Narcolepsy - a sleep-wake disorder and beyond

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HABILITATIONSSCHRIFT

Narcolepsy – a sleep-wake disorder and beyond

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To my husband Charles and my son George

SUMMARY

Narcolepsy is a chronic sleep-wake disorder characterized by excessive daytime sleepiness (EDS) and sudden loss of muscle tone triggered by emotions (cataplexy). Further symptoms include sleep paralysis, hypnagogic hallucinations and fragmented night-time sleep. Diagnosis of narcolepsy is clinical and supported by three different biological markers: first, the electrophysiological documentation of two or more sleep onset REM periods on the multiple latency test; second, the genetic predisposition with HLA DQB1*0602 allele positivity and third, the loss of the hypothalamic peptide hypocretin (also called orexin).

As much as several etiological, pathophysiological and clinical characteristics of narcolepsy were clarified over the past 15 years, lots of questions remain and newly emerge. The majority of them are related to the discovery of the hypocretin system and the hypocretin deficiency in narcolepsy. Hypocretins are highly excitatory neuropeptide hormones, produced in the perifornical area of the lateral hypothalamus. They have been implicated in maintenance of wakefulness, emotional processing, especially reward, energy homeostasis and learning.

In our previous work, we tried to link hypocretin deficiency in narcolepsy to external factors, such as traumatic brain injury. We studied manifestations of narcolepsy, which spread beyond the classical sleep-wake disorder symptoms, including a specific form of cataplexy, time perception and emotional processing, with emphasis on humour, reward and emotional learning. Using clinical, electrophysiological and neuroimaging methods, we tried to find the functional, metabolic and structural correlates of these manifestations, and link them to hypocretin deficiency. We also applied manipulation of sleep pressure in order to study homeostatic mechanisms of narcolepsy.

This cumulative habilitation thesis is based on the following publications:

1. Poryazova R, Hug D, Baumann CR (2011). Narcolepsy and traumatic brain injury – cause or consequence? Sleep Med 12:811
2. Poryazova R, Werth E, Parrino L, Terzano MG, Bassetti CL (2011). Cyclic alternating pattern in narcolepsy patients and healthy controls after partial and total sleep deprivation. Clin Neurophysiol 122:1788-1793
3. Poryazova R, Khatami R, Werth E, Bassetti CL (2009). Weak with sex: sexual intercourse as a trigger for cataplexy. J Sex Med 6:2271-2277
4. Poryazova R, Mensen A, Bislimi F, Huegli G, Baumann CR, Khatami R (2013). Time perception in narcolepsy in comparison to patients with Parkinson's disease and healthy controls – an exploratory study. J Sleep Res (Epub ahead of print)
5. Schwartz S, Ponz A, Poryazova R, Werth E, Boesiger P, Khatami R, Bassetti CL (2008). Abnormal activity in hypothalamus and amygdala during humour processing in human narcolepsy with cataplexy. Brain 131:514-522
6. Ponz A, Khatami R, Poryazova R, Werth E, Boesiger P, Schwartz S, Bassetti CL (2010). Reduced amygdala activity during aversive conditioning in human narcolepsy. Ann Neurol 67:394-398
7. Ponz A, Khatami R, Poryazova R, Werth E, Boesiger P, Bassetti CL, Schwartz S (2010). Abnormal activity in reward brain circuits in human narcolepsy with cataplexy. Ann Neurol 67:190-200
8. Poryazova R, Schnepf B, Werth E, Khatami R, Dydak U, Meier D, Boesiger P, Bassetti CL (2009). Evidence for metabolic hypothalamo-amygdala dysfunction in narcolepsy. Sleep 32:607-613
9. Schaer M, Poryazova R, Schwartz S, Bassetti CL, Baumann CR (2012). Cortical morphometry in narcolepsy with cataplexy. J Sleep Res 21:487-494

