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DREAM RECALL IN EPILEPTIC PATIENTS WITH PARTIAL AND GENERALIZED SEIZURES

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Keywords: focal and generalized epilepsy, dream experience, frequency of dream recall, cognitive functioning

Study objectives: The frequency of dream recall (DR) has proven, in studies on patients with different types of brain disease [1, 2], to be indicative of the relationships between sleep disturbances and functioning of cognitive processes involved in dreaming. In this study we attempted to ascertain whether DR frequency of patients with focal epilepsy is higher than that of patients with generalized seizures, under the assumption that temporo-occipital cortical areas are involved in dream production.

Methods: The study included 61 epileptic patients (34 males, 27 females), who were consecutively admitted over a 12-month period to the Epilepsy Centre of the University Hospital of Pisa, and received chronic anticonvulsant drugs for at least 1 year. They were all right-handed, without either neurological deficits other than seizures or psychiatric disorders, and without impairment in several tests of global cognitive functioning and specific memory abilities. Forty patients (18 male, 22 female, aged 34.2 ± 12.2 years) were diagnosed as having focal epilepsy, and 21 patients (9 male, 12 female, aged 24.6 ± 6.2 years) as having idiopathic generalized epilepsy with tonic–clonic seizures. Patients with focal epilepsy had EEG abnormalities located in the right temporal lobe (11 patients) and in the left one (29 patients). Brain CT scan showed lesions in 14 out of 40 patients with focal epilepsy, while in no patients with generalized epilepsy. The overall frequency of DR was evaluated using morning diary as a technique of self-observation of dream recall frequency [3]. Each patient was instructed to fill out on his/her sheet at morning for 60 consecutive days whether he/she recalled in a detailed way one or more dream experiences developed during the night. Patients were given no suggestion as to the peculiar characteristics of contents and formal properties of dream experiences, in order to prevent any bias in selecting the mental experiences to be classified as 'real' dreams and, thus, to be scored in dream diary [4]. The patients were also requested to fill out in another sheet before going to bad whether seizure occurred during the day. Two adjusted individual averages, which represented the daily frequency of seizures with respect to success or failure in dream recall, were computed.

Results: No indicator of cognitive functioning significantly differed in the two groups of patients. The ability of DR was ascertained in nearly all patients with either focal or generalized epilepsy. However, the mean frequency of DR over the 60 days was significantly higher in patients with focal epilepsy (33.05 ± 17.94) than in those with generalized epilepsy (14.95 ± 13.99) ($F_{1,59} = 16.168$, $P < 0.001$). This effect did not depend on (i) the side of the epileptic focus, (ii) the presence of a cerebral lesion detectable at a CT scan, and (iii) the occurrence of seizure in the previous day.

Conclusions: The present findings indicate that the ability of recalling dream experiences is maintained in epileptic patients, and that DR is more frequent in patients with partial than generalized seizures. The high DR frequency in patients with partial seizures, regardless of the side of epileptic focus, corroborates the hypothesis of a specific involvement of temporo-occipital cortical areas of both hemispheres in activating the visuo-perceptual and visuo-constructive components of dream experience.

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SURGERY VERSUS NASAL CPAP IN PATIENTS WITH SLEEP DISORDERED BREATHING – COMPARISON OF SUCCESS RATES IN CONSIDERATION OF COMPLIANCE

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Keywords: obstructive sleep apnoea, CPAP, surgery

Objectives: Treatment with nasal continuous positive airway pressure (nCPAP) is proven to be highly successful in eliminating sleep disordered breathing (SDB). Yet compliance is still insufficient in many cases. In contrast palatal surgery for SDB has lower success rates but 100% compliance. The aim of our study was to assess whether responder rates of nCPAP therapy are lowered substantially when objective compliance is taken into account maybe equalising results obtained by palatal surgery.

Methods: We retrospectively reviewed the records of 220 men and 36 women (mean age 51.4 years, mean BMI 29.5 kg m^{-2}, mean RDI 26 h) who were treated from 1996 to 1999 either with nCPAP ($n = 149$) or upper airway surgery ($n = 107$). All the patients were treated with Laser assisted Uvulopalatoplaty or Uvulopalatopharyngoplasty. In 91 patients nasal surgery was combined with palatal surgery. All the patients had a standard polysomnography before and 6 months after treatment. Compliance was measured using the built-in time counter of the nCPAP-devices. The mean RDI per night adjusted for compliance was calculated for each patient.

Results: nCPAP was used during 69 ± 27% of the night reducing mean RDI from 32 h to 7 h during the PSG nights. It raised to 14 h if adjusted for compliance. Surgery reduced mean RDI from 18 h to 14 h. 57% of nCPAP- vs. 34% of the operated patients had at least a 50% reduction of RDI. It was additionally pushed below 20 h in 48 vs. 32% of the cases. If RDI also had to be less than 10 h, responder rates dropped to 32 vs. 30%. Patients with a BMI below 26 kg m^{-2} experienced a responder rate above 50% by surgery which was significantly higher than with nCPAP.

Conclusions: Cure rates of SDB by nCPAP are much lower than published previously when adjusted for compliance. However, they are still superior to those obtained by palatal surgery. Only patients with a low BMI are suitable for a primarily surgical approach.

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COMORBIDITY, GENDER AND AGE AT ONSET OF FIRST SYMPTOMS IN NARCOLEPTIC PATIENTS IN GERMANY

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Keywords: narcolepsy, comorbidity, onset of first symptoms, narcolepsy register, gender

Introduction: For the understanding of aetiology and natural history of narcolepsy it is helpful to assess comorbidity and to define the onset of first symptoms. A study of hospital records from 1980 to 2001 performed to prepare a German narcolepsy register emphasized the importance of these items.

Methods: Records of 106 randomly chosen narcoleptic patients of the Hephata Klinik, all presenting cataplexies and excessive daytime sleepiness plus HLA DR 2 positive typing, using data from the most recent hospital admission were analysed. Missing data was taken from prior admissions or filled up with information from Narcolepsy Questionnaires. Patients: 46 women age 40.2 ± 13.2 years, BMI 29.1 ± 5.9 kg m^{-2}, 60 men age 35.5 ± 15.0 years, BMI 28.4 ± 4.0 kg m^{-2}.

Results: Most frequent comorbid diseases are parasomnias, headache and migraine, depression, dysthymia, and obesity. Excessive daytime sleepiness (EDS), cataplexy and sleep attacks each are present in 96–98%, the narcoleptic tetrade in 44.9% of the patients. First essential symptoms of narcolepsy evolved mainly between the ages 10–20, 30–45, in a few between 50 and 55. The distribution of age at onset of first symptoms also shows two peaks. In patients with early manifestation the latency between onset of EDS and cataplexy was much longer than in those with late manifestation (8.8 vs. 3.1 years). Since the chance of narcolepsy to be diagnosed increases significantly after the onset of cataplexies the latency between manifestation of first symptoms and diagnosis is much longer in the group with early manifestation (19.4 vs. 7.9 years, $P < 0.0001$). In women first symptoms started earlier than in men (22 vs. 24 years, not significant), cataplexy started earlier than in men during first manifestation period, and less women had EDS in the second manifestation period.

Conclusions: Comorbidity with parasomnias is more frequent than described before and indicates a motor disorder beyond REM-sleep. Other comorbid diseases do not seem to be more frequent than described in literature. The bimodal distribution of first symptoms fits recent findings [1, 2]. Our results may implicate the existence of two different types of narcolepsy, depending on the age of manifestation of first symptoms.

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DREAMING AND MEMORY FUNCTIONING DURING SLEEP IN PATIENTS WITH TEMPORAL LOBE EPILEPSY

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Keywords: temporal lobe epilepsy, dream experience, REM and NREM sleep, memory functioning

Study objectives: Dream experiences elaborated in REM and NREM sleep by patients with temporal lobe epilepsy (TLE) were examined in this study. Sleep alterations, often associated to TLE, may concur to memory impairments exhibited by these patients, under the assumption that disturbed or insufficient sleep has negative influence on memory functioning [1]. According to the recent view of memory consolidation [2], dream experience may be seen as one of the outcomes of the reprocessing of new information during sleep. Therefore, the characteristics of dream experiences should provide pertinent indication to understand the functioning of memory processes involved in dreaming and supposedly in consolidation of new information. By examining the frequency and structural organization of REM- and NREM-dream experiences on a sample of right- and left- (R- and L-) TLE patients without apparent cognitive deficits, we attempted to estimate the basic characteristics of dream experiences and cast some light on memory functioning during REM and NREM sleep.

Methods: Twelve patients (six with R-TLE and six with L-TLE) were included in the study. All patients (four males, eight females) were receiving chronic pharmacological treatment and were cognitively unimpaired, as assessed by several tests of global cognitive functioning and specific memory abilities. Sleep was recorded for three consecutive nights. The first night was intended for adaptation to laboratory condition, the second (baseline) one for evaluation of sleep organization, the third (experimental) one for dream collection. In the last night, sleep was interrupted after 5 min of continuous sleep during each period of REM sleep and stage-2 NREM sleep subsequent to the first REM period. Upon each awakening, patients were asked to report the interrupted dream experience. After reporting, they were asked to go back to sleep. This procedure was repeated until the definitive awakening in the morning. Only contentful reports (i.e. with at least one sentence describing contents of dream experience) were taken into account for evaluation of the frequency of dream recall. Dream reports were then analysed using a story grammar adapted for Italian, which allows to identify the story-likeness of dreams in terms of contextual and hierarchical organization, and sequential development (or report length) [3].

Results: R-and L-TLE patients did not differ with respect to any sleep parameter or indicator of waking cognitive functioning and provided fully comparable frequencies of dream recall. Instead dream recall frequencies were significantly lower in NREM than REM sleep (being, respectively, 31.94 and 83.87%: $F_{1,10} = 44.113$, $P < 0.001$). Dream recall frequency of TLE patients was substantially lower for NREM sleep compared to that of healthy individuals (about 50%, as reported in the literature, 4), while was very similar for REM sleep (about 80% in both cases). The overall structural organization of dream experiences did not significantly differ either with respect the type of sleep and the side of epileptic focus.

Conclusions: The capacity of dreaming appears to persist in TLE patients regardless of the side of epileptic focus, but with a greater between-stage variation than in healthy individuals. This finding indicates that memory processes involved in dreaming are much less effective during NREM sleep not only compared to REM sleep of TLE patients, but also to NREM sleep of healthy individuals. This indication seems reliable, as sleep parameters in the baseline night were in keeping with the normalization and stabilization of sleep architecture by long-term antiepileptic medication. The lower effectiveness of NREM sleep may depend on difficulty of consolidation during sleep or retrieval of dream contents after awakening. The former possibility is more coherent with the general hypothesis of a relationship between the impairment of memory functioning in waking and that observed during sleep. Pertinent data to distinguish in favour of either hypothesis may be gathered by applying well-established behavioural paradigms on samples of TLE patients stratified by severity of disease and degree of sleep disturbances.

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BRAIN TOPOGRAPHY AND THE NEURONAL TRANSITION PROBABILITY MODEL

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Keywords: brain topography, modelling NREM structure, global sleep, spectral power time-courses

The very characteristic overall structure of the early NREM episode, as seen in its spectral power evolution, has been reported by a number of groups including ourselves. In 1997 we conceived the neuronal transition probability (NTP) model [1, 2], the only one that provides a mechanism by which the systematic peaking of sigma power before delta power, the S-shape of the typical delta power rise and the mirror image relation between delta and beta, can be physiologically explained. The model is simple, coherent and mathematically formulated, with basic tenets that are firmly anchored in neuronal oscillation measurements. The probability parameters are physiologically meaningful, summarising essential characteristics of the NREM episode (rate of going towards and away from deep sleep). Inherent in the model is a fixed-size source population of neurones that generates the spectral time-courses and dictates them to the thalamus and cortex. This implies the global propagation of a spectral-power-evolution template valid at all sites downstream from the brainstem, including all sites on the scalp. The aim of the present study is to lend support to this implication and thus to the model itself, by confirming that there is a high correlation between corresponding EEG frequency-band results, when measured at different topographic sites.

Methods: This study is based on data obtained from 10 healthy volunteers aged between 20 and 30 years. All night sleep EEG was recorded using three bipolar derivations (F4–Cz, C4–T4 and Pz–O2), one horizontal EOG and one submental EMG. For each subject, the three EEG signals were digitized at a 256 Hz-sampling rate and power spectra computed by fast Fourier transform for consecutive 4-s epochs over a frequency range 0.5–35 Hz. This range was then divided into four bands: delta, sigma, beta1 and beta2. The time series data for each frequency band at the three different sites were log-transformed and cross-correlation coefficients calculated using the combined data from all subjects.

	DELTA (0.5–4.0 HZ)			*SIGMA (11.0–15.0 HZ)*			*BETA1 (15.0–18.0 HZ)*			*BETA2 (18.0–35.0 HZ)*		
	F4–Cz	*C4–T4*	*Pz–O2*	*F4–Cz*	*C4–T4*	*Pz–O2*	*F4–Cz*	*C4–T4*	*Pz–O2*	*F4–Cz*	*C4–T4*	*Pz–O2*
F4–Cz	1.00			1.00			1.00			1.00		
C4–T4	0.92	1.00		0.87	1.00		0.85	1.00		0.83	1.00	
Pz–O2	0.89	0.94	1.00	0.66	0.81	1.00	0.73	0.82	1.00	0.69	0.83	1.00

Results: In all three frequency bands and for all subjects, a marked similarity was observed in the temporal evolution of the power curves at the frontal, central and parietal sites, exemplified by a perfect temporal coincidence in the occurrence of the main power peaks and troughs. This was objectively confirmed by the generally very high and statistically very significant (Bonferroni $P < 0.0001$) cross-correlation coefficients, illustrated in the table for the first NREM episode. Correlation coefficients of similar level and statistical significance were obtained for NREM episodes 2–4. These results are obtained despite the observed differences in EEG power at the different sites.

Conclusions: The very high correlations between measurements at different topographical sites provide convincing support for the validity of the NTP model in its implication that a single neuronal source population generates the observed NREM power time courses in all frequency bands of the EEG. In our view, the NTP model mechanism provides global control of the shape and timing of the observed spectral evolutions, while the thalamus 'cortex' interaction determines the detailed wave forms and the site-dependent and frequency-dependent signal amplification that results in the EEG seen at different regions of the scalp. Thus, differences in power at different localities would simply reflect variation in transmission amplification.

Acknowledgement: Supported by the Swiss National Science Foundation grant 3100-050765.97.

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THE AROUSAL SYSTEM AND ITS RELATION TO THE OVERALL STRUCTURE OF NREM

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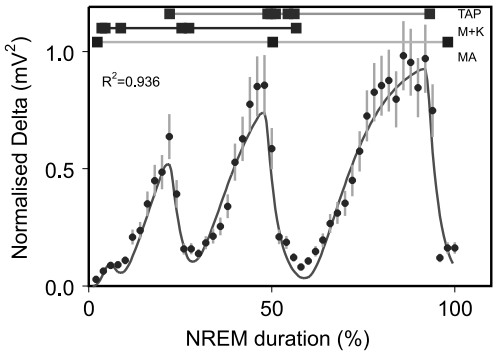
Keywords: arousals, NREM structure, neuronal transition probability model, sleep–wake switch

In a recent study [1] we reported that over the early non-rapid eye movement (NREM) episode, getting to deep slow-wave sleep and returning to rapid eye movement (REM) sleep is by no means as straightforward as presented in the literature where it is often described as a single excursion to deep sleep and back. This is an oversimplification resulting from the common use of averaged data that conceal an important reality: the episode in fact systematically exhibits repeated alternations towards and away from deep sleep. Our neuronal transition probability (NTP) model [2] accurately reproduces the shape of these alternations, in simple neurophysiological terms. Inherent in the model is a fixed-size neuronal population whose statistical tendency to move towards or away from deep sleep is toggled by an external switch. The population generates the observed spectral time-courses and dictates them to the thalamus and thence to the cortex. We hypothesized that this repeated switching between sleep deepening and sleep lightening within the NREM episode is provided by the arousal system in interaction with the sleep-promoting neurones. This interaction would then play a decisive triggering role throughout the NREM episode and not just at sleep onset and wake onset as implied by Gallopin *et al.* [3] The aim of the present study is to examine this hypothesis.

Methods: First the NREM episode duration for each subject was normalized to 100% and average power calculated for each 2% time bin for the delta (0.5–4.0 Hz), sigma (11.0–15.0 Hz), and beta (15.0–30.0 Hz) bands. The NTP model was then fitted to the data for each NREM episode in turn, using the coefficient of determination r^2 as criterion for goodness of fit, and graphed. Using the current definitions for the various types of EEG arousal events [4, 5], the times of occurrence of each type of arousal event were then displayed on the same graph. The relation between the timing of these events and the detailed structure of the NREM episode were examined in 20 healthy subjects. By observing the time-course comportment in the vicinity of each event, we distinguished which of these events was associated with

the dominance of the sleep-promoting neurones (leading to sleep deepening) and which with the dominance of the wake-promoting neurones (leading to sleep lightening).

Results: Preliminary results clearly suggest that during NREM sleep Transitory Activation Phases (TAPs) together with ASDA defined microarousals, appear to trigger shifts away from deep sleep (illustrated in the figure for one typical subject) while K-bursts and delta-bursts, which occur systematically on the rising limb of the delta power curve, appear to encourage shifts towards deep sleep.



