RS, Palkovits M. Brainstem projections of neurons located in various subdivisions of the dorsolateral hypothalamic area-an anterograde tract-tracing study. *Front Neuroanat*. 2014;8:34.
38. Ritzenthaler T, Lhommeau I, Douillard S, et al. Dynamics of oxidative stress and urinary excretion of melatonin and its metabolites during acute ischemic stroke. *Neurosci Lett*. 2013;544:1-4.
39. Frank MG, Cantera R. Sleep, clocks, and synaptic plasticity. *Trends Neurosci*. 2014;37(9):491-501.
40. Sharma R, Sahota P, Thakkar MM. Melatonin promotes sleep in mice by inhibiting orexin neurons in the perifornical lateral hypothalamus. *J Pineal Res*. 2018:e12498.
41. Xu H, Li H, Woo SL, et al. Myeloid cell-specific disruption of Period1 and Period2 exacerbates diet-induced inflammation and insulin resistance. *J Biol Chem*. 2014;289(23):16374-16388.
42. Ritzenthaler T, Nighoghossian N, Berthiller J, et al. Nocturnal urine melatonin and 6-sulphatoxymelatonin excretion at the acute stage of ischaemic stroke. *J Pineal Res*. 2009;46(3):349-352.
43. Ayer RE, Sugawara T, Chen W, Tong W, Zhang JH. Melatonin decreases mortality following severe subarachnoid hemorrhage. *J Pineal Res*. 2008;44(2):197-204.
44. Koch C, Massimini M, Boly M, Tononi G. Neural correlates of consciousness: progress and problems. *Nature Reviews Neuroscience*. 2016;17(5):307-321.