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1. INTRODUCTION

Narcolepsy with cataplexy (NC) is characterized by excessive daytime sleepiness (EDS) and sudden loss of muscle tone, triggered by emotions (cataplexy)[1]. Additional symptoms include hypnagogic hallucinations, sleep paralysis and fragmented night-time sleep. NC is a debilitating sleep-wake disorder, affecting between 25 and 50 per 100 000 people. Diagnosis of NC is clinical, yet a striking association with different biological markers has been observed. First, the presence of two sleep onset REM periods on multiple sleep latency test supports the diagnosis of NC[2]. Second, there is a strong association with the HLA system, with the genetic marker HLA DQB1*0602 being found in 98% of patients with NC compared to only up to 35% of healthy controls[3]. Third, a decrease or loss of the hypothalamic peptide hypocretin (also called orexin) is found in NC[4-6].

The discovery of the hypocretin system[7, 8] and its impairment in NC[4] triggered extensive studies in human subjects and in animal models. Hypocretins have been implicated in maintenance of wakefulness[9], emotional processing, especially reward[10, 11], energy homeostasis[12] and learning[13]. Narcolepsy with cataplexy is more than a sleep-wake disorder with manifestations extending beyond sleep-wake impairment.

NC is associated with hypocretin cell loss, yet the aetiology of this loss remains unclear. Because of the strong association with the HLA system autoimmune mechanisms have been discussed[14], however, neurodegenerative processes might also be involved[15]. External factors, such as traumatic brain injury (TBI) might also play a role, as excessive daytime sleepiness belongs to the most common sleep-wake disturbances after TBI[16].

Hypocretin cell loss alludes to hypothalamic dysfunction in NC. Human post-mortem studies have shown a drastic reduction of hypocretin peptides[4, 17] and hypocretin-producing

neurons and axons in the hypothalamus[4, 18, 19] as well as signs of gliosis[4]. These neurons have widespread excitatory projections in the brain[20] and interact with other neurotransmitter systems. An important interplay is the one with the dopaminergic system. Hypocretin efferents from the lateral hypothalamus innervate midbrain dopaminergic nuclei, and dopamine cell bodies express hypocretin receptors[20, 21]. Activation of hypocretin receptor-expressing neurons in the ventral tegmental area (VTA) leads to direct activation of mesolimbic dopamine neurons and is probably associated with the development of rewarding effects[22]. Intra-VTA administration of hypocretin increases dopamine levels within the prefrontal cortex[23]. Prefrontal cortex dopaminergic neurotransmission plays an important role in a variety of higher-order cognitive and affective processes, including learning under appetitive and aversive conditions[24], working memory[25], and attention[26].

Another important area abundant in hypocretin projections is the amygdala. In canine narcolepsy, neurodegeneration and gliosis have been reported not only in the hypothalamus, but also in the amygdala[15]. Different observations suggest the possibility of an involvement of the amygdala in cataplexy. For example, electrophysiological studies in narcoleptic dogs demonstrated changes in neuronal firing in the amygdala during cataplexy[27]. The amygdala is also strongly activated during REM sleep[28] and involved in emotional processing[29].

Pathophysiologically, dysfunctional REM sleep regulation is suggested in NC, including a disinhibition of REM sleep (as suggested by the occurrence of sleep onset REM periods), the appearance of dissociated REM-like states such as cataplexy (REM atonia during wakefulness) and hallucinations (REM mentation during wakefulness). However, NREM sleep regulation is also essential in NC as insufficient NREM sleep intensity with rapid decline of slow wave activity and increased sleep fragmentation during the second sleep cycle in NC have been reported[30].

Based on the available evidence, it is plausible to assume an impairment of a number of neuronal structures and networks in human narcolepsy, such as hypothalamus, amygdala, ventral tegmental area and prefrontal cortex. Knowing the functional involvement of these structures, changes in higher cognitive functions (including time perception), and emotional processing (including reward, humour and aversive conditioning) can be expected in human narcolepsy. Using clinical assessment, different behavioural paradigms, electrophysiological and neuroimaging techniques and combining these different methods allows to further explore the various manifestations of NC and their morphological substrate.