Conclusions: These results lend further support to the validity of our NTP model for the global control of the evolution of sleep, and in particular to our hypothesis that the interaction between the sleep promoting VLPO neuronal population and the wake-promoting systems in the brainstem, provides the sleep–wake switch that is an integral part of the model. This switch toggles repeatedly during the NREM episode, and gives rise to its systematic oscillatory structure.

Acknowledgement: Supported by the Swiss National Science Foundation grant 3100-050765.97.

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DREAM RECALL, REMS AND SPECTRAL EEG COMPONENTS IN BLIND AND SIGHTED

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Keywords: dream recall, REMs, EEG frequency bands, blind

Objectives: To evaluate the EEG spectral content and rapid eye movements (REMs) associated with dream recall in sighted subjects and congenital blind.

Methods: 10 congenital blind (CB) subjects (age: 28.2 ± 5.2; five males and five females) were studied and compared with a control sighted group (CS) (age: 28.2 ± 5.5; three males and five females). In all subjects medical or psychiatric diseases were excluded. During two consecutive nights polysomnographic recordings were performed at subjects home; both groups were subjected to periodic awakenings (period = 90 min) and requested to dictate any dream recall to a voice activated tape recorder. For this study, only REM sleep awakenings were selected, whenever they were preceded by a stable REM epoch of at least 5 min duration. Power spectra were obtained for C4-A1 and O2-A1 EEG channels, by means of the Fast Fourier Transform and values from conventional EEG bands were selected; REMs were detected by visual inspection on both EOG channels (EOG-H, EOG-V) and further classified as occurring isolated or in bursts. Dream recall was defined by the existence of a dream report. The two groups were compared using *t*-test and also the two-way ANOVA and a posthoc Fisher test (for the features diagnosis (blind vs. sighted) and dream recall (yes or no) as a function of time).

Results: REM awakenings were obtained in 10 CB and six CS subjects. The average of REM awakenings per subject and the recall ability were identical in both groups (REM awakenings: 1.6 for CB and 1.7 for CS; 60% of recall in both groups). CB had a lower REM density than CS (CB: 3.7 ± 3.4 vs. CS: 17. 4) ± 8.5; $t = -5.622$; $P < 0.0001$); the same applied to REM bursts ($P = 0.0001$) and isolated eye movements ($P = 0.0015$). In the two-way ANOVA, REM bursts and REM density were significantly different for positive dream recall ($P = 0.0125$ and 0.0082, respectively), mainly for the CB group and for diagnosis ($P = 0.001$ and 0.0002, respectively); furthermore for both features significant results were obtained for the interaction of time, recall and diagnosis ($P = 0.0139$ and 0.0191, respectively); the interaction of recall and time was however, stronger ($P = 0.0089$ and 0.0074).

Spectral EEG bands: Higher delta power in O2 was associated with no dream recall ($P = 0.0279$), whereas higher sigma was associated with the presence of dream recall both in C4 and O2 ($P = 0.0092$ and 0.0012, respectively). Furthermore, in C4 the differences obtained concerned mainly the two diagnostic groups with CB having higher values of delta ($P = 0.0268$), theta ($P = 0.0083$) and sigma activity ($P = 0.025$) and CS higher values of alpha ($P = 0.0014$) and beta activity ($P = 0.0072$).

Conclusions: In line with previous findings the data show that blind have lower REMs density. Both groups have also differences in the C4 spectral EEG components of REM preawakening epochs: CB showed an increase in the lower frequency bands (delta and theta) and in sigma activity (spindles) and CS have higher fast activities (alpha and beta). However the ability of dream recall in Congenital blind and Sighted controls is identical. In both groups dream recall is associated with an increase in REM bursts and density, higher sigma activity in C4 and O2 and lower delta activity in O2. REM bursts also show differences in the temporal profile. REM dream recall is associated with changes in EEG frequencies and increased REMs activity.

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THE ROLE OF DORSO-MEDIAL AMYGDALA IN THE REGULATION OF SLEEP–WAKEFULNESS CYCLE

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Keywords: dorso-medial amygdala, sleep–wakefulness cycle, paradoxical sleep

Objectives: Sleep and wakefulness (W) are considered as different motivated behaviours of the organism. Therefore sleep–wakefulness cycle (SWC) could be viewed as dynamic process within the various stages of emotional tension [1]. According to the fact that dorso-medial

amygdala is in a close relation with the motivational-emotional processes in W [2], it is of special interest to study the participation of this brain structure in SWC and, especially, in paradoxical sleep (PS) regulation. The purpose of the present research is analysis of the influence of bilateral dorso-medial amygdala lesion on the SWC structure in general, and development of motivational-emotional processes within different SWC phases, in particular.

Methods: Adult cats ($n = 4$) were chronically implanted with metal electrodes for both the SWC polygraphic registration (EEG, EMG, EOG and ECG) and bilateral lesion of dorso-medial amygdala by anodic electrolysis (parameters: 5 mA, 30 s). The animals were observed for 3–4 weeks before the dorso-medial amygdala lesion, and during 1 month – following the lesion. Alterations registered in the SWC and behaviour, following the dorso-medial amygdala lesion, were compared with the respective baseline indices. Data were treated statistically by Student's *t*-criterion. Morphological control of lesion was made.

Results: The neurophysiological analysis has shown that dorso-medial amygdala lesion causes: an increase of 'passive' W, decrease of slow wave sleep (SWS), followed by its fast recovery and PS elimination during first period (5–7 days after lesion). In the recovery period increasing of SWS is not accompanied by enhancement of PS duration. Even after the recovery of SWC phases ratio, the following parameters are still altered: decrease of motivational–emotional level, reduction of 'active' W, increase of 'non-emotional' PS stages onset (on expense of 'emotional PS stages' reduction), decrease of theta-rhythm amplitude and increase of delta-rhythm amplitude, decrease of the quantity of rapid eye movements, deceleration of the heart-rate during PS and increase of EEG-awakenings from PS.

Discussion: Following dorso-medial amygdala lesion disturbance of brain mechanisms is observed, which take part in 'active' W and PS formation [3]. These alterations are long-lasting events and their restoration occurs slowly that hampers PS rebound even in the time course of significant accumulation of the PS need. Total PS elimination in the first postlesion period and its development without rebound during recovery period, indicates the specific facilitatory effect of dorso-medial amygdala on the brain mechanisms responsible for 'active' W and PS regulation.

Conclusion: Dorso-medial amygdala is significant in modulation and organization of 'active' wakefulness and paradoxical sleep.

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HORMONAL RESPONSE TO A MELATONIN INDUCED SHIFT IN SLEEP TIMING

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Keywords: melatonin, hormones, circadian

Objectives: Appropriately timed exogenous melatonin can shift the timing of endogenous melatonin, core body temperature and sleep. To what extent it influences other aspects of the circadian and endocrine systems remains unclear. Further investigation of human endocrine responses to melatonin is indicated for reasons of safety. We investigated the effects of melatonin administration for 8 days on endogenous melatonin, core body temperature, plasma cortisol, pituitary and reproductive hormones. Melatonin was administered daily at the onset of a 16-h sleep opportunity in dim light (< 5 lux).

Methods: Eight healthy male subjects aged 24.4 ± 4.4 years (SE) completed two 14-day study periods. The study was carried out in a light proof, sound attenuated, temperature and humidity controlled facility. Prior to the study subjects were required to abstain from caffeine for 7 days and maintain a regular sleep–wake cycle for 10 days as verified by actigraphy (sleep 23:00 h–07:00 h ± 30 min). On arrival in the unit subjects were allowed a baseline sleep opportunity (23:00 h–07:15 h). This was followed by a 29-h constant routine in < 5 lux with hourly blood samples and performance tests. A 9-day extended sleep protocol was then imposed with a sleep opportunity 16:00 h–08:00 h < 5 lux and wake 08:00 h–16:00 h in 300 lux. Melatonin (1.5 mg, surge-sustained release, Penn Pharmaceuticals Ltd.) or placebo was administered orally in a randomized, double-blind, cross-over design at 16:00 h for the first 8 days. During the sleep opportunity subjects were required to remain in bed without access to recreational material such as books, television or radio. On day 9 all subjects received placebo at 16:00 h before the final extended sleep opportunity. This was followed by a second constant routine and a 16-h recovery sleep opportunity. Polysomnography (PSG) was recorded during all sleep opportunities and constant routines. Core body temperature and actigraphy/light were measured throughout. Blood samples collected during the first constant routine were assayed for melatonin and cortisol, whilst samples collected during the second constant routine were assayed for melatonin, cortisol, TSH, prolactin, growth hormone, FSH, LH and testosterone.

Results: Phase advances after melatonin administration were: core body temperature minimum 4.72 ± 01.00 h (SE), melatonin onset (midrange crossing method) 5.04 ± 00.33 h (SE), cortisol onset (midrange crossing method) 4.26 ± 00.65 h (SE). Phase advances after placebo condition were: core body temperature minimum 1.55 ± 00.63 h (SE), melatonin onset (midrange crossing method) 2.15 ± 00.67 h (SE), cortisol onset (midrange crossing method) 1.74 ± 00.66 h (SE). All phase advances were significantly greater after melatonin than after placebo ($P < 0.05$) In the data of the five subjects analysed to date there was no significant difference in the mean hormone concentrations between the melatonin and placebo treatments for TSH, FSH, LH, prolactin, growth hormone and testosterone.

Conclusion: Melatonin induces robust phase advances of similar magnitude in rhythms with a major circadian component. There were no significant effects on reproductive hormones, GH and PRL. These preliminary data suggest that substantial changes in circadian timing can be induced by short-term melatonin administration without compromising anterior pituitary function.

Acknowledgement: This work was supported by Medical Research Council/Ministry of Defence Grant G9810584 and Stockgrand Ltd (University of Surrey).

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HOW TO MAKE THE DIAGNOSIS OF NARCOLEPSY: OLD (MSLT < HLA) AND NEW (CSF HYPOCRETIN-1) TOOLS

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Keywords: hypocretins, orexin, HLA, narcolepsy, cataplexy, MSLT

Objectives: Narcolepsy is characterized by sleepiness and abnormal REM sleep. The most specific symptom is cataplexy, a sudden loss of muscle tone triggered by emotions. Diagnostic procedures for the syndrome include clinical confirmation of cataplexy, HLA typing and sleep recording studies, most notably the Mutliple Sleep Latency Test (MSLT). We analysed cerebrospinal fluid (CSF) hypocretin-1 (orexin-A) and HLA typing in various sleep disorders to estimate the diagnostic value of low CSF hypocretin-1 for narcolepsy.

Methods: Clinical data, HLA typing, MSLT and CSF hypocretin-1 (direct assay and after extraction) were studied in 275 patients with various sleep disorders (Narcolepsy, Idopathic Hypersomnia, Obstructive Sleep Apnoea Syndrome, Restless Leg Syndrome and Insomnia). Atypical hypersomnia cases such as familial cases, narcolepsy without cataplexy, narcolepsy without DQB1*0602, recurrent hypersomnias and symptomatic cases (e.g. Parkinson's disease, depression, Prader–Willi syndrome, Niemann × Pick Type C and others) were also included, together with 295 controls (67 healthy and 228 with various neurological disorders). Signal detection analysis was used to determine CSF hypocretin-1 levels best predictive for narcolepsy.

Results: DQB1*0602 frequency was increased in narcolepsy with typical cataplexy (93 vs. 17% in controls), narcolepsy without cataplexy (56%) and idiopathic hypersomnia (52%). Low hypocretin-1-values (direct assay = 110 pg mL^{-1}) were diagnostic for narcolepsy. Values above 200 pg mL^{-1} were considered in the normal range for healthy subjects. Almost all patients with low hypocretin-1 levels were HLA-DQB1*0602 positive subjects with narcolepsy–cataplexy. These subjects had moderately increased Body Mass Index, normal CSF leptin levels but did not always have abnormal MSLTs. Rare narcolepsy subjects without cataplexy, without DQB1*0602 and/or with secondary narcolepsy had low levels. Ten subjects with hypersomnia had intermediate levels, seven of whom were narcolepsy cases (often HLA negative, of secondary nature and/or with atypical or no cataplexy), and one of whom was a periodic hypersomnia case (one of three total, during an episode). Normal controls and subjects with sleep disorders other than hypersomnia/narcolepsy were all in the normal range. Subjects with neurological disorders very rarely had low levels (one comatose subject with myxoedema and three subjects with Guillain–Barre syndrome). More frequently ($n = 30$) intermediate levels were observed with various acute pathologies such as head trauma and encephalitis, though most subjects ($n = 194$) with neurological disorders had normal levels.

Conclusions: Measuring CSF hypocretin-1 levels is the definitive diagnostic test, providing it is interpreted within the clinical context; It may be most useful in subjects already treated with psychoactive drugs and/or with other concurrent sleep disorders such as sleep apnoea syndrome.

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ABSTRACT WITHDRAWN

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DIURNAL VARIATION OF CSF HYPOCRETIN-1 (OREXIN-A) IN CONTROL AND DEPRESSED SUBJECTS

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Keywords: depression, hypocretin, orexin

Objectives: CSF hypocretin-1 have not been studied in mood disorders. Patients with narcolepsy, a condition associated with depression, have low hypocretin-1. Hypocretins have excitatory effects on monoaminergic transmission. Depression, a condition associated with short REM sleep latency and HPA axis abormalities, is treated with monoaminergic reuptake blockers. In rats, hypocretin release is higher during the active phase and may consolidate wakefulness at specific times. Hypocretin release is also activated by sleep deprivation, possibly an manipulation with antidepressant effects. Finally, hypocretins may activate the HPA axis. We examined CSF hypocretin-1 in control and depressed subjects over the 24-h period.

Methods: 14 controls (six males, 41 ± 4 years) and 15 depressed subjects (five males, 39 ± 3 years, HDRS = 19.5 ± 1.1; three bipolar type 1, 4 bipolar type 2, others with unipolar or first depressive episode) were included. CSF was drawn continuously for 24 h using an indwelling intrathecal catheter under entrained light-dark, supine conditions. Depressed subjects were studied before and after 5 weeks of sertraline ($n = 10$, 3 non-responders) or bupropion ($n = 5$, 3 non-responders). Hypocretin-1 was measured using a direct RIA in 10–30 min samples across the 24 h. Repeated ANOVA and sinusoid curve fitting were used to assess diurnal variation and estimate phase and amplitude, respectively. Other analyses involved *t*-tests across groups.

Results: Hypocretin-1 did not vary with age, sex and diagnosis. Levels varied slightly across the diurnal cycle, with lower levels at 1–2 pm ($P < 0.001$). Amplitude was reduced in depression (before and to a lesser extent after treatment) (Table 1). Hypocretin-1 decreased after sertraline (−14%, $P < 0.01$) but not bupropion (−4%, NS). Neither treatment-induced decreases nor changes in day/night differences (e.g. circadian amplitude) correlated with antidepressant response. Interestingly, day/night differences correlated before and after treatment, suggesting a stable trait in depressed subjects.

Table 1. Sinusoidal curve fitting in control and depressed patients

	Controls	*Depressed, Pre-*	*Depressed, Post-*
Phase of hypocretin minimum (mean ± SD)	13:58 ± 0:22	12:54 ± 1:45	13:09 ± 1:26
Amplitude (1/2 peak to trough, mean ± SD)	5.72% ± 0.55	1.77% ± 0.81	2.83% ± 1.06

Conclusion: CSF hypocretin-1 fluctuates moderately (< 15%) but significantly across the 24 h. Lumbar sampling at anytime is adequate

to diagnose narcolepsy. Lower values around 1–2 pm, possibly reflect decreased release in the late part of the dark period and a delayed and dampened oscillation. Less likely, levels may be reflecting spinal release and/or would be opposite with what was observed in nocturnal rodents. We also found that sertraline but not bupropion slighly decreased levels, suggesting serotoninergic influences on hypocretin. Results are consistent with other findings in depression indicating that multiple diurnal physiological measures are dampened. Interestingly, relief of depression was not correlated with restored rythmicity; Depressive mood and hypocretin variation are independent, with the caveat that longer treatment may lead to more significant changes in diurnal variation.

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SUSTAINED INSPIRATORY AIRFLOW LIMITATION – A PREFERRED MEASURE OF PARTIAL UPPER AIRWAY OBSTRUCTION

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Keywords: sustained inspiratory airflow limitation

Background: Inspiratory airflow limitation can be readily identified by inspecting the signal from a pneumotachometer or a nasal (or nasal-oral) pressure transducer. Furthermore, patients with very mild obstructive sleep apnoea syndrome (OSAS) often exhibit sustained episodes of Inspiratory Airflow Limitation (SIAL) without any obvious changes in tidal volume, oxygen saturation, oesophageal pressure, snoring, or brief arousals from sleep. Often, SIAL episodes may be resolved by a return to normal breathing without any obvious arousal or change in sleep state.

Methods: We have investigated the utility and relevance of this phenomenon by comparing SIAL with (a) the appearance of overt obstructive apnoeas (OA) and obstructive hypopnoeas (OH), (b) changes in oxygen saturation, (c) respiratory-event-related arousals from sleep associated with short-term (<4 min) falls in intra-oesophageal pressure (EP-RERAS), (d) respiratory-event-related arousals from sleep associated with short-term (<4 min) episodes of IAL-RERAS, (e) sustained episodes of low intra-oesophageal pressures not associated with arousals from sleep, (f) change in phase of rib-cage and abdominal inductive plethysmography, and (g) episodes of snoring measured by a standardized A-weighted industrial sound meter. These measures are compared by evaluating their response to various CPAP pressures during an automatic nasal-CPAP titration procedure. The automatic CPAP titration procedure is designed to establish a pressure sensitivity profile (PSP) by evaluating the patient at airflow pressures that are automatically, progressively and repetitively adjusted below, at, and above the optimum pressure during attended polysomnography (*J. Sleep Res.*, 1998, 7: S178).