Figures title and legends

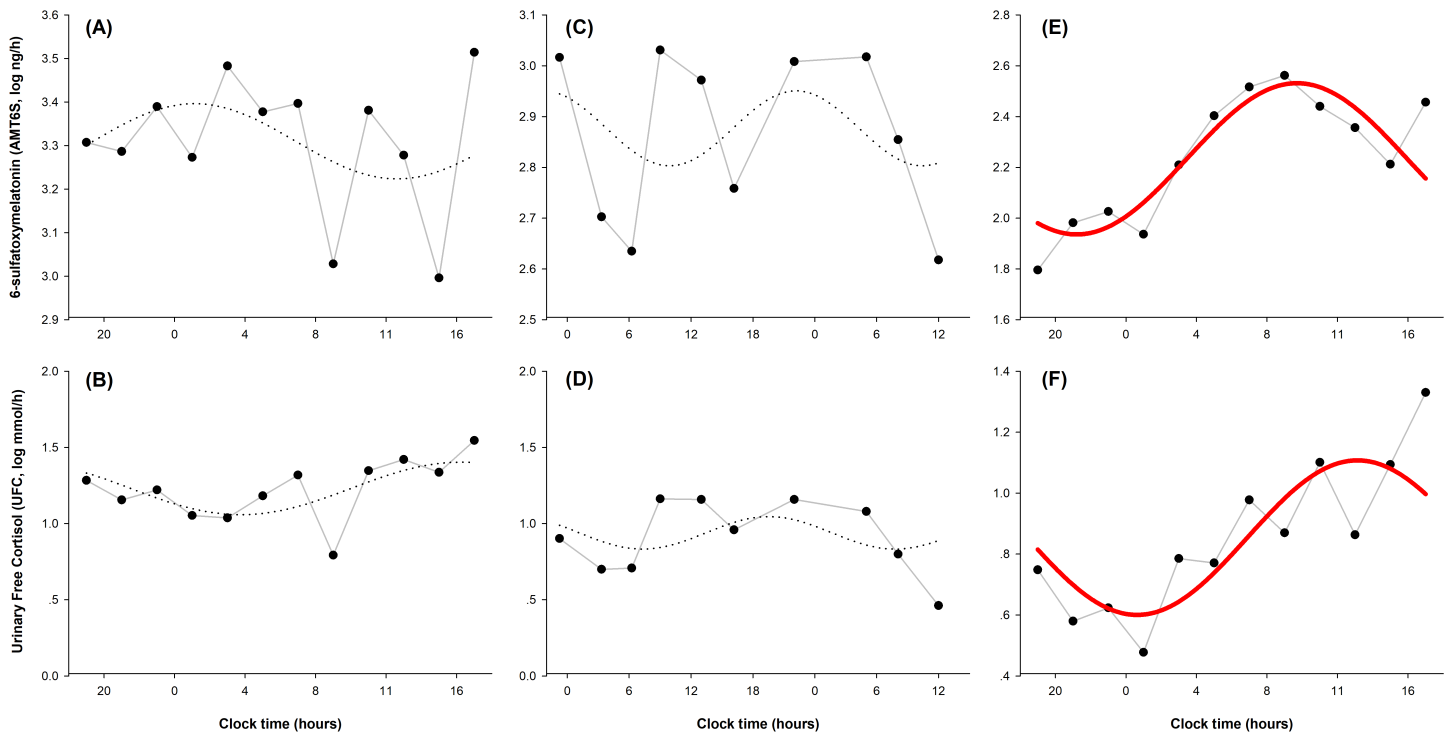


Figure 1: Biological results for Patient 1 presented from left to right according the date of assessment.

X-axis: clock time in hour; Y-axis: hormonal concentrations, log transformed (upper-part: urinary sulfatoxymelatonin; bottom-part: urinary free cortisol). Non-significant sinusoidal fits (*dotted line*) are found when Patient 1 is comatose in the 1st month (A: AMT6S – B: UFC with $p=0.698$ and 0.231 respectively) or in vegetative state in the 6th month (C: AMT6S – D: UFC with $p=0.824$ and 0.148 respectively). At the 8th month, when he is in minimally conscious state, a significant sinusoidal regression is found (*solid line*) for AMT6S (E: $R^2=0.76$, $p=0.008$) and for UFC (F: $R^2=0.61$, $p=0.04$).

The peak of AMT6S was delayed in the morning (at 9:00 AM, ie. after the end of the period previously cited in²⁰ with a mean acrophase of the young population at 4:06 AM, SD = 1:25). The peak of UFC was delayed in the afternoon (1:00 PM, ie. at the end of the period

previously cited in²⁰ with a mean acrophase of the young population at 11:28 AM, SD = 2:09).

The phase angle between cortisol and melatonin (~4 hours) was normal.