2. AIMS OF THE STUDIES

The overall aim of the presented research was to clarify different etiological, pathophysiological and clinical aspects of NC.

The specific aim of the presented studies can be summarized as follows:

1. To explore the link between NC and traumatic brain injury (*study 1*)

2. To investigate NREM sleep homeostasis using manipulation of sleep pressure (*study 2*)

3. To assess a special form of cataplexy, namely cataplexy during sexual intercourse (orgasmolepsy) (*study 3*)

4. To assess time perception in NC (*study 4*)

5. To assess emotional processing in NC and its neural basis (*studies 5-7*)

6. To identify metabolic correlates of hypothalamus, amygdala and tegmental dysfunction in NC (*study 8*)

7. To quantify structural brain changes associated with narcolepsy (*study 9*).

3. MATERIALS AND METHODS

3.1 Clinical assessment

We assessed all NC patients, participating in the nine studies, presented in this work, using a detailed clinical interview and different validated questionnaires. Diagnosis was based on complaints and symptoms, neurological examination, video-polysomnography and multiple sleep latency test. All patients were positive for HLA DQB1*0602. As a part of the diagnostic process, cerebrospinal fluid hypocretin measurements were available in the majority of the patients.

We used several questionnaires including the Zurich sleep questionnaire, the Epworth sleepiness scale, the Sleep Apnoea Scale of the Sleep Disorders Questionnaire, the Ullanlinna Narcolepsy Scale, the Swiss Narcolepsy Scale, and the Stanford Cataplexy Questionnaire.

The Zurich sleep questionnaire includes 69 questions addressing sleep–wake habits and complaints. The answers provide information on symptoms/signs suggestive of sleep apnoea, EDS, narcolepsy, different parasomnias, insomnia, and disturbances of the sleep–wake rhythm. Possible answers include “yes” and “no” or provide a rating on a 5-point scale depending on the frequency of occurrence (“almost always”, “often”, “occasionally”, “seldom” or “never”).

Four validated scores are included in the questionnaire. The Epworth sleepiness scale is a scale for assessment of subjective EDS based on eight questions on the urge to sleep in various life situations[31]. Each question is rated on a 4-point scale, ranging from 0 to 3 points, thus resulting in a maximum sum score of 24 points. A score of 10 or above is considered abnormal.

The Sleep Apnoea Scale of the Sleep Disorders Questionnaire is a screening instrument for obstructive sleep apnoea. It consists of 12 items for sleep-related breathing disorders, and includes the well-known risk factors of age and body mass index. Cut-off scores of 32 for women and 36 for men (range is 12–60) provide a sensitivity of 85% and a specificity of 72% for males and 88% and 81% for females, respectively[32]. The 11-item Ullanlinna Narcolepsy Scale is a widely used screening instrument for narcolepsy[33]. A cut-off of 14 has been shown to have a high sensitivity (100–96%) for patients with NC but specificity is variable (99–56%)[34]. The Swiss Narcolepsy Scale is based on five questions; a score <0 had a sensitivity and specificity for NC of 96 and 98%, respectively[34]. The Stanford Cataplexy Questionnaire contains 51 items on cataplexy, including questions on cataplexy triggers, localization, duration, and frequency of the attacks[35].

In *studies 4-9,* psychometric assessment was also performed using standard tools, including the Beck Anxiety inventory, Beck Depression Inventory and Speilberger State-Trait Anxiety Inventory (in *studies 5-9*).

In *study 1*, the subjects who reported TBI, and in *study 3*, the subjects who reported cataplexy during sexual intercourse (orgasmolepsy), were contacted to obtain more details about their complaints.