Results: Of the phenomena evaluated during this study, SIAL was the last to become normalized with increasing CPAP pressure in patients with OSAS. Changes in SIAL also correlated most closely to the ideal CPAP pressure as indicated by the 95th centile value of a 3–4 week AutoSet-T habituation-desensitization–titration-compliance (HDTC) test (*Sleep*, 2001, 24: A311). Furthermore, the absence of a flow limitation waveform was often helpful in excluding non-obstructive events following spontaneous arousals or periodic leg movements (PLMS).

Conclusions: Although SIAL was found to be the most sensitive measure of partial upper airway obstruction during this study; it is not known whether the phenomenon is associated with increased cardiovascular morbidity, mortality, or functional impairment. The relationship of SIAL to factors such as beat-to-beat blood pressure, pulse transit time, autonomic function, and hormonal or metabolic parameters, may be important. Furthermore, by identifying SIAL phenomena, many polysomnographic records scored as being within normal limits according to published AASM criteria, can be classified as having unequivocal partial upper airway obstruction.

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SUBJECTIVE SLEEP COMPLAINTS AND QUALITY OF LIFE IN PATIENTS ON MAINTENANCE HAEMODIALYSIS USING THE KIDNEY DISEASE QUALITY OF LIFE QUESTIONNAIRE

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Keywords: sleep complaints, quality of life, haemodialysis, kidney disease quality of life questionnaire

Background: Previous studies reported high prevalence of sleep disorders in patients on chronic haemodialysis. Sleep complaints may contribute to the impaired quality of life of those patients. The aim of our study was to asses quality of life and frequency of sleep complaints using the 'Kidney Disease Quality of Life Questionnaire' (KDQoL) and to assess the correlation between sleep complaints and overall quality of life in patients on maintenance haemodialysis.

Patients and methods: 137 patients on chronic haemodialysis (HD) (50% male, median age 58 years, 21–84 years) completed the questionnaire. The KDQoL is a self-administered disease-specific quality of life scale based on the SF-36 scale. We analysed the association between the presence of sleep problems and overall QoL scores. The age and sex-distribution of the two groups were comparable. The data are presented as mean ± SD.

Results: 40% of patients were defined as poor sleepers based on their answer to the question: 'do you get the amount of sleep you need?'. 37 patients (27%) reported daytime sleepiness ('having trouble staying awake during the day?'). 59 patients (43%) reported interrupted night sleep ('do you wake up during the night and have trouble falling asleep again?'). The patients' SF-12 Physical Health Composite score (SF12Ph) was 36 ± 10 and SF-12 Mental Health Composite score (SF12Mh) was 44 ± 12. These values are comparable to results published from different dialysis populations. The composite 'sleep score' calculated from the four sleep-related questions showed a moderate, but statistically strongly significant correlation with SF12Ph, SF12Mh and the score obtained for 'energy/fatigue' ($r = 0.409$, $r = 0.334$ $r = 0.477$, respectively; $P < 0001$ for all variables). Both the mean SF12Ph and SF12Mh values were statistically significantly lower in poor sleepers than in patients who reported satisfying sleep (33 ± 9 vs. 38 ± 11 and 41 ± 13 vs. 47 ± 11 for SF12Ph and SF12Mh, respectively).

Conclusion: We confirmed that there is high prevalence of sleep complaints in patients on haemodialysis. Sleep disorders may contribute to impaired quality of life of these patients.

Acknowledgement: This work was supported by a grant from the Ministry of Health (ETT: 240/2000).

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SLEEP EFFECTS OF BILATERAL SUBTHALAMIC STIMULATION IN PARKINSON'S DISEASE

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Keywords: Parkinson's disease, sleep, subthalamic stimulation

Sleep disturbances are frequently observed in Parkinson's disease. The most common are insomnia, REM sleep behaviour disorder, restless legs syndrome… Physiopathology of these sleep's troubles are various: parkinsonian symptoms (tremor, rigidity, akinesia), pain, nycturia, treatment and also probable non-dopaminergic neurodegenerative lesions in sleep–wake regulatory centres

Objectives: Bilateral subthalamic stimulation is an alternative treatment in Parkinson's disease. We have studied sleep–wakefulness cycle before and after chirurgical treatment in nine parkinsonian patients.

Methods: One month before implantation of electrodes in subthalamic nucleus, we have recorded polysomnography in nine patients during two nights. Three months after intervention, polysomnographic recordings were made with (all patients) and without stimulation (if patients have giving informed consent). All recordings are scored by the same examiner. Statistic analysis was realized with a non-parametric, apparied test (Wilcoxon test).

Results: Before intervention, all patients suffered from sleep fragmentation, insomnia. Total sleep time are reduced. After intervention, when bilateral subthalamic stimulation are 'on', sleep parameters are significantly improved: total sleep time, duration of slow wave sleep and of paradoxical sleep, sleep efficiency. When bilateral subthalamic stimulation are 'off', sleep disturbances are similar with those observed before cerebral stimulation.

Discussion and conclusion: In view of these results we can conclude that sleep disturbances are significantly improved by the bilateral stimulation of subthalamic nucleus. This effect is concomitant of improvement of motor disturbances. No patient suffered of nocturnal dystonia after chirurgical treatment and parkinsonian symptoms are significantly reduced. Moreover, all antiparkinsonian treatments are diminished. It is another explanation of sleep's improvement. However, we can not exclude a direct effect of stimulation in sleep–wakefulness regulation centres.

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THE DEVELOPMENT OF AN EFFECTIVE PRIMARY CARE TREATMENT OF SEVERE SLEEP DISORDERS IN CHILDREN WITH A LEARNING DISABILITY: A RANDOMIZED CONTROLLED TRIAL

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Keywords: children, learning disabilities, behavioural treatment, media-based treatment

Objectives: Severe sleep disorders are extremely common and persistent in children with learning disabilities. In contrast to medication, behavioural treatments for sleep problems are very successful, even in long-standing cases. At present, access to behavioural treatments is poor largely because of resource limitations. This study assessed the relative efficacy of a brief treatment for sleep problems in children with learning disabilities, for use in the primary care setting.

Methods: Subjects were screened for severe sleep problems and severe learning disabilities and eligible children aged between 2 and 6 were randomly allocated to one of three experimental groups.

- Conventional delivery: behavioural treatment delivered face-to-face ($n = 20$)
- Written information: behavioural treatment delivered by booklet ($n = 22$)
- Wait list control group: no treatment then subjects were randomized to one of the above groups ($n = 24$)

Subjects were assessed 6 weeks postintervention and durability of the treatments was evaluated 6 months later.

Results: According to parent report, the groups receiving the treatment conventionally were statistically indistinguishable from those receiving it from the booklet ($H = 36.975$, d.f. $= 2$, $P < 0.01$) and both groups improved compared with controls. Further improvements were noted 6 months post-treatment.

Conclusions: As a front-line treatment, behavioural methods should always be considered for child settling and night-waking problems even with supposedly hard-to-treat groups such as those with learning disabilities. The delivery of this treatment by media-based methods seems to be generally as good as conventional ones in the majority of cases. In view of the considerable personal, social, health and (to the nation) economic benefits of successful and early treatment of these sleep disorders, it has been seen as highly desirable to devise effective behavioural treatments which require less time and professional expertize for use in primary care.

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GENETICS OF THE RESTLESS LEGS SYNDROME

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Keywords: RLS, genetics, dopamine

Epidemiological studies have shown that the prevalence of RLS may vary from 5 to 10% in the caucasian population. Numerous studies have suggested a substantial genetic contribution in the aetiology of the primary form or RLS. Familial aggregation has been repeatedly reported since its original description by Ekbom in 1944 with more than 40% of the idiopathic cases showing a positive family history. In a recent study of a small sample of monozygotic twins, 83% of twin pairs were concordant for RLS, suggesting that a significant portion of the familial aggregation may be due to genetic factors. The pathogenesis of RLS remains largely unknown. However, the clinical improvement observed with dopaminergic agents (levodopa and dopaminergic agonists) as well as evidences provided by brain imagery studies support the hypothesis of a dopaminergic dysfunction in RLS. Several candidate genes involved in the dopaminergic transmission and metabolism were studied: dopaminergic receptors D1 to D5, dopamine transporter, tyrosine hydroxylase, and dopamine beta-hydroxylase. This study was performed on 92 patients with RLS and 182 controls matched for ethnic background and no significant difference was found in the genotypic or allelic distribution between groups. To map genes that play a role in the vulnerability to RLS, a genome-wide scan was conducted in a large French–Canadian family. Significant linkage was established on chromosome 12q for a series of adjacent microsatellite markers with a maximum two points lod score of 3.42. Results

suggest a pseudo dominant pattern, in which the true mode of inheritance is autosomal recessive. Several candidate genes have been mapped within the region of interest. Amongst these, is the gene encoding the tridecapeptide neurotensin (NTS). NTS is reported to act as a neuromodulator of the dopaminergic transmission. This is particularly relevant since, as mentioned before, several lines of evidence implicate the dopamine system in the pathogenesis of RLS. Other studies will be needed to further identify the RLS gene, through recruitment and analysis of new affected families, to confirm this result and to refine the genetic interval to a size more suitable for positional cloning.

Acknowledgements: Supported by the National Institute of Health (USA) and the Canadian Institute of Health Research.

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DOPAMINE, SLEEP AND PLMS

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Keywords: dopamine, sleep, PLMS

Periodic limb movements in sleep (PLMS) are stereotyped repetitive movements of the lower and upper extremities that occur during sleep. These motor activities were originally documented in the lower limbs especially in patients afflicted with the restless legs syndrome (RLS). They were later reported in a wide variety of disorders and in normal subjects especially amongst elderly individuals. Several observations are consistent with the view that PLMS results from DA dysfunction. Placebo-controlled studies showed that levodopa and DA agonists, suppress PLMS whereas gamma-hydroxybutyrate, a short acting blocker of DA release, was found to increase PLMS, in both RLS and narcoleptic patients. Another series of evidences supporting the DA hypothesis of PLMS comes from brain imagery using both SPECT and PET technology, although most of these studies were performed in patients with RLS and PLMS and not in patients with PLMS alone. Other evidences in favour of the DA hypothesis of PLMS come from the study of PD and levodopa-responsive dystonia, two conditions associated with decreased DA transmission. A recent study of unmedicated PD patients revealed an increase of PLM indices during both sleep and wakefulness in comparison to healthy controls of the same age. The prevalence of PLMS is also higher in at least three sleep disorders, namely: RLS, narcolepsy and RBD. There are several indications that these three conditions are associated with impaired DA transmission. Conversely, Ancoli-Israel *et al.* have studied the prevalence of PLMS in schizophrenia, a condition characterized by an increase in DA in subcortical regions. These authors concluded that elevated DA in subcortical areas in schizophrenia may be protective against PLMS. Finally, neurophysiological data suggest that PLMS may result from suprasegmental disinhibition at the brain stem and spinal cord levels. The presence of descending DA pathways in the central nervous system has been well identified and these neuronal pathways may be involved. In animals, experimental lesions of DA neurones in the diencephalon (A11) and in the striatum by 6-hydroxydopamine (6-OHDA) resulted in increased motor activity. PLM were seen in striatal-lesioned animals. These results are concordant with the hypothesis that DA, is involved in the pathophysiology of PLMS.

Acknowledgements: Supported by the National Institute of Health (USA) and the Canadian Institute of Health Research.

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ANXIETY CORRELATES WITH IMPAIRED ADAPTATION TO POLYSOMNOGRAPHY IN PATIENTS WITH ALZHEIMER'S DISEASE

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Objective: First night effect (FNE) is characterized by alteration of the sleep structure consisting in decreased sleep efficiency and percentage of delta and REM sleep associated to increased sleep latency (REM, NREM) and percentage of stages 1 and 2. Previous data show that FNE is present in patients with Alzheimer's disease (AD), although it is less pronounced than in normal aged people. It is also known that normal individuals with anxious personality traces have impaired adaptation. Our purpose is to study to which extent anxiety can be associated with impaired adaptation in patients with AD.

Methods: 12 female patients with mild to moderate AD followed in the São Paulo Hospital (65–86 years old, body mass index < 30) underwent polysomnography in two subsequent nights. Females alone were chosen to avoid sexual differences of personality trace. Polysomnographic parameters studied were sleep latency, REM sleep latency, percentage of stages 1, 2, delta, and REM, sleep efficiency, PLMs and RDI. Anxious state and trace was assessed by IDATE (Brazilian version) test. IDATE scores were associated to polysomnographic parameters by Spearman non-parametric correlation.

Results: Anxiety trace scores correlated inversely with delta sleep adaptation ($P < 0.05$). There was no significant correlation with other adaptation parameters. Anxiety state scores did not correlate significantly with adaptation.

Conclusions: These data show that anxiety influences FNE in demented patients, especially concerning delta sleep adaptation.

Acknowledgement: Supported by FAPESP and AFIP.

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SLEEP QUALITY BY SLEEP APNOEA PATIENTS BEFORE AND AFTER CPAP THERAPY

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Keywords: SAS, CPAP, PSG

According to present day knowledge, the main problems of sleep apnoea syndrome (SAS) that causes worsening of life quality and possible health problems like stroke, myocardial infarction, high blood pressure and others, are bad sleep quality and low blood oxygenation due to irregular breathing. The group of patients suffering from SAS was examined in sleep laboratory. They were divided to the subgroup of sleep apnoea without other health problems and the subgroup of patients suffering from sleep apnoea and high blood pressure. These patients were treated by continuous positive pressure (CPAP) for 3 years. The main aim of the study was to evaluate sleep architecture and daily activity of the patients. There were 90 patients suffering from SAS examined in this study before and during the therapy by CPAP after 3, 12 and 36 months. The complete polysomnography (PSG) and Epworth scale of sleepiness (ESS) were evaluated. The Mann–Whitney *U*-test, Wilcoxon rank sum *W*-test and Wilcoxon matched-pairs signed ranks test were used for statistical evaluation. The therapy wih CPAP improved both parameters – daily activity and sleep architecture by all the patients. Meaningful improving with rebound effect of some parameters was seen after 3 months therapy and after mild worsening stayed stable till last control after 36 months in all the patients suffering from sleep apnoea. The

differences in sleep architecture between both subgroups of patients suffering from SAS were functionally unimportant before therapy, but they were significantly different from normal. The importance of complete PSG in exact evaluating of CPAP therapy effect is emphasized.

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CHANGES IN BRAIN MORPHOLOGY ASSOCIATED WITH OBSTRUCTIVE SLEEP APNOEA

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Keywords: sleep apnoea, neurocognitive deficits, hypoxia, magnetic resonance brain scans

Objectives: Obstructive sleep apnoea is associated with cognitive deficits, including impairment of memory, learning, attention and executive functions. The extent to which these deficits are attributable to the episodic hypoxia or the frequent arousals from sleep, acting independently or synergistically is unclear. In rats, chronic exposure to intermittent hypoxia during sleep results in cellar damage within the CA1 region of the hippocampus (*J. Neurosci.*, 2001, 21: 2442–2450), a region known to be closely associated with learning and memory. We hypothesized that cognitive deficits in obstructive sleep apnoea would be associated with the focal loss of grey matter within the hippocampus and in other cortical areas associated with executive functions.

Methods: T1-weighted, magnetic resonance brain scans (Siemens 1.5T Vision, 3d MP-RAGE, TI = 300 ms, TE = 4 ms, TD = 300 ms, $1 \times 1 \times 2$ mm) were performed in seven right handed male obstructive sleep apnoea patients recruited from our sleep clinic; group median (range), age 50 (28–65) years, AHI 28 (25–40) events/ h, minimum nocturnal O_2 saturation 71 (61–79)%, Epworth sleepiness score 12 (4–17). We also studied seven control subjects, matched for age, sex and handedness. Brain images were normalized into standard stereotaxic space and grey matter concentration determined voxel by voxel (SPM99, *Neuro Image*, 2001, 14: 21–36).

Results: The voxel-based morphometry on the obstructive sleep apnoeic patients revealed a significant reduction in grey matter concentration in the left hippocampus, which was maximum at $x = -34$; $y = 20$; $z = -20$, Talaraich co-ordinates, ANOVA, $P = 0.001$ corrected for multiple comparisons based on *a priori*, bilateral, hippocampal regions of interest (see Fig. 1). No further focal grey matter reductions were seen in other brain regions (no *a priori* region of interest, $P < 0.05$ corrected for multiple comparisons for entire brain). In the control subjects no reductions in grey matter concentration were found.

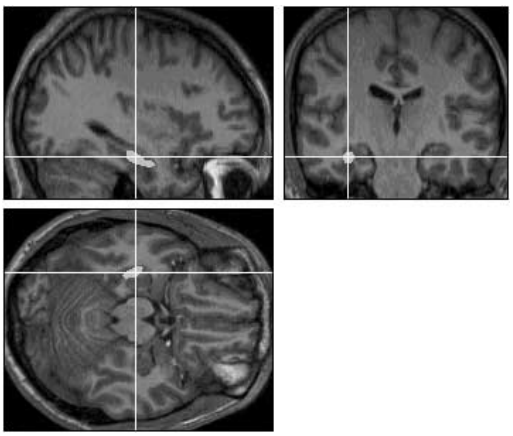

Figure 1. Region of interest in left hippocampus ($P = 0.001$, $f = 83$).