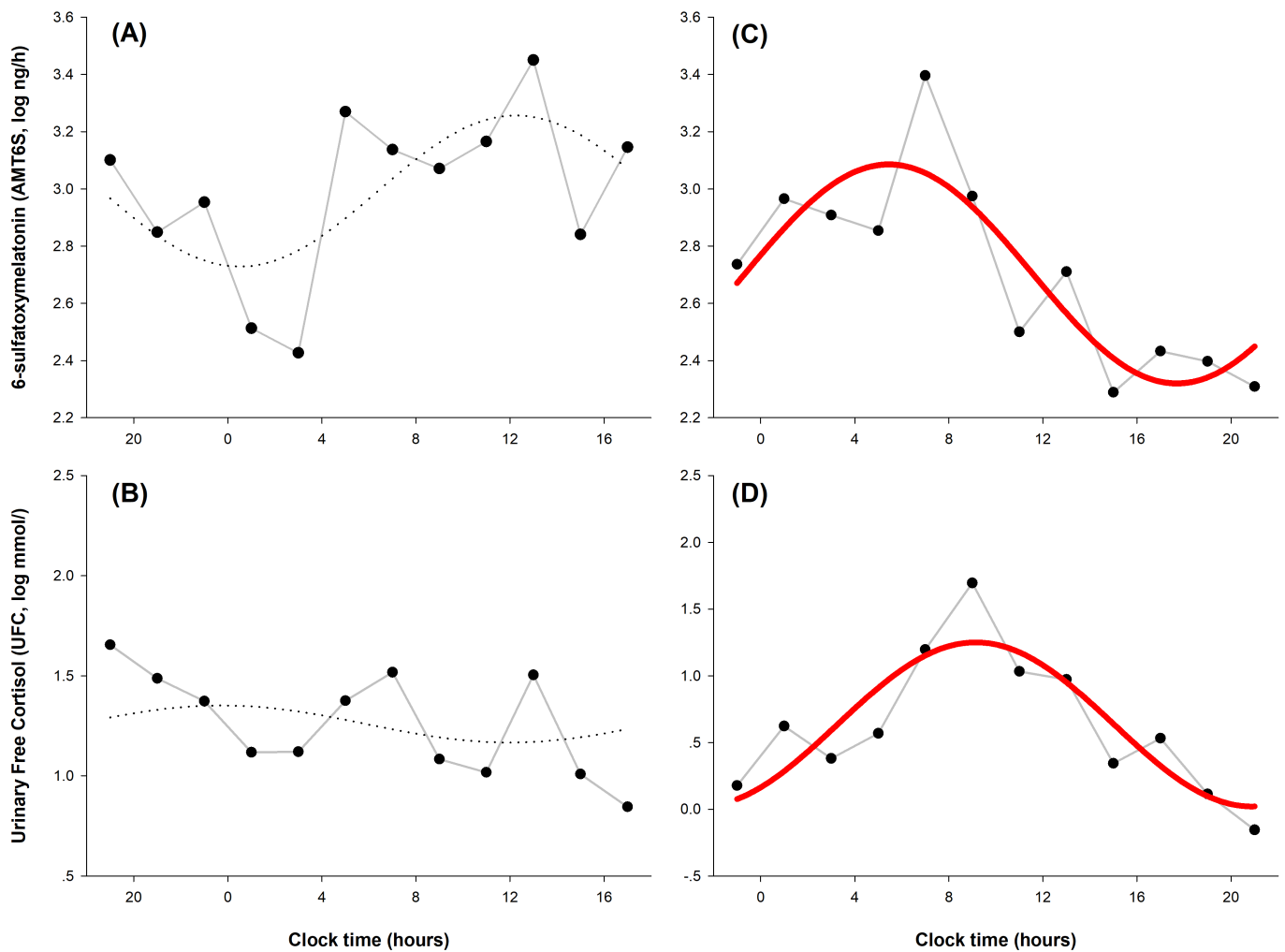


Figure 2: Biological results for Patient 2 presented from left to right according the date of assessment.

X-axis: time in hour; Y-axis: hormonal concentrations, log transformed (upper-part: urinary sulfatoxymelatonin; bottom-part: urinary free cortisol). Non-significant sinusoidal fits (*dotted lines*) are found while Patient 2 is in vegetative state during the 1st month (A: AMT6S – B: UFC with $p=0.191$ and 0.86 respectively). In the 7th month, when Patient 2 is in minimally conscious state, we find a significant sinusoidal regression (*solid line*) for AMT6S (C: $R^2=0.73$, $p=0.001$) and for UFC (D: $R^2=0.78$, $p=0.005$). Although slightly delayed compared to subjects normally entrained to the environmental light-dark cycle, AMT6S was found to peak during the second half of the night (5:00 AM, ie at the end of the period previously cited in²⁰ with a

mean acrophase of the young population at 4:06 A, SD = 1:25), while UFC peaked early in the morning (9:00 AM, ie at the beginning of the period previously cited in²⁰ with a mean acrophase of the young population at 11:28 AM, SD = 2:09). The phase angle between the two rhythms is normal (~4 hours).