Healthy subjects, age and gender matched to our NC patients, served as controls in *studies 2 and 4-9*. Healthy subjects served as controls also in s*tudy 3.* NC patients were compared not only to healthy control subjects but also to patients with EDS of different origin in *study 3* and to another group of patients with reported hypocretin cell loss, namely patients with Parkinson's disease, in *study 4.*

3.2 Behavioural tests

In *studies 4-7* different behavioural paradigms were used:

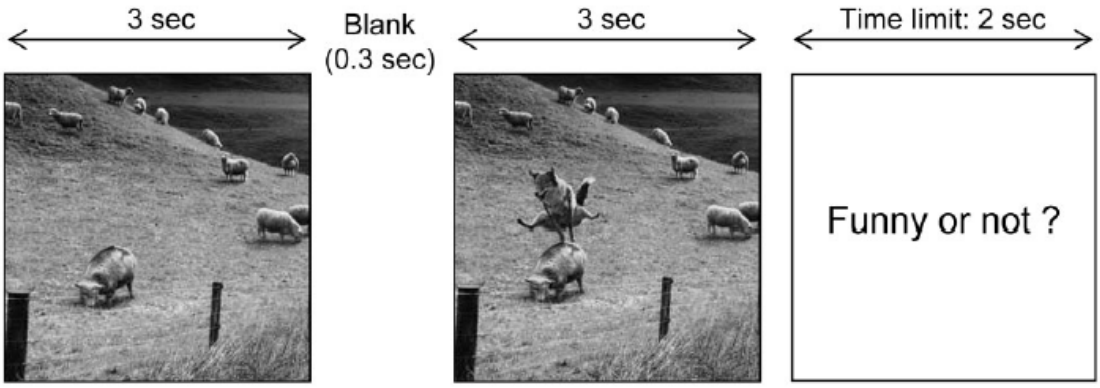
In *study 4,* all subjects performed a short time production task, where they had to produce an interval of 1, 2 or 5 s. They were asked to press a button on the keyboard when they thought the interval of 1, 2 or 5 s had elapsed. Counting was not explicitly prohibited. Each target time duration was produced separately. The task consisted of six blocks of 30 estimations each, with two blocks for each target duration (Fig. 1). The sequence of target durations was varied randomly between participants.

Fig. 1: Schematic representation of the task:

Each target duration (1, 2 and 5 s) was estimated separately twice in blocks of 30 estimations. The sequence of the target durations was randomly varied between subjects. To test for changes in time estimation over the time course of the experiment, the mean duration of the first five estimations was compared to the mean duration of the last five estimations in each block for each target duration (presented in light grey).

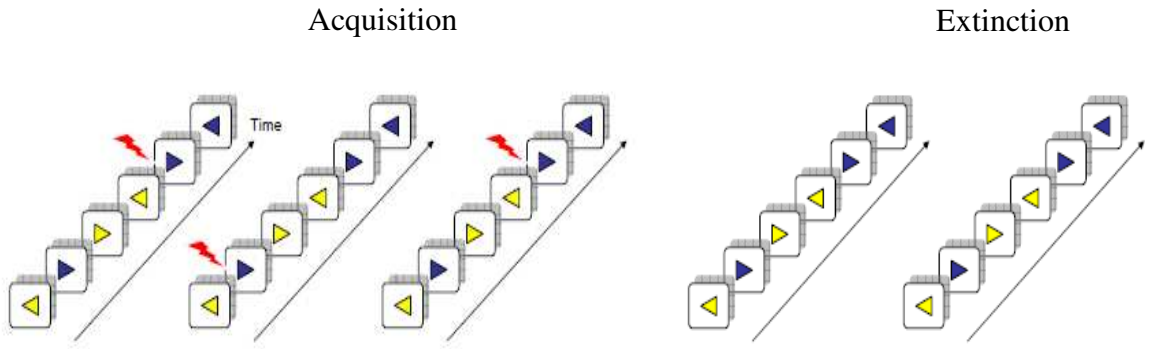
In *study 5,* a humour judgment paradigm was used, combined with functional magnetic resonance imaging (fMRI). Minisequences, consisting of a first neutral picture followed by a second picture with an either humorous or a neutral new element were presented during fMRI assessment (Fig. 2). On each trial, the participants judged whether they found the sequence funny or not.