Conclusions: We conclude that obstructive sleep apnoea is associated with changes in brain morphology. We speculate that these changes are most likely due to hypoxia and that reversal of cognitive deficits following continuos positive airway pressure treatment may be associated with reversal of grey matter loss.

Acknowledgements: Wellcome Trust Fellowship, Breathlessness Research Charitable Trust.

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INFLUENCE OF AMITRYPTILINE AND MOCLOBEMIDE ON THE SLEEP–WAKEFULNESS CYCLE IN RATS

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Keywords: rats' sleep–wakefulness cycle, amitryptiline, moclobemide, paradoxical sleep

Introduction: Most of the antidepressants induce selective suppression of paradoxical sleep (PS) in the sleep–wakefulness cycle (SWC) due to significant elevation of the monoamines' concentration in the brain. However, administration of the majority of these drugs with an aim to treat depression is limited because their withdrawal results in significant rebound of PS, which, in its turn, leads to farther aggravation of the patient's state [1]. In a search of correct therapeutic strategies the problem of appropriate timing of the drugs' administration within the day-and-night period has been discussed [2]. Therefore, detailed investigation of effects of a certain drugs on the SWC in animals seems to be important and adequate for this purpose. In the present work comparative study of effects of different kinds of antidepressants – tricyclic blockator of the monoamines' reuptake, amitryptiline and reversible MAO inhibitor, moclobemide – on the SWC has been performed.

Methods: Experiments were carried out in the rats weighing 250–350 g ($n = 6$ per each group). Duration of SWC recording was 12 h (10:00 am–10:00 pm) in both baseline and experimental observations. The EEG, EMG, and EOG indices of SWC were registered. Effects of amitryptiline at the doses of 1.6, 3, 6, 10, 12 mg kg^{-1}, and of moclobemide at the doses of 10 and 20 mg kg^{-1} were studied. Three 4-h time spans were evaluated statistically: 10:00 a.m–2:00 pm – action period of drug; 2:00–6:00 pm – first recovery period; 6:00–10:00 pm – second recovery period.

Results: The multiparametric neurophysiological analysis of the data obtained has shown that SWC alterations induced by different doses of amitryptiline have unidirectional character and are strictly dose-dependent. Under the influence of the antidepressants the latencies of sleep onset and development of the first PS are increased. At the initial period of action amitryptiline elicits total deprivation of sleep. In the period of 10:00 am–2:00 pm the percentage of waking (W) increases, the share of deep slow wave sleep (DSWS) increases significantly, and PS suppress or is deprived completely. In the recovery periods the compensatory changes do occur, manifested in decreased total duration of W, increased percentage of DSWS, and significant rebound of PS. On the background of moclobemide action, the sharp increase of W, decrease of DSWS, and total deprivation of PS are the case. In the recovery cycles W decreases, DSWS increases sharply, and PS appears and gradually returns to the baseline value.

Discussion: Comparative evaluation of amitryptiline and moclobemide effects on SWC has shown that in the action period they equally abolish development of PS. In the recovery period both of these drugs equally increase the duration of DSWS. Essential difference between the effects of these drugs is following – in a case of amitryptiline an inevitable rebound of PS does occur, while in a case of moclobemide, SWC normalizes without significant rebound of this state. In addition it was shown that for achieving of desirable results – deprivation of PS – the time of an antidepressant administration is of certain importance.

Conclusions: Preference of moclobemide over amitryptiline in treatment of depression and importance of drug administration timing are emphasized.

Acknowledgement: Research supported by the Japanese Government, under the ISTC grant G-391.

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QUANTITIVE DESCRIPTORS OF NREM SLEEP MICROSTRUCTURE

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Keywords: EEG automatic analysis, sleep microstructure, arousals, cyclic alternating pattern

Objectives: The aim of the research has been to apply a method based on the automatic computation of five band – related descriptors to the quantitative analysis of the various microstructure phenomena occurring during NREM sleep.

Methods: The following frequency bands of the EEG signal were considered: delta (0.75–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), sigma (12.5–14.5 Hz) and beta (15–25 Hz). For each band activity, two amplitude averages were computed, the one (M) over an interval lasting 64 s and the other (m) over a shorter interval lasting 2 s. Each band – related descriptor was then calculated every 0.5 s as the ratio (m–M)/M. The criterion for the detection of a microstructure phenomenon simply consisted in the overcoming of the threshold value of 1 (the descriptors were non-dimensional). Each detected phenomenon was characterized by the following features: frequency bands involved, peak delay for the bands involved, intensity (defined as the peak value of the descriptors) and time length (whose measure was based on the application of a second threshold, equal to 0). The method was applied to the F4–C4 trace of the sleep EEG of 10 normal young subjects.

Results: The following phenomena were automatically recognized and characterized: K complexes, arousals, A-phases of the Cyclic Alternating Pattern, alpha bursts and spindles. It was possible to discriminate between the different types of A-phases (A1, A2 and A3), to distinguish between arousals (visually recognized according to the ASDA criteria) inside an A-phase and outside an A-phase and to characterize the arousals according to the bands involved (one or more amongst theta, alpha and beta). The frequencies (numbers of occurrences divided by time) of the various microstructure phenomena provided quantitative parameters able to describe the general properties of the sleep microstructure over the various NREM sleep stages. As expected, there were significant differences in the values of the frequencies comparing Stages 2 and 4.

Conclusion: The automatic method, based on the computation of band – related descriptors during the night, makes it possible to detect and characterize significant microstructure phenomena. The results obtained are in good agreement with those of the visual analysis; furthermore, the method introduces conceptually simple criteria, while the criteria for the visual recognition and classification are sometimes uncertain and difficult to apply.

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CURRENT DENSITY DISTRIBUTION DURING PHYSIOLOGICAL SLEEP

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Keywords: EEG, LORETA, sleep, human

Objectives: The aim of the study was to estimate distribution of intracerebral electric current densities during physiological sleep, as compared with waking.

Methods: The all-night sleep data were pooled from 10 healthy volunteers (4 M, 6 F, mean age: 30.9 ± 8.52, range: 22–50 years). Following two nights of sleep recording, multiple sleep latency test (MSLT) was administered to obtain EEG records of quiet wakefulness. Twenty-one channels from standard EEG sites according to international 10–20 system were digitized at sampling rate 128 Hz and recomputed to average reference. For each sleep stage, identified according to the criteria of Rechtschaffen and Kales (1968), and for each quiet wakefulness period all artifact-free 4 s EEG epochs were taken for Fast Fourier Transform (FFT) analysis. Comparisons between sleep stages and quiet wakefulness were computed within following frequency bands: delta (1.5–6.0 Hz), theta (6.5–8.0 Hz) and beta1 (12.5–18.0) Hz. Low Resolution Electromagnetic Tomography (LORETA) [2, 3], a novel source localization method that computes three dimensional distribution of cortical current density in the Talairach brain space, was then applied. The LORETA images were statistically compared by voxel-by-voxel paired *t*-tests.

Results: Within beta1 frequency, LORETA demonstrated greater current density in medial prefrontal (Brodmann areas 9 and 10) cortices. This localized activity was seen during all NREM sleep stages. For

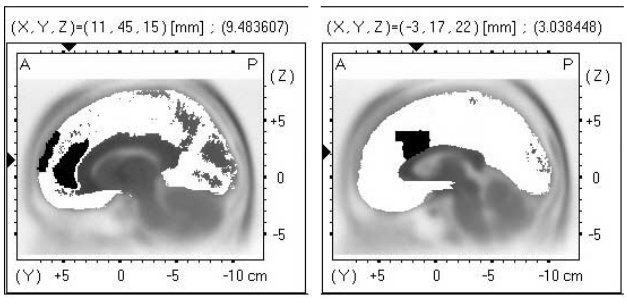

Figure 1. LORETA significant differences between regional brain electrical activities during waking and sleep are labeled black. Left: stage 2, EEG frequency band betal (12.5-18.0 Hz), Brodmann area 10, $P < 0.001$. Right: sleep stage 4, EEG frequency band delta (1.5-6.0 Hz), Brodmann area 24, $P < 0.05$. The arrows indicate voxels where extreme *t* value (top right of every image) was found.

delta frequency, greater current density appeared to occur most consistently in Brodmann area 24, also throughout all stages of NREM sleep (Fig. 1). Moreover, significant greater current densities within delta frequencies were systematically detected in Brodmann area 6. In this preliminary topographical approach, paralimbic and neocortical sites, including anterior cingulate, were identified. These parts of distributed anatomical networks were previously posited as involved in changes in the state of consciousness throughout the sleep–wake cycle. Greater current density within delta range in the anterior cingulate is of special interest because limbic disengagement during slow wave sleep may be a precondition of recuperation related to sleep [1].

Conclusions: Greater current density within delta frequencies in Brodmann area 24 was the most consistent finding throughout all NREM sleep stages.

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WRIST ACTIVITY, SLEEPINESS AND STRESS IN RELATION TO ANXIETY AND DEPRESSION

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Keywords: HAD-S, sleepiness, stress, activity

Objective: The aim of the study was to investigate whether measures of subjective sleepiness, stress and wrist activity were related to scores on the Hospital Anxiety and Depression-scale(HAD-S), in a non-clinical group.

Methods: 28 healthy subjects (14 men, 14 women. Mean age: 27.1 years, ±1.1) with normal sleep habits participated. The HAD–scale [1] was used to evaluate presence and severity of generalized anxiety and depression. Karolinska Sleepiness Scale was used to measure sleepiness (1 = very alert to 9 = very sleepy, fighting against sleep). A similar 9-point scale was used to measure stress. This was carried out once per week, during 3 weeks. The ratings were made every second hour from 08:00 h until bedtime. Only ratings in between 10:00 to 20:00 h were included in the analyses. Wrist activity (non-dominant hand) was measured with Actiwatch AWL-32K Extended Memory, Cambridge Neurotechnology. Mean levels were obtained for the 2 h preceding the subjective ratings of sleepiness and stress. At 21 h, subjects rated themselves on the HAD-scale, consisting of 14 items. Four subgroups were created based on those ratings: no symptoms (NO; $n = 9$), anxiety only (A; $n = 5$), depression only (D; $n = 5$) and both (AD; $n = 9$) with ratings on and above the median as cut-off points (Median Anxiety = 5.12, SE = 0.5, Median Depression = 2.7, SE = 0.33). Data was analysed using repeated measures ANOVA (Huynh Felt corrected), with group and gender as between factors. Day and time of day was within factors.

Results: There was a trend towards a significant main effect of group for stress ($P < 0.10$), but not for sleepiness or activity. Sleepiness was however, higher ($P = 0.0501$) in subjects rating higher on depression ratings. The main effect of time of day was significant for activity ($P = 0.002$) as for stress ($P < 0.05$), with a trend towards a difference for sleepiness ($P = 0.067$), which was due to higher ratings of sleepiness during the latter part of the day in the D and AD groups. A significant interaction effect was found for activity ($P < 0.05$). The levels differed at the 10 h and the 20 h-ratings, with higher levels of activity in the A and AD groups. The opposite was the case during the other time points of the day. Sleepiness was higher at 18 and 20 h in the D and AD groups. For stress there were significantly higher ratings of the D and AD groups throughout the day with the exception of the 14 and the 20 h ratings ($P < 0.05$). There were no differences in relation to gender. There were no 'day' effect on the HAD-S ratings.

Conclusions: The results show that subjects above the median in anxiety and depression ratings have a higher wrist activity level, and rate higher on stress during most parts of the day. Sleepiness was also higher during the latter part of the day (18 and 20 h) in subjects rating above the median on depression items and the combination of depression and anxiety. Higher wrist activity and stress in healthy subjects rating higher on hospital anxiety and depression scale.

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THE DEVELOPMENT OF INFANTS' CIRCADIAN SLEEP-ACTIVITY RHYTHM AND MOTHERS' CIRCADIAN RHYTHM

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Keywords: activity rhythm, infant, actigraph, autocorrelogram

Objectives: In many studies of sleep-logs, infants' circadian sleep-wake rhythm is established during the first 3 months after their birth. However, it has been unclear when and how infants obtain their circadian sleep-wake rhythm. In the present study, infants' developing circadian sleep-activity rhythm and mothers' circadian rhythm in the postpartum period were examined using actigraph monitoring. In particular, we discuss the development of sleep-activity in a group of infants based on autocorrelograms calculated from continuous objective data.

Methods: The subjects were 11 primipara (mean age 28.8 ± 2.6 years) and their infants (seven girls and four boys). Actigraphic recordings for the infants and their mothers were made with epochs of 1 min over three continuous days during Weeks 3, 6, 9 and 12 after birth. The infants wore Actiwatches (Mini-Mitter Co., OR, USA) on their legs, while the mothers wore them on their left wrists. The mothers were instructed to write both their and their infants' sleep logs and feeding logs every day. In this study, the circadian rhythm of sleep-activity for the infants and their mothers was examined by autocorrelograms for three continuous days for each week. The group mean correlation coefficients were calculated to facilitate interpretation of a tendency in all subjects, and t tests were applied to evaluate whether or not a 0.05 significance level for the tests had been reached. We also evaluated the amplitude of the 24-h peaks for the four occasions, Weeks 3, 6, 9 and 12, using a one-way ANOVA with repeated measures.

Results: A 24-h peak of a mean autocorrelogram of the infants' movements was significantly detected at week 3. The amplitude of this 24-h peak gradually became larger from weeks 6–12 [$F(3, 30) = 4.963$, $P < 0.006$)]. An 11-h peak was also observed at week 3. This 11-h peak was thought to be a semi-circadian rhythm. Regarding the

mothers, the amplitude of a 24-h peak on a mean autocorrelogram at week 3 was the smallest of all other weeks, and it became larger from weeks 3–12 [$F(3, 30) = 6.072$, $P < 0.002$)]. This meant that the mothers' circadian rhythm at week 3 was influenced by their interrupted sleep at night to take care of their infants.

Conclusions: The infants' circadian sleep-wake rhythm has begun from week 3. The amplitude of the 24-h peak for mean autocorrelograms seemed to become an index of the development of infants' circadian sleep-wake rhythm. The mother-infant synchronization was probably the first factor in the entrainment of infants' circadian sleep-wake rhythm. The infants' circadian sleep–wake rhythm has begun from week 3, and its amplitude based on mean autocorrelograms increased from weeks 3–12. circadian sleep-activity rhythm, infant, actigraph, autocorrelogram.

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NOCTURNAL PAROXYSMAL DYSTONIA AND EPILEPTIC NOCTURNAL WANDERINGS WITH A TEMPORAL LOBE ORIGIN: A STEREO-EEG STUDY

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Introduction: Nocturnal paroxysmal dystonia (NPD) and epileptic nocturnal wanderings (ENW) are commonly considered as manifestations of nocturnal frontal lobe epilepsy (NFLE). The aim of this study is to describe the clinical seizures characteristics, the interictal and ictal electroencephalogram findings obtained with both scalp and stereotactically implanted intracerebral electrodes (stereo-EEG) in five drug-resistant adult epileptic patients with NPD and/or ENW in which a temporal lobe origin has been demonstrated.

Patients and methods: They were three females and two males with complex partial seizures occurring exclusively or predominantly (>95%) during nocturnal sleep and with a seizures frequency ranging from 5 to more than 50 per month. The localization of the epileptogenic zone (EZ) was defined by preliminary video-EEG and MR studies. Moreover in all the cases a video-stereo-EEG investigation by means of stereotactically introduced intracerebral electrodes, implanted under general anaesthesia, was performed.

Results: The overall clinical pattern (resulting from both anamnestic features and video recordings) suggested a frontal lobe origin of these seizures but interictal and ictal EEG features were consistent with a temporal lobe origin. For this reason an individualized stereo-EEG exploration was conducted. Stereo-EEG ictal recordings during nocturnal NREM sleep demonstrated an initial epileptic discharge localized (in various combination for each patient) to the following temporal structures: amygdala, hippocampus, parahippocampus, temporal pole and mainly basal temporal neocortex. Such modifications preceded of some seconds the first clinical sign in all the cases. The hyperkinetic phase (i.e. paroxysmal dystonia, emiballistic movements, paroxysmal wanderings, etc.) appeared when the discharge in the temporal region became recruiting and involved some extratemporal structures, such as the cingulate gyrus and frontal regions, with a rhythmic spike discharge. On this basis an individualized temporal resection was conducted in all the subjects. After surgery three patients are seizure free and one is almost seizure free. In the last, non-cured patient the right speech dominance avoided a complete resection of the EZ.

Discussion: This study suggests a possible temporal lobe origin of NPD and ENW. The detailed analysis of the ictal semiology allowed us to identify some clinical signs which, despite the complexity of this clinical picture, may indicate a temporal ictal discharge.