Figure 3

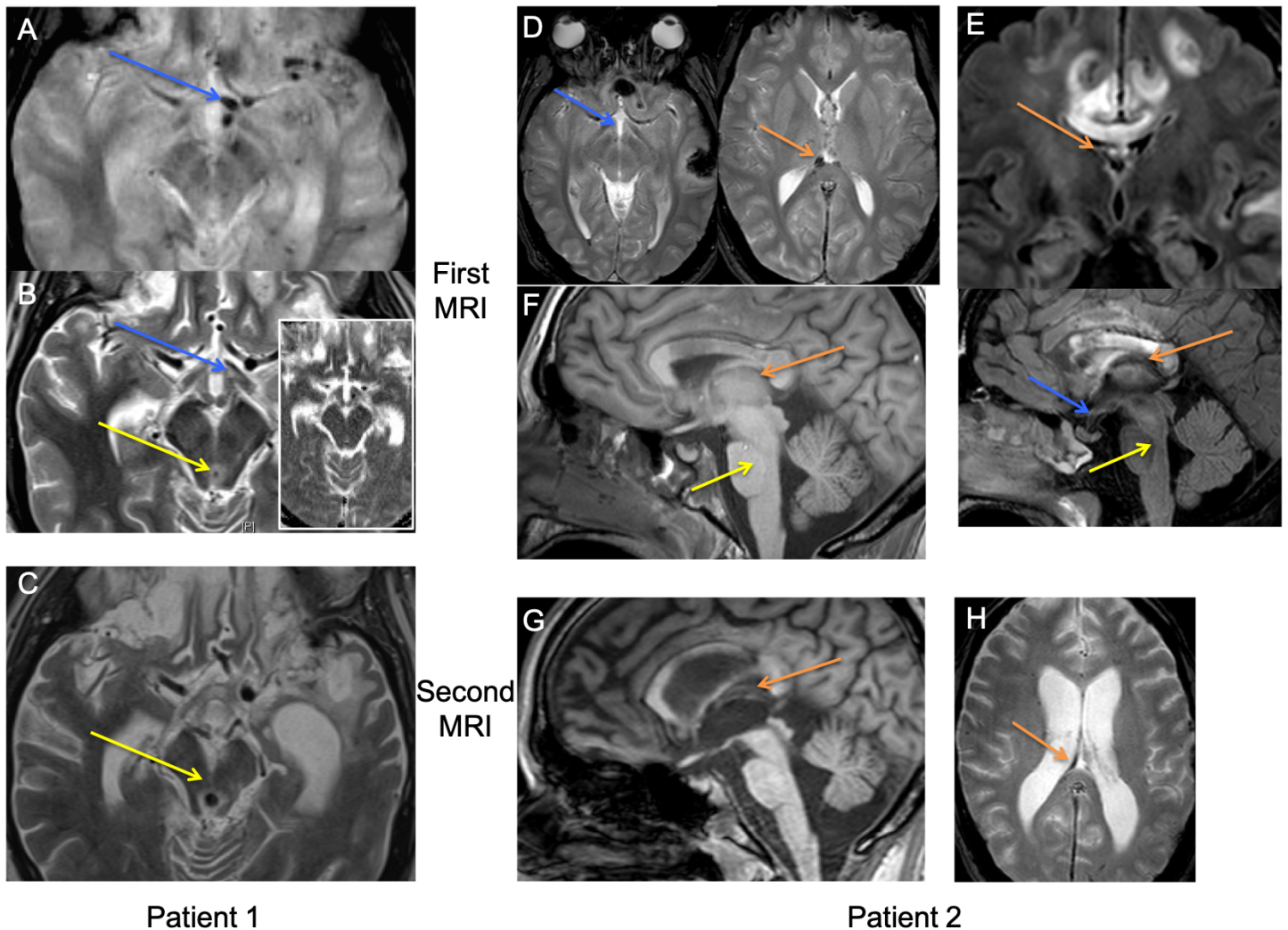


Figure 3: Structural imagery of Patient 1 (left-side) and Patient 2 (right-side) according to their initial (upper-part) or follow-up (bottom-part) MRI assessment (colour code: Blue for hypothalamic structures; yellow for brainstem structures; orange for habenular commissure).

The MRI of Patient 1 was assessed after 30 days and after 8 months post-TBI using a 1.5T Siemens Avanto system (Siemens AG®, Erlangen, Germany).

A: T2-Gradient Echo hypointensity (microbleeds) of the anterior and lateral part of the left hypothalamus (blue arrow). B: Initial hyperintensity on SET2 sequence that could be attributed to vasogenic oedema (in the white insert: no decreased ADC) of the peri-aqueducal

grey matter (yellow arrow) and left antero-lateral hypothalamus (blue arrow) C: Mesencephalic atrophy during MRI longitudinal analysis using SET2 (increased 3rd ventricle diameter, large mesencephalic aqueduct and shrinking of the mesencephalum compared to initial MRI (yellow arrow: peri-aqueducal grey matter).

The MRI of Patient 2 was assessed after 7 days and after 5 months post-TBI using a 3T Philips Achieva system (Philips Healthcare®, Best, The Netherlands).

D: T2-Gradient Echo hypointensity (microbleeds) of right habenular commissure (orange arrow) without visible lesion on the hypothalamus (blue arrow) and on the hypothalamo-spinal tract (yellow arrow). E: FLAIR hyperintensity in coronal cross-section (upper part) and in sagittal cross-section (bottom part) of centro-diencephalic and centro-telencephalic structures, involving bilaterally cingular gyrus, corpus callosum, fornix and habenular commissure (orange arrow: habenula hyperintensity; blue arrow: normal hypothalamus; yellow arrow: normal brainstem). F: Anatomical sagittal cross-section in T1-weighted imaging showing a large hypointensity of the corpus callosum (orange arrow: position of the habenular commissure). G: MRI longitudinal analysis of the sagittal cross-section in T1-weighted imaging (orange arrow: position of the habenular commissure after diffuse diencephalic atrophy). H: MRI longitudinal analysis of the right habenular microbleeds using T2-Gradient Echo (orange arrow).