Fig. 2: Stimulus sequence. Each trial consisted of a first neutral picture followed by a second picture with a new element which was either neutral or humorous. Subjects judged if the sequence was funny or not after the presentation of the second picture.



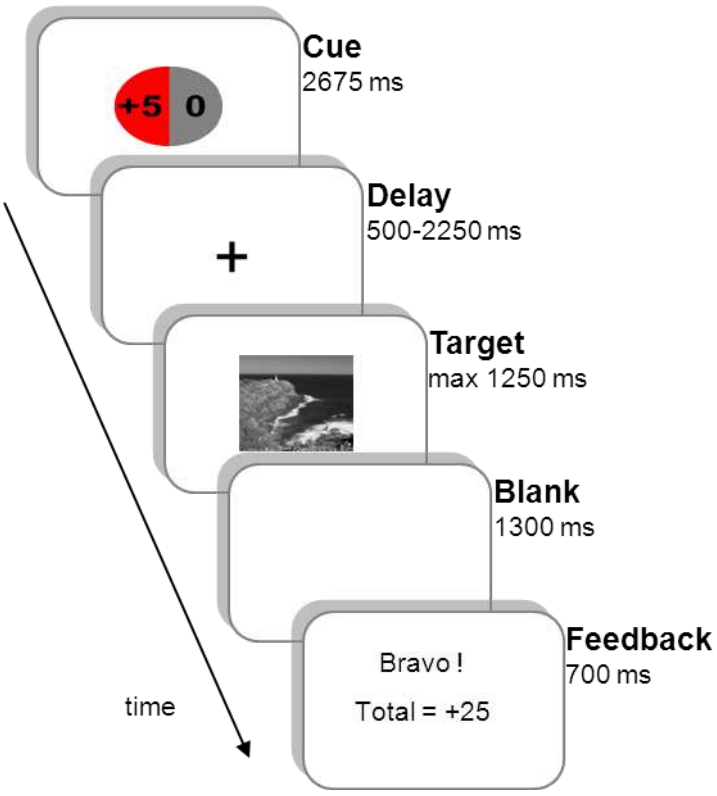
In *study 6,* an aversive conditioning paradigm was combined with fMRI. On each trial, one triangle was displayed at the centre of the screen and the subjects had to decide as quickly and as accurately as possible whether the triangle was pointing to the left or to the right. Subjects were required to respond on each trial, which allowed us to check whether they paid attention to each stimulus and did not fall asleep. Triangles were either blue or yellow in colour. One colour (conditioned stimulus) signalled a possible upcoming aversive unconditioned stimulus, which was a brief, mildly painful electrical stimulation delivered on one finger on half of the conditioned stimulus trials (partial reinforcement conditioning paradigm). The other colour was never associated with any other stimulation (nonconditioned stimulus). The acquisition phase was followed by extinction phase, where conditioned stimulus was not paired with the unconditioned stimulus anymore (Fig. 3).

Fig. 3: Aversive conditioning experimental paradigm:



In *study 7,* a game-like task was combined with fMRI. The subjects could win (or lose) points if they rapidly pressed on a key while a visual target was briefly shown (Fig. 4). During each trial, the subjects were presented with a cue indicating the potential gain (+1 or +5 points) or potential loss (-1 or -5 points) associated with that particular trial. After a variable delay, the visual target that required a rapid key press was briefly presented on the screen. A feedback display, telling the subjects whether they had won or lost, was presented at the end of each trial. The paradigm design allowed different comparisons: cue and feedback presentation, large and small trials, success and failure, gain and no gain.