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REGIONAL CEREBRAL GLUCOSE METABOLISM DURING SLEEP IN HUMANS

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Keywords: sleep, imaging, brain, glucose

The brain's appetite for glucose during sleep is regionally specific. As a correlate of neuronal function, we can trace these patterns across the sleep–wake states to provide insights into the neuronal mechanisms of sleep generation and of sleep modulation. Further, such studies provide knowledge about the work that sleep may be doing, thereby offering insights regarding the functional roles of sleep. We have made extensive use of the [^{18}F]-FDG PET method for studying sleep. The primary advantage of this method is that subjects do not have to be confined in a scanning device at the time of sleep. Rather, given the relative irreversible metabolism of deoxyglucose in cells, a scan collected 60–90 min after injection of a radioisotope reflects metabolic activity from the time of injection to 10–20 min postinjection. This significantly minimizes subject attrition and has allowed us to visualize brain mechanisms during sleep in healthy subjects, in depressed subjects, in insomniacs, and in elderly subjects up to the age of 89. This method has also allowed for repeated assessments in subjects following treatments. The results of these studies suggest that there are several major systems of central nervous system integration across behavioural states. First, is a generalized arousal system. This includes not only brainstem reticular activating system, but also includes primary limbic structures such as the amygdala, hippocampus, parahippocampal cortex, ventromedial prefrontal cortex, the basal forebrain/hypothalamus as well as occipital cortex. Recent evidence suggests that a baseline level of function in this system may represent an individual trait that determines the degree to which they are able to generate slow wave sleep. This ventral brainstem/limbic and related paralimbic system is preferentially active in waking and REM sleep and acts in opposition to cortical structures as they deactivate in slow wave sleep. Heteromodal association cortex represents a second major system in relation to behavioural states. This system is relatively active in waking and relatively inactive in sleep, but predominantly during NREM sleep. This higher order, neuronal network is an online system that appears to be the primary domain of conscious, waking behaviour. A third major system consists of a broad region of paralimbic mesocortex, including the cingulated gyrus. This system is preferentially activated during REM sleep when heteromodal association cortex is not. Anterior portions of this, extending into medial prefrontal cortex and the ventral striatum are most active in REM sleep. The functional significance of this remains to be seen, although this network has been intimately linked with emotion regulation, attentional processes, self-monitoring and error detection. Intriguingly, waking function in more dorsal portions of paralimbic mesocortex, including the dorsal cingulate and extending into prefrontal cortex appears to be directly related to the production of slow wave sleep at night. Recent studies show that the declines in slow wave sleep production across ageing are related to a significant loss of function with ageing in these areas. Given that slow wave sleep loss begins in the third and fourth decades of life, these changes in prefrontal cortex and dorsal paralimbic

mesocortex may represent among the earliest signs of brain maturation, or deterioration associated with ageing. In summary, relatively consistent patterns of cerebral organization across behavioural states are emerging based on evidence from functional brain imaging studies of sleep. Further investigation of these patterns in human pathology will shed additional insights into the behavioural significance of these patterns as well as provide knowledge regarding targets of treatment in human pathological states.

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ALTERATIONS OF THE N1/P2 AUDITORY EVOKED COMPONENT DURING THE SLEEP–WAKE CYCLE

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Keywords: auditory evoked potentials, stimulus intensity, information processing in sleep

Objective: As opposed to early auditory evoked potentials the late event-related potentials are altered during sleep [1, 2]. In the awake condition the first large negative wave after 100 ms, the late N1 component, correlates with stimulus intensity [3]. Generally, the N1 is thought to reflect a very early orienting, predominantly automatic response of the cortex to external stimuli, which seems to continue to exist in sleep too [e.g. 4].

Method: Following an adaptation night in our EEG-laboratory, 10 healthy subjects underwent full ambulatory polysomnography [5], in combination with multichannel-EEG for a single night. Series of sinus tones of various intensities, ranging from 50 to 100 dB, were applied during pre-sleep, sleep and post-sleep stages.

Results: Overall there was a reduction of loudness intensity of the N1/P2 amplitude from early to late NREM sleep in comparison to some augmentation in REM sleep. Topographical data of additional dipole source analysis of the N1/P2 component will also be presented in detail.

Conclusion: Presence of stimulus intensity response is based on highly precise functioning of the neuronal projection to temporocortical areas. Therefore, differences of the N1/P2 intensity dipole components in NREM and REM sleep stages represent a further argument for preservation of early cortical information processing, especially of stimulus intensity patterns, in sleep [6, 7].

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REM SLEEP REBOUND AFTER TOTAL OR SELECTIVE REM SLEEP DEPRIVATION ASSESSED IN AN INTERMITTENT REM SLEEP DEPRIVATION PROTOCOL IN THE RAT

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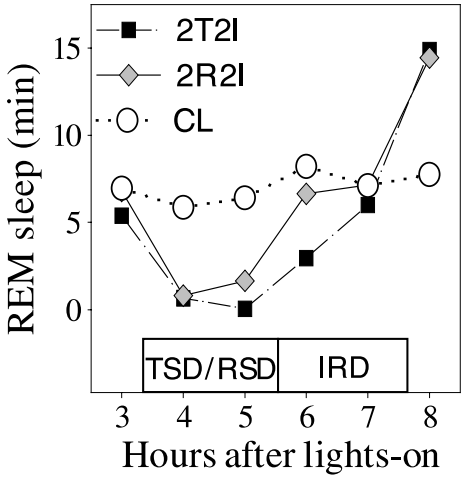
Keywords: REM sleep homeostasis, REM sleep deprivation, total sleep deprivation

Objectives: In a previous work [1], by combining total sleep deprivation (TSD) with selective REM sleep deprivation (RSD) we showed that both the build up of pressure to enter REM sleep and the REM sleep rebound depend on the time elapsed without REM sleep, regardless of the presence of the NREM sleep allowed during deprivation. We designed an intermittent REM sleep deprivation protocol (IRD) consisting of alternating short windows of selective REM sleep depivation and spontaneous sleep (see Methods), to measure REM sleep rebound in parallel with the temporal course of REM sleep pressure build up [2]. The aim of the present work is to compare the time courses of REM sleep rebound after 2 h of TSD or RSD followed by an intermittent REM sleep deprivation schedule.

Methods: Five adult male Sprague–Dawley rats were chronically implanted and maintained under a 12 : 12 LD schedule in individual isolation chambers. A real-time acquisition system sampled (at 250 Hz), stored and displayed polygraphic signals. Visual state scoring (wakefulness, NREM sleep or REM sleep) was performed with a time resolution of 15 s. After two baseline days each rat was subjected to two protocols starting with: (a) 2T2I protocol: 2 h TSD followed by 2 h of IRD; the following protocol was (b) 2R2I protocol: 2 h of RSD followed by 2 h of IRD. IRD consisted in the alternation, for four successive times, of 10 min of spontaneous sleep (REM sleep permission window), and 20 min of selective REM sleep deprivation (REM sleep deprivation window). Finally, a control (CL) session, that matched interventions during 2R2I, was also run. Protocols and control sessions were started at hour 4 after lights-on. One recovery day was left between trials. ANOVA for factors protocol and hour, and paired *t*-tests were applied.

Results: During TSD (hours 4 and 5, protocol 2T2I) REM sleep suppression was almost total. During RSD (hours 4 and 5, protocol 2R2I) REM fell under 10% of baseline. During the first hour of IRD (hour 6 after lights on), REM sleep expression in 2T2I was significantly lower than in the CL condition, whereas in 2R2I, REM sleep expression did not differ from CL or baseline. At hour 8 after lights-on (first hour of recovery) both 2T2I and 2R2I showed a REM sleep rebound that more than doubled baseline and CL (see Figure, baseline data not shown). In 2R2I, the fraction REM/Total sleep time in REM permission windows is higher respect to CL already at hour 6, whereas REM/total sleep time in REM permission windows during hour 6 is lower for 2T2I respect to CL and 2R2I. REM sleep rebound begins earlier after selective REM sleep deprivation than after total sleep deprivation.



Acknowledgements: Part of this research was supported by grants FONDECYT 3010028 (A.O.) and 1990631. E. Brunetti is a Doctoral Fellow of the Institutode Ciencias Biomédicas (ICBM).

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O

WHEEL-RUNNING DOES NOT CHANGE PHASE PREFERENCE OF REST-ACTIVITY RHYTHM IN A NOCTURAL OCTODONTID

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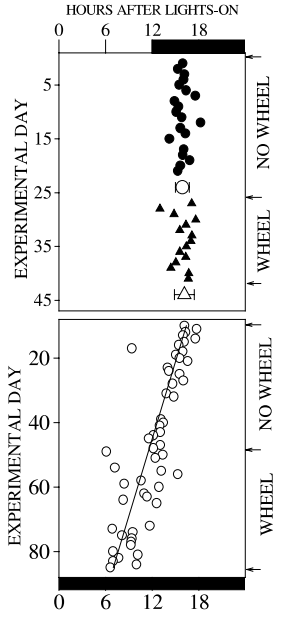
Keywords: circadian rhytms, wheel running, non-photic entrainment, octodon bridgesi, octodon degus

Objectives: It has been described that wheel-running (a non-photic stimulus) produces a rapid diurnal-nocturnal inversion in phase preference in *Octodon degus* (RODENTIA: HYSTRICOGNATHI). This phenomenon occurs in entrained and free-running individuals that exhibit a diurnal chronotype [1]. *Octodon degus* is a crepuscular semi-fossorial specie, that inhabits xeric habitats of central Chile. Under controlled conditions, *Octodon bridgesi* (Waterhouse, 1844), a close relative of *O. degus* [2] that lives in the temperate forest of centre-south Chile, expressed a nocturnal phase preference in the rest-activity rhythm without crepuscular activity bouts [3]. Here, we describe the effect of wheel-running exposure on rest activity rhythm in *O. bridgesi*.

Methods: Two male adult *O. bridgesi*, were captured in the field and installed in cages (30 × 40 × 30 cm) contained in individual isolation chambers (65 × 60 × 60 cm), under a 12 : 12 light–dark schedule (300 lx) and at 23°C, with food and water *ad libitum*. A running-wheel was inside the cage, that could be locked or unlocked (NO WHEEL and WHEEL condition, respectively). An automated acquisition system recorded and stored with a 15-s resolution the activity detected by a movement transducer placed at the base of the animal's cage. After a period of adaptation, each animal was subjected to four conditions (A) entrained to a 12 : 12 LD schedule with running-wheel locked (LD-NO WHEEL condition); (B) entrained with unlocked running-wheel (LD-WHEEL condition); (C) free-running (constant darkness, DD) with running-wheel locked (DD-NO WHEEL condition), and (D) free-running with unlocked running-wheel (DD-WHEEL condition). Angular statistics (cosinor rhythmometry) were applied for hourly incidence of 15-s bins with the highest 20% activity events. Acrophases (mean angles) are expressed in hours respect to lights-on (zeitgeber-time).

Results: Figure 1 depicts acrophases of 22 consecutive days under LD-NO WHEEL condition (filled circles) followed by 16 days under LD-WHEEL condition (filled-triangles). The animal concentrates the activity phase during the objective night with means at hour 15.8 ± 0.9 and 16.1 ± 1.3 in LD-NO WHEEL (open circle ± SD) and LD-WHEEL (open triangle ± SD), respectively. It was found a mild reduction in rest-activity rhythm amplitude under LD-WHEEL condition. During free running, it was observed a period shorter than 24 h in the two individuals studied. Figure 2 show acrophases (open circles) of one of the animals maintained in constant darkness. After 44 days in DD-NO WHEEL, the animal is exposed to DD-WHEEL condition. After a transient interval of high variability, achrophases in DD-WHEEL condition tend to fall at the phase predicted (line, $t = 23.87$) from the achrophases obtained during DD-NO WHEEL period (days 1–44).



Discussion: *O. brigesi* and *O. degus* being phylogenetically close relatives, evolved under ecological pressures that determined different chronobiological strategies. Octodon emerges as a Genus of high interest for comparative chronobiological studies. Wheel-running does not change phase preference nor rest-activity rhythm period in *Octodon bridgesi*.

Acknowledgements: Research supported by grant FONDECYT 3010028. Thanks are due to Drs Rodrigo Vásquez, Bárbara Saavedra and Ennio Vivaldi.

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LOW CSF HYPOCRETIN/OREXIN LEVEL IN A CASE OF EXCESSIVE DAYTIME SLEEPINESS WITH SLEEP-ONSET REM PERIODS

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Keywords: excessive daytime sleepiness, narcolepsy without cataplexy, hypocretin-1

Objectives: Hypocretins/orexins are hypothalamic neuropeptides involved in sleep and energy homeostasis. It has been reported that cerebrospinal fluid (CSF) hypocretin-1 level is dramatically decreased in most narcolepsy-cataplexy cases. Narcolepsy is a disorder associated with excessive daytime sleepiness (EDS), cataplexy and unusually rapid transitions to rapid eye movement (REM) sleep, however, controversies arise in patients without cataplexy. The aim of the study was to examine the low CSF hypocretin-1 level in a case of excessive daytime sleepiness with sleep-onset REM periods without cataplexy.

Methods: Subject is a 28-year-old women who developed EDS at the age of 15. Despite obtaining 9 h sleep each night, she began to fall asleep easily during classes in schooldays. She currently obtains 6 h sleep each night and takes 30 min nap after lunch almost every day. She experienced traffic accidents three times due to sleepiness while driving car or motorcycle. She did not experience any cataplexy episodes. At night, she had infrequent episodes of sleep paralysis. Physical and neurological examination was normal. Polysomnography (PSG) was recorded to search for any sleep disorders that may cause EDS. A multiple sleep latency test (MSLT) was recorded after the PSG recording. Human leucocyte antigen (HLA) marker for narcolepsy was examined. CSF was sampled and kept frozen before measurement. Hypocretin-1 in CSF was measured using radioimmunoassay kits (Phoenix Pharmaceuticals, Belmont, CA, USA) as previously reported (Nishino *et al.* 2001). The patient gave informed consent for the lumbar puncture and CSF hypocretin measurement.

Results: PSG did not show any abnormality that may cause EDS. A MSLT revealed a mean sleep latency of 2.1 min with four sleep-onset REM periods. HLA-DR2 was positive. Although this patient did not have any cataplexy events, these findings met the minimal ICSD criteria of narcolepsy. CSF hypocretin-1 was 53 pg mL^{-1} and was below normal range (250–350 pg mL^{-1}).

Conclusions: Making the diagnosis of narcolepsy in patients with EDS without cataplexy is controversial. Although previous study indicated that CSF hypocretin-1 levels in narcolepsy without cataplexy were not significantly low (Kanbayashi *et al.*, in press), our result showed decreased CSF hypoctretin-1 level in a case of EDS without cataplexy who met the minimal ICSD criteria of narcolepsy. Further research is needed in assessing the significance of CSF hypocretin among patients with EDS without cataplexy.

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DAYTIME SLEEPINESS, FATIGUE AND COGNITIVE FUNCTION IN MILD SLEEP-DISORDERED BREATHING

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Keywords: sleepiness, fatigue, cognitive functions, sleep-disordered breathing

Objective: Daytime functioning impairment related to severe sleep-disordered breathing (SDB) are frequently reported. However, few studies have investigated cognitive function in mild SDB (respiratory disturbance index, RDI > 5 and $< 30\ h^{-1}$) in which a consistent hypoxemia is rarely associated with respiratory events. The goal of the study is to assess neuropsychological functioning in relation to respiratory efforts and sleep microstructure in mild SDB.

Subjects and methods: 18 patients, 12 men and six females, aged 33.3 ± 10.8 years presenting with mild SDB, and nine normal controls, two men and seven females, aged 34.2 ± 12.4 years were selected in this study. Tree consecutive polygraphic nights were performed. The first night was an habituation night, the second night (N2) the base line night, and the third night (N3), was devoted to respiratory events assessment by means of oesophageal pressure (EP). In N2 and N3, EEG spectral analysis by Fast Fourier Transformation (FFT) and an integrated digital filtering analysis (IDFA) were performed in order to assess alpha, theta, sigma and delta bands, and sleep spindle density. Sleep was scored according to the criteria of Rechtschaffen and Kales (1968) with the help of IDFA and arousals according to the criteria of ASDA (1992). Respiratory events (RE) were detected according to the flow and the variation of EP value. Finally, respiratory efforts were assessed by the inspiratory–expiratory delta pressure (Δp) value at the beginning (Δp1) and the end (Δp2) of each RE. Statistical analysis consisted of ANOVA for repeated measures, analysis of covariance, *t*-test and stepwise linear regression analysis. Daytime functioning was assesses the day following N2. Sleepiness evaluation was done by means of the Epworth sleepiness scale (ESS) and the multiple sleep latency test (MSLT). Fatigue was assessed by two fatigue scales (Chalder fatigue scale, CFS and fatigue severity scale, FSS). Neuropsychological testing was performed by an exhaustive neuropsychological in order to assess the general functioning, the vigilance, the attention, the verbal and visual abilities, the memory and the executive function.

Results: Sleep was more fragmented in SDB patients than in controls and sigma power density was lower in patients than in controls. Patients were more sleepy and expressed more fatigue than controls, respectively, in sleepiness (13.3 ± 0.98 vs. 6.1 ± 1.38; $P < 0.01$) and Chalder fatigue scales (7.43 ± 0.8; 3 ± 1.04; $P < 0.02$) but did not significantly differ on the MSLT. Patients performed worse in neuropsychological tests than controls in tasks involving executive and motor function: trail making test form B, Purdue pegboard test (non-dominant hand NDH, simultaneous hands SH, and assembly), similarities (WAIS-R) and immediate recall of the complex figure test (Rey–Osterieth, CFT). In stepwise linear regression, assembly was best predicted by the arousal index in slow wave sleep, mean of SaO_2 nadir, SaO_2 nadir, RDI while NDH and SH were predicted by (Δp2). Similarities test was best

predicted by the RDI and the time required for immediate recall of CFT was best predicted by arousal and micro-arousal indexes, Dp2 and ESS score. On the other hand, CFS and ESS were predicted by sigma activity and SaO_2 nadir.

Conclusion: As EP values and sleep fragmentation parameters were significantly correlated and may contribute to sleepiness (as expressed by the ESS score) and/or fatigue, we hypothesize that the executive function deficit in our study may be due to a particularly state, typical of mild SDB and characterized by a combination of both fatigue and sleepiness possibly due to prefrontal and frontal cortex dysfunctioning and which cannot be explored by the MSLT.

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SLEEP ARCHITECTURE, SLOW WAVE ACTIVITY AND SLEEP SPINDLES IN MILD SLEEP-DISORDERED BREATHING

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Keywords: sleep disordered-breathing, slow wave activity, sleep spindles

Objectives: Sleep-disordered breathing (SDB) are characterised by an important sleep fragmentation related to numerous respiratory events. Sleep architecture in mild SDB ($5 < RDI < 30\ h^{-1}$) is not well known. We hypothesized that a less great number of respiratory events could fragment sleep enough to modify slow wave activity (SWA) and sleep spindle index. The aim of this study was to evaluate sleep architecture and EEG power density in mild SDB by means of spectral analysis and spindle density analysis with the help of integrated digital filtering analysis (IDFA).

Methods: 18 mild SDB subjects (six females and 12 males), aged 18–56 years with and 18 controls (11 females and seven males) aged 18–52 years were included in a 3-night polysomnographic recording protocol. The first night was an adaptation night and in night 2 (N2) and night 3 (N3), EEG spectral analysis by Fast Fourier Transformation (FFT) and an integrated digital filtering analysis (IDFA) were performed in order to assess SWA, alpha (8–12 Hz), theta (5–7 Hz), sigma (12.25–15 Hz) and delta (0.5–4.75 Hz) bands, and sleep spindle density. In addition, oesophageal pressure monitoring was performed in the third night (N3) for a best recognition of respiratory events in both groups. Sleep was scored according to the criteria of Rechtschaffen and Kales [1] with the help of IDFA and arousals according to the criteria of ASDA [2]. For each subject, SWA and EEG bands were calculated by sleep cycle, non-REM and REM period. Moreover, the cycle values of SWA, expressed as the percentage of the sleep night recording, were plotted against time at cycle midpoint and an exponential decay function with a horizontal asymptote was fitted to the data by a non-linear regression procedure. Statistical analysis consisted of ANOVA for repeated measures, analysis of covariance, *t*-test and non-linear regression analysis.

Results: Sleep analysis showed a significant higher number of awakenings < 1 min (21.2 ± 5.0 vs. 15.2 ± 5.5), and arousal indexes in total sleep time (18.0 ± 6.5 vs. 7.8 ± 6.0), in NREM sleep (24.2 ± 7.9 vs. 17.6 ± 6.0) and in SWS (7.6 ± 3.7 vs. 3.8 ± 1.4) than in controls. SWA and theta band decreased significantly from the first to the fourth cycle in both subjects. Theta and sigma bands were significantly lower in patients than in controls in each sleep cycle and during the whole night ($128.81 \pm 12.69\ \mu V^2\ Hz^{-1}$ vs. $167.5 \pm 16.4\ \mu V^2\ Hz^{-1}$ and $23.67 \pm 2.9\ \mu V^2\ Hz^{-1}$ vs. $37.73 \pm 5.6\ \mu V^2\ Hz^{-1}$, respectively). Moreover, the temporal course of SWA showed an exponential decay in both patients and controls but the time constant of the curve was significantly slower in patients (142.31 ± 42.24 min) than in controls (100.65 ± 26.47 min). Furthermore, in both groups, the sleep spindle index (SSI) was significantly lower in patients than in controls for the whole night (154.16 ± 123.14 vs. 210.55 ± 96.93), and in both SWS (99.55 ± 126.7 vs. 153.50 ± 111.46) and stage 2 (224.52 ± 169.29 vs. 319.90 ± 123.07). Finally, there was no interaction between groups and cycles or stages.

Conclusion: Sleep architecture in mild SDB subjects is characterized by a high degree of sleep fragmentation related to respiratory events. This could result in a different time course of SWA and a decreased sleep spindle index in SDB subjects when compared to controls. Sleep fragmentation could interfere with the reticulo-thalamic forward loop resulting in a modification in the genesis of SWA and a decrease of sleep spindle density.

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SECONDARY NARCOLEPSY IN PATIENTS WITH PARANEOPLASTIC ANTI-MA2 ANTIBODIES IS ASSOCIATED WITH HYPOCRETIN DEFICIENCY

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Keywords: narcolepsy, Ma proteins, hypocretin, paraneoplastic

Objective: The vast majority of narcoleptic patients suffer from the 'idiopathic' form of the disorder, which is characterised by decreased CSF and hypothalamic levels of hypocretin, possibly due to an autoimmune-degeneration of hypocretin-producing cells [1, 2]. Besides idiopathic narcolepsy, undetectable levels of hypocretin-1 have been identified in the CSF in a few severe cases of Guillain–Barré Syndrome, a well-known autoimmune disorder [2]. Narcolepsy with cataplexy has recently been described in patients with immunity to Ma2 [3]. Ma2 is the major autoantigen of a family of onconeuronal proteins that are targets of immune responses associated with paraneoplastic hypothalamic and brainstem encephalitis. We hypothesised that the frequent hypersomnia of these patients could be related to decreased synthesis of hypocretin-1. We therefore examined the levels of hypocretin-1 in the CSF of these patients.

Methods: CSF samples of six patients with anti-Ma2 associated encephalitis were obtained at the time of symptom presentation, and kept frozen until use. Analysis of hypocretin-1 was done in unextracted samples, using an standardised radioimmunoassay with a detection limit of $100\ pg\ mL^{-1}$ [2]. Investigators examining the hypocretin levels were blinded from clinical information of the patients.

Results: Clinical and demographic data are summarised in Table 1. Four patients had excessive daytime sleepiness (EDS); cataplexy was not documented. Analysis of CSF demonstrated low or undetectable levels of hypocretin-1 in the four patients with EDS. In contrast, the two patients without a sleep disorder in their history had normal levels of hypocretin-1 (Table 1).

Table 1. Clinical and demographic data

Patient no.	*Age/sex*	*Neurologic symptoms*	*Tumour type*	*Localisation of MRI abnormalities*	*Hcrt-1 (pg mL^{-1})*
1	45/M	EDS, depression, memory loss, seizures, weight gain	Non-seminomatous germ-cell tumour of testis	Mesiotemporal, ventricular enlargement, left medial temporal lobe atrophy	Undetected
2	22/M	EDS; seizures, left facial twitching, abnormal taste	Mixed germ-cell tumour of testis	bilateral temporal	Undetected
3	82/F	Personality change, nystagmus, opsoclonus, gait difficulty	Poorly differentiated non-small-cell cancer of the lung	Brainstem, periventricular region basal ganglia	237
4	67/M	EDS, diplopia, balance difficulties, weakness in left hand	Non-small-cell cancer of the lung	Right thalamus, superior colliculus, medial temporal regions (enhancing)	Undetected
5	38/M	EDS, lethargy, abulia, endocrine dysfunction, urinary incontinence, hyperthermia	Seminoma	Hippocampus and midbrain	Undetected
6	53/F	Dementia, parkinsonism. Vertical gaze limitation, nystagmus, hyperreflexia, gait difficulty	Ovarian carcinoma	Normal	218

Conclusions: (1) Some patients with anti-Ma2 associated encephalitis harbour low or undetectable levels of hypocretin-1 in the CSF. (2) This finding correlates with the presence of hypersomnia, suggesting a paraneoplastic immune-mediated hypothalamic/hypocretin dysfunction. (3) Anti-Ma2 associated encephalitis is the first identified immune-mediated disorder of the CNS which may result in low hypocretin levels; because this is a small retrospective series, further studies with larger number of patients and evaluation by sleep specialists are warranted.

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LIPID PEROXIDATION AND OSMOTIC FRAGILITY OF RED BLOOD CELLS IN SLEEP-APNEA PATIENTS

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Keywords: lipid peroxidation, sleep apnoea, osmotic fragility

Objectives: Cyclical alterations of arterial oxygen level with oxyhaemoglobin desaturation developing in response to apneas followed by resumption of oxygen saturation during hyperventilation, are observed in obstructive sleep apnea (OSA) patients. These hypoxia/reoxygenation episodes may cause an imbalance between reactive oxygen species and the anti-oxidant reserve that is important for detoxification of these molecules (1). Reactive oxygen species are generated as by-products of oxidative metabolism in mitochondria in aerobic cells. They are toxic to biomembranes and eventually lead to the peroxidation of lipids (2). On the basis of these considerations, we aimed to test the hypothesis that OSA may be linked with increased oxidative stress, lipid peroxidation and osmotic fragility of red blood cells.

Method: Lipid peroxidation was expressed in terms of thiobarbituric acid derivatives (TBARS). Sixteen subjects were included the study. Of these, six subjects were polysomnographically diagnosed as obstructive sleep apnea syndrome. Ten subjects were served as control subjects. After all subjects gave written informed consent, blood samples were collected in the morning between 08:00 and 09:00 a.m. following the polysomnography. Blood samples were immediately taken to the laboratory. Glutathione, TBARS levels and osmotic fragility of red blood cells were assessed manually. Glutathione and TBARS measurements were made in erythrocytes.

Results: The results are given in Table 1. Our results did not show any statistically significant difference in Glutathione, TBARS levels and osmotic fragility of red blood cells between OSA patients and normal controls.

Table 1. Bmi: Body-mass index; AHI: apnoea-hypopnoea index; TST < 90%: total sleep time percent with oxygen saturation below 90%

Group	*Age*	*BMI*	*AHI*	*TST < 90%*	*Glutathione*	*TBARS*
OSAS ($n = 6$)	45 ± 8	31 ± 3	37 ± 16	15.3 ± 15.0	105.7 ± 38.6	3.1 ± 2.3
Control ($n = 10$)	51 ± 14	29 ± 2	2 ± 1	0.9 ± 1.3	100.6 ± 62.1	3.2 ± 2.8
Statistics	$P > 0.05$	$P > 0.05$	$P < 0.001$	$P < 0.01$	$P > 0.05$	$P > 0.05$

Conclusions: OSA and control groups were matched in terms of age and body-mass index. OSA patients did not show increased lipid peroxidation revealed by TBARS levels and osmotic fragility of red blood cells in comparison with normal control subjects. Obstructive sleep apnea is not linked with increased oxidative stress, lipid peroxidation and osmotic fragility.

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ADAPTATION TO NOCTURNAL INTERMITTENT HYPOXIA IN SLEEP DISORDERED BREATHING: 2,3 DPG LEVELS

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Keywords: 2,3 diphosphoglycerate, nocturnal intermittent hypoxia, sleep apnoea

Objectives: The oxygenation of tissues depends on several factors such as blood flow, oxygen carrying capacity of the blood and the affinity of the haemoglobin for oxygen. The oxygen affinity of the haemoglobin is modified by three intracellular co-factors: Hydrogen ion, carbondioxide and 2,3 diphosphoglycerate (DPG). In human red blood cells, 2,3 DPG appears to be an important regulator of haemoglobin function. Elevated levels of 2,3 DPG have been noted in various states of hypoxia [1]. However, the current knowledge about physiological consequences of intermittent hypoxia and the adaptation mechanisms with special emphasis on 2,3 DPG is fairly limited. In this study, we investigated the 2,3 DPG levels as an adaptation to nocturnal intermittent hypoxia in sleep disordered breathing patients.

Methods: Eleven patients with polysomnographically diagnosed as sleep disordered breathing were included to the study: five obstructive sleep apnea (OSA) patients with apnea–hypopnea index greater than five (mean AHI: 35); six upper airway resistance (UAR) patients with apnea–hypopnea index lower than five (mean AHI:2) and respiratory disturbance index (RDI) greater than 10 (mean RDI:18). Seven healthy controls were also participated. After giving informed consent, all participants underwent a venous blood sampling performed between 07:00 and 08:00 a.m. after an overnight fast. Blood 2,3 DPG levels were measured using spectrophotometry with commercially available kit (Sigma Diagnostics-665PA). The 2,3 DPG values obtained for whole blood also used to calculate 2,3 DPG levels on the basis of packed cells as follows: [(Blood 2,3 DPG mmol mL^{-1})/(hematocrit percentage)] $\times$ 100. Comparisons between OSA, UAR and control groups were performed by using Kruskal Wallis test and Mann–Whitney *U* test.

Results: Patients with OSA and upper airway resistance syndrome (UARS) were not differed in terms of age and body mass index (BMI). The respiratory parameters and 2,3 DPG levels in OSA, UARS and control groups were given in the table. The results did not show any difference in 2,3 DPG and packed cell 2,3 DPG levels between patients with OSA, UARS and normal controls.

Groups	*Age*	*BMI*	*AHI*	*TST < 90%*	*2,3 DPG (μmol mL^{-1})*	*Packed Cell 2,3 DPG (μmol mL^{-1})*
OSAS ($n = 5$)	51 ± 7	31 ± 4	35 ± 20	18.2 ± 15.5	1.76 ± 0.51	3.86 ± 1.35
UARS ($n = 6$)	50 ± 15	28 ± 3	2 ± 1	1.5 ± 1.5	1.70 ± 0.19	3.96 ± 0.58
Mann Whitney U			$P < 0.01$	$P < 0.05$		
Controls ($n = 7$)	26 ± 7	24 ± 4			1.71 ± 0.12	3.99 ± 0.64
Kruskal Wallis	$P < 0.05$	$P < 0.05$			$P > 0.05$	$P > 0.05$

Conclusions: 2,3 DPG levels in hypoxic and non-hypoxic sleep disordered breathing did not show any significant change in nocturnal intermittent hypoxia. Further studies are needed with increased number of patients to clarify the role of 2,3 DPG. 2,3 Diposphoglycerate levels did not show any significant change in response to nocturnal intermittent hypoxia.

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HOMEOSTATIC REGULATION OF SLEEP AFTER DAILY TORPOR IN DJUNGARIAN HAMSTERS

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Keywords: daily torpor, EEG, sleep regulation, sleep deprivation

Objectives: Sleep, daily torpor and hibernation have been considered homologous processes. After daily torpor in Djungarian hamsters and after hibernation episodes in ground squirrels an increase in slow-wave activity (SWA, 0.75-4.0 Hz) in the non-REM (NREM) sleep EEG was found, which is similar to the well known SWA increase after sleep deprivation (SD) [1, 2, 7]. To investigate whether SWA after torpor was homeostatically regulated, hamsters were subjected to SD immediately after emerging from torpor. After this combined intervention, a SWA rebound was found, indicating that a sleep debt incurred during torpor, continued to increase during the SD and thereafter recovered [3]. Our aim was to further investigate the homeostatic property of SWA after torpor, by partially suppressing SWA during recovery.

Methods: Sleep was recorded in Djungarian hamsters (Phodopus sungorus) ($n = 6$ males, $n = 2$ females) implanted with EEG (frontal and parietal vs. cerebellum) and EMG electrodes and a brain thermistor to record brain temperature. The build-up of SWA during NREM sleep was reduced by disturbing the animals when they exhibited NREM sleep with high amplitude slow waves (NSD). This intervention was performed during 1.5 h either immediately after emergence from torpor (T + NSD, $n = 8$) or following 4 h SD (SD + NSD, $n = 8$). In addition, on a separate day, the animals were exposed to 4-h SD by gentle handling (SD, $n = 6$) at the beginning of the light period. They were recorded also during a 24-h baseline and a day with undisturbed torpor ($n = 8$). EEG power spectra were computed for 4-s epochs. The three vigilance states NREM sleep, REM sleep and waking were scored for 4-s epochs.

Results: SWA in NREM sleep increased significantly after torpor, *T* + NSD, SD + NSD and SD in both the parietal and frontal derivation. During the NSD, NREM sleep and SWA were significantly reduced compared to recovery after undisturbed torpor or 4-h SD. But the amount of NREM sleep did not differ between SD + NSD and *T* + NSD. Moreover, there was no significant difference between the conditions in the amount of NREM sleep or SWA in NREM sleep during the initial 1.5-h of recovery, with the exception of recovery after SD, where SWA reached higher initial values than in all other conditions. Slow-wave energy (SWE) was significantly lower during both NSD's compared to the first 1.5-h interval after torpor, and did not differ between SD + NSD and *T* + NSD. After 7.5 h no differences in SWE emerged between torpor, *T* + NSD and SD + NSD. Several differences between the conditions were observed in the time-course of SWA after its initial increase. After SD alone recovery was faster than after SD + NSD. A frontal predominance of SWA in NREM sleep was found, compared to the parietal derivation, both during recovery after torpor alone and after SD. After SD the fronto-parietal difference was restricted to the first 30 min of recovery, whereas after torpor it lasted 2 h.

Conclusions: Our results are in accordance with previous data demonstrating that torpor and SD have comparable effects on sleep and SWA in Djungarian hamsters. The SWA increase does not disappear after an additional NSD, supporting the view that a homeostatic process is involved in sleep regulation after daily torpor. The larger increase of SWA after SD in the frontal compared to the parietal derivation, is comparable to the regional differences observed in humans, rats and several strains of mice after SD [4, 5, 6]. This frontal predominance was encountered also after arousal from torpor, providing another feature which is similar between SD and torpor.

Acknowledgement: Supported by Swiss National Foundation grant 3100-053005.97.

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DECREASE IN THE CAROTID SHARE OF CEREBRAL BLOOD FLOW DURING REM SLEEP

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Keywords: cerebral blood flow, carotid artery blood flow, vertebral artery blood flow, carotid blood steal, REM sleep

Objectives: Previous studies of the effects of short-lasting bilateral common carotid artery occlusion on hypothalamic temperature in cats and rabbits [1, 2] have shown indirectly that during REM sleep a spontaneous decrease in the common carotid artery blood flow occurs which is buffered by an autoregulatory increase in the vertebral artery blood supply to the brain. Such circulatory changes raise the brain temperature as a result of a consistent decrease in both systemic and selective brain cooling [1, 2]. The present study was aimed at obtaining direct evidence of the haemodynamic changes in the common carotid artery bed affecting cerebral circulation during REM sleep.

Methods: Adult rabbits (New Zealand) were used. Chronic implantation of (i) extradural screw EEG and wire EMG electrodes and (ii) a flowmeter, placed around the common carotid artery, was carried out under general (Clonazepam: 0.5 mg kg^{-1} i.m.; sodium pentobarbital: 40 mg kg^{-1}) anaesthesia. Ear pinna temperature was also recorded by means of surface thermistors (Yellow Springs) since it indicates blood flow changes in the systemic heat exchangers of the head. The experimental sessions lasted 5–6 h at 25 ± 2°C in a sound-attenuated chamber. A grass polygraph recorded the variables under study.

Results: During the whole duration of undisturbed NREM sleep the average common carotid blood flow decreased slowly (on average up to −20% with respect to quiet waking) showing also small oscillations in amplitude. In contrast, at REM sleep onset the average common carotid blood flow diminished rapidly (on average up to −47% with respect to NREM sleep). During the REM sleep episode the flow remained low showing either small oscillations or one or a few abrupt and short-lasting surges depending on the duration of the episode. Pulsatile common carotid blood flow showed a decrease in pulse amplitude and frequency, and even short periods of clear-cut bradycardia. A transient rebound in blood flow occurred on arousal from REM sleep.

Conclusions: The present results support in particular the inferences drawn from previous studies, indirectly showing that systemic haemodynamic events underlie hypothalamic temperature changes during REM sleep [1, 2], and are in general consistent with the alteration in cardiovascular homeostatic regulation characterizing this sleep stage [3]. The blood flow decrease in the common carotid artery is so conspicuous as to bring about easily a blood 'steal' at the expense of the amount of carotid blood flowing into the circle of Willis [1, 2]. This occurrence also entails an increase in vertebral artery blood flow in REM sleep with respect to NREM sleep as an autoregulation response to the metabolic demand of the cerebral bed suddenly raised by the drop in the carotid artery share of cerebral blood flow [1, 2].

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EFFECTS OF MATERNAL CIGARETTE SMOKING ON INFANT AROUSAL RESPONSES TO SOMATOSENSORY AND CHEMOSENSORY STIMULI

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Keywords: sleep, arousal, smoking, infant

Objective: A failure to arouse to an asphyxial challenge has been implicated in the final pathway leading to the Sudden Infant Death Syndrome (SIDS). Maternal cigarette smoking is a major risk factor for SIDS. Therefore, we investigated the effects of maternal smoking on infant arousal responses to both somatosensory (air-jet) and chemosensory (mild hypoxia) stimuli.

Methods: Ten infants born to non-smoking (NS) mothers and nine infants born to smoking (S) mothers were each studied at 16–32, 68–80 and 155–175 days (d) of postnatal age. Mothers who smoked consumed 12 ± 2 (mean ± SEM) cigarettes per day while pregnant and were still smoking 14 ± 2 cigarettes per day at the time of the infant's first study. All infants were studied in both active (AS) and quiet sleep (QS) using daytime polysomnography. Each infant was born between 38 and 41 weeks gestation and was within the normal range for birth-weight. The NS and S group did not differ in demographic data, including birth-weight, and age and weight at each study. All infants were exposed to both somatosensory (a pulsatile jet of air to the nostrils) and chemosensory (15% O_2 inhalation) stimulation at each study. Hypoxia tests were terminated at either arousal, SpO_2 reaching 85% or at 5 min of hypoxia. Arousal responses to the air-jet and hypoxia were quantified using arousal threshold and arousal latency, respectively. Sleep-state effects were determined at each age within both groups using paired *t*-tests. Age effects were determined within each sleep-state for both groups using one-way repeated measures ANOVA with Student–Newman–Keuls post-hoc analysis. Arousability to both forms of stimulation was compared between NS and S infant groups (matched for sleep-state and age) using independent samples *t*-test. Significance was taken at $P < 0.05$.

Results: *Somatosensory stimuli*: A rousability of infants to the air-jet was significantly depressed in QS compared to AS in both NS and S groups at each study age, except at 16–32 days in the NS group. While postnatal age had no effect on arousability in S infants, NS infants were more arousable at 68–80 days than at 16–32 days in AS and less arousable at 155–175 days than at 16–32 days in QS. There was no significant difference in arousal threshold between NS and S infants when matched for age and sleep-state. *Chemosensory stimuli*: In accordance with responses to air-jet stimulation, arousability to mild hypoxia was significantly depressed in QS compared to AS in both NS and S infant groups at each study age. Postnatal age had no effect on arousability in either group. When matched for age and sleep-state there was no difference in arousal latency between NS and S infants.

Conclusions: Arousal responses to both somatosensory and chemosensory stimuli are similarly affected by sleep-state and are not affected by maternal cigarette smoking in infants up to 6 months postnatal age.

Acknowledgement: Supported by the Sudden Infant Death Research Foundation (South Australia) and SIDassist

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OBSTRUCTIVE SLEEP APNEA SYNDROME AND DRIVING CAPACITY IN CITY BUS DRIVERS

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Keywords: sleepiness, sleep apnea, driving

Objectives: Excessive daytime sleepiness (EDS) in drivers is a serious safety risk. In obstructive sleep apnea syndrome (OSAS) the risk of a traffic accident is significantly increased. We have reported a 20.3% prevalence of OSAS and 7.9% prevalence of severe OSAS with EDS (oxygen desaturation index, ODI4 > 30 and S1-latency in MWT < 12.9 min) in city bus drivers. We have further analysed the implications on driving performance and clinical decision-making.

Methods: 421 bus drivers of the City Transportation Department, Helsinki, Finland answered to a modified Basic Nordic Sleep Questionnaire. Twenty-two subjects with suspected OSAS [loud intermittent snoring every or nearly every night, and either sleep apneas > 1 night a week or snoring 20 years or longer, with Epworth Sleepiness Scale (ESS) score > 8] and 16 age-matched controls (snoring once a week or less often, ESS < 8) were studied by all-night polysomnography (PSG), MWT and driving simulator test (STISIM, Systems Technology Inc., USA). Definitions: EDS: S1-latency < 12.9 min in MWT, OSAS: ODI4 > 10, severe OSAS ODI4 > 30. The subjects' histories of traffic accidents in the past 10 years were reviewed.

Results: 17/22 with suspected OSAS and 4/16 of controls had OSAS on PSG. The performance in the driving test and the past history of accidents with or without OSAS did not differ. ESS score did not correlate significantly with ODI4. The S1-latency was shortest in those with OSAS and longest with no OSAS when ESS > 10. The sensitivity and specificity for severe OSAS with EDS of 1) ESS > 10 were 60 and 66%, and of 2) self-reported snoring every day or almost every day and reported apneas at least once a week, 100 and 40%. MWT S1-latency increased with age. ODI4 correlated significantly with reaction time ($F = 4.498$, $P = 0.042$), but the reaction times of subjects with OSAS and EDS did not differ from those with OSAS but no EDS, nor with controls with EDS.

Conclusions: The results illustrate the difficulty of clinical conclusions in professional drivers with OSAS and/or EDS. Questions on habitual snoring and self-reported apneas at least once a week did single out all drivers with severe OSAS and EDS, but the specificity was low. ESS > 10 was not sensitive in differentiating OSAS patients from controls, nor the driving performance, or the accident history. A significant correlation between ODI4 and the mean reaction time was found, but it did not differentiate those with or without EDS. Better tools to screen for sleep laboratory examinations and to estimate driving capacity in professional drivers are warranted.

Support: The Finnish Work Environment Fund supports this study.

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MATURATION OF THE NEONATAL SLEEP EEG – QUANTITATIVE CHANGES

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Keywords: neonatal sleep Eeg; maturation; quantitative changes

Objective: Rapid brain maturation of the foetus during the last weeks of pregnancy is reflected – beside other changes – in changes of EEG activity. In this communication a quantitative description of maturational changes of newborn EEG by the use of a computer supported analysis will be presented.

Method: 32 healthy sleeping newborn infants (11 in 32, 10 in 36 and 11 in 40 weeks of conceptional age) were recorded polygraphically (EEG 8 channels, ECG, respiration, EOG, EMG) in standardized conditions using a digital EEG apparatus in the course of 90–120 min. In each infant, a 5 min sample of EEG record from the middle part of both quiet sleep and active sleep were analysed off-line using an automatic

method developed by [1]. Numerical data of 13 characteristics describing amplitude, power in five frequency bands, average frequency and variability of EEG signal were obtained in each channel. Averaged data of each age group were compared with those of other age groups.

Results: Statistically significant developmental changes were found in nearly all characteristics. The voltage and the variability of amplitude decreased with the maturation. The power in delta and alpha bands diminished with increasing conceptional age whereas the power in theta band increased. The steepness and the sharpness of the curve were lower in full-term than in pre-term infants. The length of low-voltage segments decreased in quiet sleep but increased in active sleep with maturation. Time percentage of middle-and of high-voltage segments during quiet sleep increased from 32 to 40 weeks of conceptional age. On the contrary, time percentage of high-voltage segments as well as the incidence of these segments in active sleep rapidly decreased with increasing conceptional age. Developmental trends of high-voltage and low-voltage activity were contrary in some characteristics.

Conclusions: Our results indicate that used quantitative characteristics describe appropriately developmental changes of sleep EEG between 32–40 weeks of conceptional age. Quantitative automatic analysis of newborn EEG may contribute to the objective assessment of brain dysfunction manifested as EEG dysmaturity.

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HYPOCRETIN LEVELS IN RAT CSF AFTER PARADOXICAL (REM) SLEEP DEPRIVATION

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Keywords: hypocretin, paradoxical sleep, sleep deprivation, CSF

Objective: The objective of this study was to examine the effects of 96 h of paradoxical (REM) sleep deprivation on CSF hypocretin-1 (HCRT-1) levels in rats at two circadian times.

Method: Male Wistar rats (3 month old) were deprived of paradoxical sleep for 96 h using the classical platform method [1]. Animals were kept under a 12:12 light dark cycle. The deprivation period was initiated at two different zeitgeber times (ZT), 7:00 a.m. (light on – ZT 0) or 3:00 p.m. (ZT 8). After 96 h of REM sleep deprivation CSF was collected in a first set of animals (Paradoxical sleep deprivation group). A second set of animals were returned to the home cages and allowed to sleep for 24 h before CSF samples were collected (sleep rebound group). The control group remained in their home cages throughout the experiments. We perform cisternal CSF taps using a 1 mL syringe connected to a 27G needle under halothane anesthesia. CSF aliquots were then frozen immediately over dry ice and stored at −80°C until analysed. For peptide level measurements, we used the commercially available 125I RIA Kit (Phoenix Pharmaceutical, Mountain View, CA, USA).

Results: As previously reported [2] basal CSF HCRT-1 levels were higher at ZT0 (end of activity/dark period) vs. ZT8 (end of rest/light period). Paradoxical sleep deprivation was associated with increased levels when CSF was collected at ZT8, not ZT0. Most strikingly, REM rebound was associated with decreased levels at both ZTs. The results can be seen in the table:

Group	*HCRT-1 (ng mL^{-1}) at ZT O*	*HCRT-1 (nhg mL^{-1}) at ZT 8*
Control	2.14 ± 0.35 (12)	1.27 ± 0.54 (9)
PS Deprivation	2.20 ± 0.35 (15)	2.18 ± 0.45 (11)*
Rebound	1.68 ± 0.43 (9)*	0.66 ± 0.09 (10)*

The values are mean ± SD. Number of subjects in parenthesis. *Statistical significant difference from the respective control. $F = 25.2$, $P < 0.05$ by Duncan's test.

Conclusion: PS deprivation increases HCRT-1 when basal levels are low, during the rest phase. REM rebound is associated with decreased levels at all zeitgeber times. The lack of increase at ZT0 parallels data found after food deprivation, suggesting a possible 'ceiling effect' for high CSF values. These data strengthen the participation of HPCT in PS regulation. Moreover the data show that 24 h of sleep recovery is sufficient to reverse the PS effect on HCRT levels, since the levels of both rebound groups (ZT0 and ZT8) are even lower than in control groups. These increased levels of HPCT after PS deprivation may account for some behavioural consequences of PS deprivation such as increased food consumption, loss of weight and antidepressant effects.

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Financial support: AFIP, FAPESP grant no. 9814303–2 and NIH grant no. NS23724.

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SLEEP LIKES STRUCTURE! INFORMATION-DEPENDENT CEREBRAL REACTIVATIONS DURING POST-TRAINING REM SLEEP

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Keywords: implicit learning, memory consolidation, REM sleep, PET

Background: Experimental observations support the hypothesis that sleep participates to the processes of brain plasticity and memory consolidation. Using positron emission tomography (PET), we showed in humans that some of the brain areas needed for the execution of a serial reaction time (SRT) task are more active during the subsequent REM sleep in subjects trained to the task than in non-trained subjects [1]. Further to the analysis of behavioural data, we surmise that these experience-dependent cerebral reactivations during post-training REM sleep relate to the re-processing of memory traces at two potential levels. On the one hand, since global reaction time (rT) improved with practice and overnight in the trained group, the experience-dependent reactivation may merely

subtend the optimization of the visuo-motor network needed to fasten the appropriate motor response to the visually displayed stimulus. On the other hand, unbeknownst to participants, the sequence of stimuli was driven by a set of complex rules during the SRT task. Albeit not consciously aware of their presence, results demonstrated that subjects took advantage of the rules of the sequence to prepare and fasten their motor response to the next stimulus, thus demonstrating unconscious learning of higher-order, abstract, knowledge about the sequence of stimuli. Therefore, cerebral reactivations during REM sleep may rather support the reprocessing of the higher-order information about the structure of the sequential material to be learned.

Objective: to specify the level of information processing to which correspond experience-dependent cerebral reactivations during post-training REM sleep.

Methods: Six subjects were trained to the SRT task between 16:00 and 20:00, then scanned during the post-training night using H215O PET. At variance with the trained group in our prior study (group 2 in [1]), the sequence of stimuli was at random during the SRT task. The experimental protocol was identical in all other aspects. Consequently, both trained groups underwent simple visuo-motor learning (i.e. global rT improvement with practice) but only the trained group in our prior study learned complex rules underlying the sequence of stimuli during the SRT task and may possibly reprocess this abstract information during post-training sleep.

Analysis: PET data were spatially transformed and analysed using SPM99 (http://www.fil.ion.ucl.ac.uk/spm). The statistical analysis included data from the [1] study and consisted in three steps: (1) finding a [group (rules trained group vs. random trained group) by condition (REM sleep vs. wakefulness)] interaction to evidence the brain areas more active during post-training REM sleep (as compared to wakefulness) in subjects having practised a structured sequence of stimuli rather than a random one; (2) finding a conjunction between this set of areas and the areas activated by the execution of the SRT task (group 1 in [1]) to ensure that these areas already participated to the SRT performance at wake; and (3) exclude from this analysis the brain areas already active during normal REM sleep, i.e. without prior SRT training (group 3 in [1]). Hence the analysis intended to evidence the brain areas both active during SRT actual practice and re-activated during post-training REM sleep as a function of the presence/absence of a structure in the sequential material.

Results: The analysis disclosed a significant effect ($P < 0.05$, corrected for multiple comparisons) whereby during post-training REM sleep, rCBF increased more in the thalamus, the occipital areas (cuneus) and the premotor cortex, in subjects trained to the probabilistic material than in subjects trained to the random sequence.

Conclusions: Our results support the hypothesis that experience-dependent cerebral reactivations during post-training REM sleep underlie the reprocessing of elaborated information about the structure of the sequential material to be learned. Moreover, it shows that cerebral reactivations are not merely experience-dependent, but also information-dependent. Indeed, the content of the material to be learned exerts an influence on the reprocessing of memory traces during sleep.

Support: FNRS, FMRE, ULg and CRC special funds.

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SLEEPING BRAIN, LEARNING BRAIN. CONTRIBUTIONS OF POSITRON EMISSION TOMOGRAPHY TO THE STUDY OF THE RELATIONSHIPS BETWEEN SLEEP AND MEMORY

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Keywords: brain plasticity, memory consolidation, cerebral reactivation, PET

Sleep has been implicated in the plastic changes that underlie learning and memory. Indications that sleep participates in the consolidation of recent memory traces come from a wide range of experimental observations, from complementary approaches in neuroscience including computational, animal and human studies, from the neurophysiological to the neuropsychological level (for review: [1, 2]). In the last decade, positron emission tomography (PET) has proven a powerful technique. This brain imaging method, which offers the advantage of recording *in vivo* the global and regional modifications of human brain haemodynamics in a non-invasive manner, has contributed in several ways to improve our understanding of normal human sleep. First, PET studies have allowed a macroscopic description and demonstrated the regional specificity of the functional neuroanatomy of human sleep stages. Second, these studies have been fruitful in supporting evidences that similar fundamental mechanisms for slow wave and REM sleep generation occur in man and non-human animals. Third, PET studies have also supported the hypothesis that sleep participates to the processes of brain plasticity and memory consolidation. In this talk, a brief overview on the PET technique and PET and sleep in general will be given, followed by a survey of PET sleep studies relevant to the topic of brain plasticity and memory consolidation during human sleep. The aim is to discuss the PET contribution to the functional neuroanatomy of normal human sleep and its potential significance in terms of underlying cognitive and plastic processes.

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EARLY IRON DEFICIENCY ANAEMIA AFFECTS THE MODULATION OF MOTOR ACTIVITY IN SLEEPING INFANTS

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Keywords: motor activity, sleep, iron deficiency anaemia, infancy

Introduction: Iron-deficiency anaemia (IDA) in infancy affects the patterns of spontaneous motor activity in waking episodes [1]. IDA infants show a reduced frequency of leg movements compared with non-anaemic controls. Since IDA alters the functioning of several central neurotransmission systems, neuronal metabolism, and both quantity and quality of myelin, it may disrupt the modulation of motor activity during sleep as well. In fact, several studies have established relationships between IDA and motor alterations while awake (restless leg syndrome) and/or asleep (periodic leg movement

syndrome). We studied the potential effects of IDA in infants by assessing the amount and patterns of motor activity as a function of sleep states.

Methods: Naturally occurring naps were recorded polysomnographically in the laboratory in a group of otherwise healthy 6-months infants who presented with iron-deficiency anaemia IDA ($n = 24$) or were non-anaemic (controls, $n = 22$). Sleep states were determined by combining usual EEG, EOG and EMG criteria; in addition, NREM stages I, II and III + IV (SWS) were subdivided by using EEG criteria. Motor activity of both upper and lower limbs (UL, LL) was recorded independently by piezo-electric crystal transducers, and processed off-line by an automated processing system. For each infant the following parameters were determined as a function of REM sleep and NREM sleep stages: (a) percentage of time spent moving (TSM), (b) duration of movements, (c) percentage of time without movements (TWM) and (e) duration of episodes without movement.

Results: In IDA infants, the TSM was higher during SWS for LL ($P < 0.02$), and the duration of movements was longer during NREM2 for UL. In contrast, the TWM was higher in controls during NREM 2 for both UL ($P < 0.05$) and LL ($P < 0.04$), and during SWS for LL ($P < 0.02$). The same was also apparent for the duration of episodes without movements during NREM 2 ($P < 0.05$) for UL, and in all NREM stages for LL (at least $P < 0.05$). During REM sleep no difference between groups was statistically significant.

Conclusions: Our results show that early IDA alters the motor activity patterns in sleeping infants. Differences between groups are restricted to NREM stages, without affecting REM sleep. Since NREM sleep is markedly restructuring throughout the first months of postnatal life, this sleep state may be more vulnerable to IDA effects. As a whole, these results suggest that iron could be critical for establishing the patterning of activity/inactivity modulation within NREM stages.

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IRON DEFICIENCY ANAEMIA IN INFANCY REDUCES EYE MOVEMENTS WITHIN REM SLEEP IN CHILDHOOD

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Keywords: REM sleep, eye movements, iron deficiency anaemia, childhood

Introduction: The organization of sleep states considered to be a functional expression of central nervous system (CNS) maturation and integrity, has been used as a sensitive predictor of neurobehavioural and cognitive development. REM sleep changes, manifested as either a modification in the duration of episodes or in the density of eye movements, appear to correlate with cognitive performance. Because iron is essential for several CNS functions (myelination, neurotramission and neuronal metabolism), the presence of iron-deficiency anaemia (IDA) during infancy might disrupt developmental REM sleep patterns. We studied the potential long-lasting effects of early IDA by assessing eye movements within REM sleep.

Methods: All-night polysomnographic recordings were done in a group of healthy 4-year-old children who were treated for IDA ($n = 24$) or non-anaemic (controls, $n = 24$) in infancy. Sleep states were determined by combining usual EEG, EOG and EMG criteria. The EOG signal was processed off-line in order to identify eye movements during REM sleep. For each individual child the number and duration of eye movements and the percentage of REM sleep spent with eye movements were determined. In order to normalize the duration of REM sleep episodes, the number of eye movements was calculated per 1-min of REM sleep.

Results: The organization of eye movements within REM sleep episodes differed between groups. The percentage of REM episodes containing eye movements was higher in controls than in former IDA children ($P < 0.0003$). The same was also true for the number of eye movements ($P < 0.004$), but without differences in their duration.

Conclusions: Our results indicate that IDA in infancy is associated with a reduction in the number of eye movements during REM sleep in childhood. These results suggest that in addition to its role in the temporal organization of REM sleep throughout the night-time [1], iron is also involved in establishing eye movement patterning within REM sleep. These REM features may well represent a contributing factor to altered developmental outcome and cognitive performance in formerly IDA children.

Support: Grants from NICHD (HD33487) and Fondecyt (CONICYT, Chile 1000657).

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INCREASED CARDIOVASCULAR MORTALITY IN OBSTRUCTIVE SLEEP APNOEA: A 7-YEAR FOLLOW-UP

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Keywords: sleep apnoea, cardiovascular, mortality

Introduction: We have previously demonstrated an increased mortality in patients with coronary artery disease and co-existing obstructive sleep apnoea (OSA) [1]. It is unclear if OSA subjects without a co-existing heart disease are at high risk of mortality.

Methods: A consecutive sleep clinic cohort of 294 middle-aged subjects (232 men, 62 women, mean age 49.1 ± 9.9, range 30–69 years at baseline in 1991) with or without OSA, and without a known heart disease, were identified. The time and the cause of death were obtained from the Swedish Hospital Discharge Register covering a 7-year period prior to 31 December 1998 as well as the National Cause of Death Registry. Effectiveness of OSA treatment initiated during the period as well as age, body-mass-index (BMI), systemic hypertension (SHT), diabetes mellitus (DM), asthma, chronic obstructive lung disease (COPD) and smoking habits were controlled.

Results: Overall mortality was 5.8% and cardiovascular mortality 2.9%. Nine out of 65 subjects (13.9%) with incompletely treated OSA died during the follow-up period, and the cause of death was cardiovascular in eight cases (12.3%). One of the 26 efficiently treated OSA subjects died (in malignancy). In the non-OSA group ($n = 203$), eight cases (3.9%) were found to be deceased, and the cause of death was cardiovascular in one case (0.5%). In a multivariate analysis, using Poisson model without regard to effectiveness of OSA treatment, OSA at baseline was a significant predictor of mortality (RR 14.83, 95% confidence interval [CI] 1.8–120, $P = 0.012$). In the OSA group,

efficient treatment reduced the mortality risk (RR 0.23, CI 0.06–0.95, $P = 0.004$) after adjustment for the univariate predictors (time interval between inclusion and death, astma or COPD, diabetes and systolic blood pressure at baseline).

Conclusion: The risk of mortality was increased in middle-aged OSA subjects without a preceding heart disease at baseline. The excess mortality was predominantly of cardiovascular origin. Efficient treatment of OSA eliminated the excess risk of cardiovascular mortality.

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A COMPARISON OF ALGORITHMS FOR THE DETECTION OF SLEEP RELATED BREATHING DISORDERS BASED ON ECG RECORDINGS

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Keywords: sleep related breathing disorders, heart rate, diagnostic methods, algorithms, obstructive sleep apnoea

Objectives: Sleep related breathing disorders are accompanied by cyclical variations of heart rate. We conducted an international competition that investigated whether sleep related breathing disorders, such as apnea, can be detected from long-term ECG recordings.

Methods: Seventy ECG recordings (401–578 min in duration) collected for diagnostic sleep studies were used for the competition. Each minute of each recording was visually labelled as a period of normal or disordered breathing by an expert. Periods of disordered breathing contained apnea or hypopnea were labelled based on oronasal airflow, respiratory movement (Respitrace), and pulse oximetry. In addition, each recording was labelled as 'control', 'borderline', or 'apnea'. The control group consisted of 20 recordings from healthy volunteers who had less than 5 min of disordered breathing. The borderline group consisted of 10 recordings from subjects who had between 5 and 100 min of sleep apnea per night. The apnea group consisted of 40 recordings from subjects who clearly had sleep apnea for 100 min or more per night. All 70 ECG recordings were sampled at 100 Hz and made available to participants at http://physionet.org/physiobank/database/apnea-ecg. The recordings were split into two groups of equal size: a training set and a test set. Participant were given the expert's minute-by-minute labels and overall classification for each recording in the training set. The competition consisted of two challenges: (a) class the 30 non-borderline recordings of the test set as 'control' or 'apnea' and (b) label each minute of all 35 test recordings as containing apnea or not. Participant submitted their record identifications and minute labels to an automatic web-based scoring program. Participant received scores for each entry via email and were allowed a limited number of resubmissions until 20 September 2000.

Results: Twelve groups entered the first challenge. Participants used a wide range of techniques including spectral analysis of heart rate, wavelet transforms, and Hilbert transforms. Some participants used time domain features. Four algorithms, which were based on Fourier and wavelet analysis, were able to identify all 30 subjects in the test set correctly. Eight participants labelled the recordings on a minute-by-minute basis. The best algorithm achieved a classification that was consistent with the expert labels on 92.6% of the minutes in the test set. The worst entry achieved a score of 84.5%.

Conclusions: This competition demonstrated that subjects with sleep related breathing disorders can be identified from ECG recordings alone with remarkable accuracy. The minute-by-minute detection accuracy of these algorithms was unexpected and probably reaches the rate of consistency among experts who have access to additional signals including respiration, nasal airflow, and pulse oximetry. Artifact and arrhythmias such as ectopic beats did not significantly affect the algorithms' accuracy. These results demonstrate the potential for commercial implementation of algorithms that recognize disordered breathing during sleep in long-term ECG systems with good accuracy.

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DIURNAL VARIATIONS IN C-FOS PROTEIN IN RAT HYPOTHALAMIC NUCLEI: EFFECTS OF GENDER AND SLEEP DEPRIVATION

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Keywords: C-FOS, oestrogen, testosteron, gender, hypothalamus, diurnal rhythm, sleep deprivation

C-fos protein is an activity dependent transcription factor and thus marker of neuronal activity. Diurnal and sleep-associated variations in the expression of C-fos protein are well documented in particular hypothalamic nuclei, such as the suprachiasmatic nucleus (SCN) and the ventrolateral preoptic nucleus (VLPO), in male rats [1]. The aim of our experiments was to study diurnal variations in c-fos expression in various hypothalamic/preoptic nuclei [arcuate nucleus (ARC), SCN, VLPO, anterodorsal preoptic nucleus (ADP), lateroanterior hypothalamic nucleus (LA), and paraventricular nucleus (PVN)] in both male and female rats. Female rats were studied during estrus and diestrus, after ovariectomy (OVX), and after ovariectomy + estradiol (10 mg kg^{-1}) treatment (OVX + E). The rats were adapted to a 12–12 h light–dark cycle, and they were sacrificed hours 8 after light or dark onset. The effects of sleep deprivation on c-fos were studied only in male rats as follows: 8-h SD starting at light onset, 5-h SD starting at light onset plus 3-h recovery, and 3-h SD in hours 6–8 after light onset (each SD group was sacrificed at hour 8 after light onset). SD was performed by gentle handling, and the EEG and motor activity was recorded throughout the SD and during the recovery. The groups of male and female rats included eight and four rats, respectively. Immunohistochemistry was performed on coronal sections through the hypothalamus using polyclonal rabbit anti-rat c-fos antibody (Santa Cruz Biotechnology, CA, USA; 1 : 10000) and the reaction was visulized by peroxidase-conjugated avidin–biotin complex (ABC, Vector Laboratories Burlingame, CA, USA). Nuclear grains were counted in 100×100 μm frames. C-fos was determined in three sections for each region, and these values were averaged. A mean count ± SE was calculated for each region in each group, and one-way

ANOVA followed by the Student–Newman–Keuls test was used for statistical comparisons. In the male rats high c-fos expression occurred during the light (sleep) period in the VLPO and the SCN, and in the dark (wake) period in the ARC and the ADP. Diurnal variations were not found in the PVN and the LA. C-fos expression was significantly decreased in both the VLPO and the SCN after 8 and 3-h SD. C-fos remained significantly suppressed in these nuclei after 3-h of recovery following a 5-h SD. In contrast, SD significantly stimulated c-fos in the PVN, ARC, ADP, and the LA, and c-fos returned to normal after 3-h recovery in each of these nuclei. In cyclic females, c-fos expression was significantly weaker than in males in the nuclei as follows: VLPO during light, SCN during light, ARC during dark, and ADP during dark. LA was the only area where c-fos expression was significantly higher in females than in males. In cycling females, diurnal variations in c-fos were significant in the SCN and ARC whereas the day–night difference did not reach the level of statistical significance in the VLPO and the ADP though the tendency was obvious. OVX was followed by significant decreases in c-fos expression in the VLPO and the SCN resulting in a complete loss of diurnal rhythm in these nuclei. In contrast, OVX had modest or no effects on c-fos expression in the PVN, ARC, LA, and ADP. Administration of oestrogen to OVX rats significantly stimulated c-fos expression resulting in a male-type expression in almost all nuclei. Thus, the differences between males and females disappeared in the ADP, ARC, and VLPO, though oestrogen also increased c-fos at night in the VLPO. The rhythm recurred in the SCN and the VLPO, and c-fos level was increased in the PVN (light) and LA. The results suggest that c-fos expression is highly testosterone/oestrogen dependent in the hypothalamic nuclei including those implicated in the regulation of sleep (VLPO) and circadian rhythm (SCN). The SD-induced increases in c-fos in the PVN and the LA might be stress-related because similar increases in c-fos did not occur at night. The high c-fos after SD in the ARC and the ADP might be associated with some aspects of wakefulness for the SD-induced c-fos did not differ from the c-fos expression occurring at night in these nuclei.

Acknowledgements: Supported by the Hungarian National Science Foundation (OTKA T-30456, and T-29979), and the National Institute of Neurological Disorders and Stroke Grants, USA (NS 27250).

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TRANSCRIPTIONAL REGULATION OF GENES RELATED TO GLYCOGEN METABOLISM BY ADENOSINE: POSSIBLE INVOLVEMENT IN THE SLEEP–WAKE CYCLE

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Keywords: adenosine, sleep deprivation, astrocyte, glycogen, hippocampus slice

Objective: In the central nervous system, glycogen is the major energy reserve and it is predominantly localized in astrocytes. We have demonstrated that NA and VIP exert, in addition to their classical glycogenolytic effects, a long-term control on glycogen levels in cultured astrocytes, giving rise to a massive glycogen resynthesis which takes place over several hours. It was also demonstrated that the same neurotransmitters induced the expression of Protein Targeting to Glycogen (PTG) mRNA which has been shown to promote glycogen synthesis in peripheral tissues [1]. Interestingly, effects occur through the activation of the cAMP pathway which is known to be also activated by adenosine in these cells. We sought to determine whether (i) adenosine, like NA and VIP, induces glycogen resynthesis and PTG mRNA expression in primary astrocytes cultures; (ii) such a mecanism exists in organotypic slice cultures where anatomical organization is preserved; (iii) PTG mRNA levels are modified by sleep deprivation, a situation known to increase extracellular adenosine in the brain [2].

Methods: In vitro *experiments*: Primary culture of cortical astrocytes were prepared from neonatal OF1 mice. Levels of PTG mRNA expression were determined by Northern blot and glycogen levels were assayed using a fluorimetric method [3]. Adenosine was applied at 100 mM during 4 h for Northern blot analysis and 8 h for glycogen assay. When required, cells were exposed for 30 min to cycloheximide (10 mM) and actinomycin D (5 mM) before adenosine treatment. Hippocampal slice cultures from 11 days old rats were prepared according to the method of Stoppini *et al*. [4]. 2-Chloro-adenosine, an analogue of adenosine, was applied at 100 mM during 3 h for Northern blot analysis. In vivo *experiments*: Two groups ($n = 6/8$) of adult male OF1 mice were used. One group was sacrificed after 6 h of 'gentle' sleep deprivation beginning at ZT0 and the control group was sacrificed at ZT6 after undisturbed sleep. PTG mRNA levels were determined in the cerebral cortex of each animal by Northern blot and *in situ* hybridization analysis. Glycogen synthase activity was also assayed in cortical samples using the method of Thomas *et al*. [5].

Results: Using primary culture of cortical astrocytes, we have observed that adenosine induces an important glycogen resynthesis with a maximum reached after 8 h of exposure. Glycogen levels were increased up to four times compared to the initial levels. It could be demonstrated that the effect was clearly concentration-dependent ($EC50 = 9.7 \pm 3.3$ mM). This effect could be blocked by addition of cycloheximide and actinomycin-D indicating that transcription and protein synthesis are required. Moreover, adenosine induced the expression of PTG mRNA in a time dependent-manner concomitant with the enhancement of glycogen levels, suggesting the involvement of this protein in glycogen resynthesis. In addition, preliminary data from hippocampal slices also show that 2-chloro-adenosine increases PTG mRNA levels. In sleep deprived animals, a twofold increase of PTG mRNA ($194 \pm 42\%$ of control, $P < 0.05$) was observed. This result was confirmed by *in situ* hybridization. Interestingly, in the same condition, the activity of glycogen synthase was increased by 2.5-fold ($P < 0.006$).

Conclusion: These data suggest that increased PTG mRNA expression by adenosine could represent a major event leading to enhancement of glycogen levels *in vitro*. Since similar data are observed following sleep deprivation, adenosine might be one of the neurotransmitters involved in cerebral energy metabolism adaptation accompanying the sleep–wake cycle in mice.

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