Fig. 4: Representation of the task:



3.3 Modulation of sleep pressure paradigm, sleep recordings and analysis

In *study 2* we used a paradigm which allowed us to modulate sleep pressure. Baseline night-time sleep (23:00-7:00 h), which served also as a screening night, and daytime sleep under either higher (no sleep in the previous night) or lower sleep pressure (allowing 4 hours of sleep from 23:00-3:00 h in the previous night) was recorded in all participants on non-consecutive occasions. Partial and total sleep deprivation (SD) were performed in the sleep laboratory on two separate nights at least 7 days apart in order to allow full recovery from previous SD. The participants were randomly assigned to begin with either partial or total SD. All participants arrived in the laboratory at 19:00 h and were kept awake either until 7:00 h the next morning (total SD, higher sleep pressure) or were allowed 4 hours of sleep (23:00-3:00 h, partial SD, lower sleep pressure). Morning recovery sleep was recorded from 7:00 h until latest 15:00 h. During baseline nights, video-polysomnography consisted of eight channel EEG (F3/A2, F4/A1, C3/A2, C4/A1, P3/A2, P4/A1, O1/A2, O2/A1), left and right electrooculography (EOG), submental electromyography (EMG), electrocardiography (ECG), respiratory flow (using a nasal cannula) and effort (using Medcare XactTrace respiratory inductive plethysmography technology belts), pulse-oximetry and left and right anterior tibialis EMG. During morning recovery sleep after total and partial SD in the previous night sleep, scoring was based on EEG, EOG, EMG and ECG. All recordings were done on Medcare Somnologica Studio. Sleep stages, periodic limb movements and arousals during baseline night were scored manually according to international criteria[2, 36, 37]. Sleep onset was defined as the first epoch of either NREM 2 or REM sleep. Cyclic alternating pattern parameters were scored and analysed for each subject[38].

Cyclic alternating pattern is a way to describe the arousability state during NREM sleep. Different subtypes have been defined: subtypes A1 – slow frequency high-voltage EEG

patterns (sequences of K-complexes and delta-bursts), subtypes A2 – rapid low-amplitude EEG patterns preceded by or mixed with slow high voltage waves and subtypes A3 – phases with fast low-voltage EEG patterns [38]. The A1 subtypes are abundant during the build-up of deep NREM sleep and play a role in NREM sleep maintenance while subtypes A2 and A3 indicate NREM sleep disruption[39, 40]. Additionally A2 and A3 subtypes occur more often before the onset of and during REM sleep, thus suggesting an association with REM-on mechanisms, active during the last part of a NREM sleep episode and during REM sleep[40].

3.4 Neuroimaging

For *studies 5-7*, whole-brain event-related fMRI data were acquired on a Philips Intera 3.0-Tesla whole-body system (Philips Medical Systems, Best, NL). The scanning parameters were optimized during pilot testing to minimize susceptibility-related signal losses in orbitofrontal cortex and inferior temporal regions. Processing and statistical analyses of imaging data were performed with SPM2 (www.fil.ion.ucl.ac.uk).

In *study 8,* ^{1}H-MRS was performed on a 3T Philips Achieva whole body magnetic resonance scanner. Single-voxel proton magnetic resonance spectra were acquired from hypothalamus (a volume of interest [VOI] of 1 cm^3 was selected to include bilateral grey matter), both amygdala (VOI of 1 cm^3), and the pontomesencephalic junction (VOI of 1.9 cm^3). Water-suppressed proton MR spectra were acquired using a PRESS single-voxel localization sequence. Second-order shimming (3T Philips Achieva) was used. Acquisition time for each VOI was 19 minutes. Levels of N-acetylaspartate (NAA), N-acetylaspartateglutamate (NAAG), NAA+NAAG (total NAA), myoInositol (mI), creatinephosphocreatine (Cr), and choline were analysed by fitting a linear combination of a basis set of metabolite model spectra to the data (LCModel) [41]. The metabolite concentrations were expressed as ratios relative to Cr peak.

As neuronal cell loss and gliosis in different regions of interest in NC have been described, we focused on NAA, a marker of neuronal integrity and on mI, a glial marker

In *study 9* cerebral MRI was acquired with a T1-weighted 3D volumetric pulse sequence using a Philips Achieva 3T scanner as a series of 180 contiguous axial slices, with a voxel size of 0.86 x 0.86 x 0.76 mm. Cortical reconstruction and volumetric segmentation were performed using published algorithms included in the FreeSurfer package (Martinos

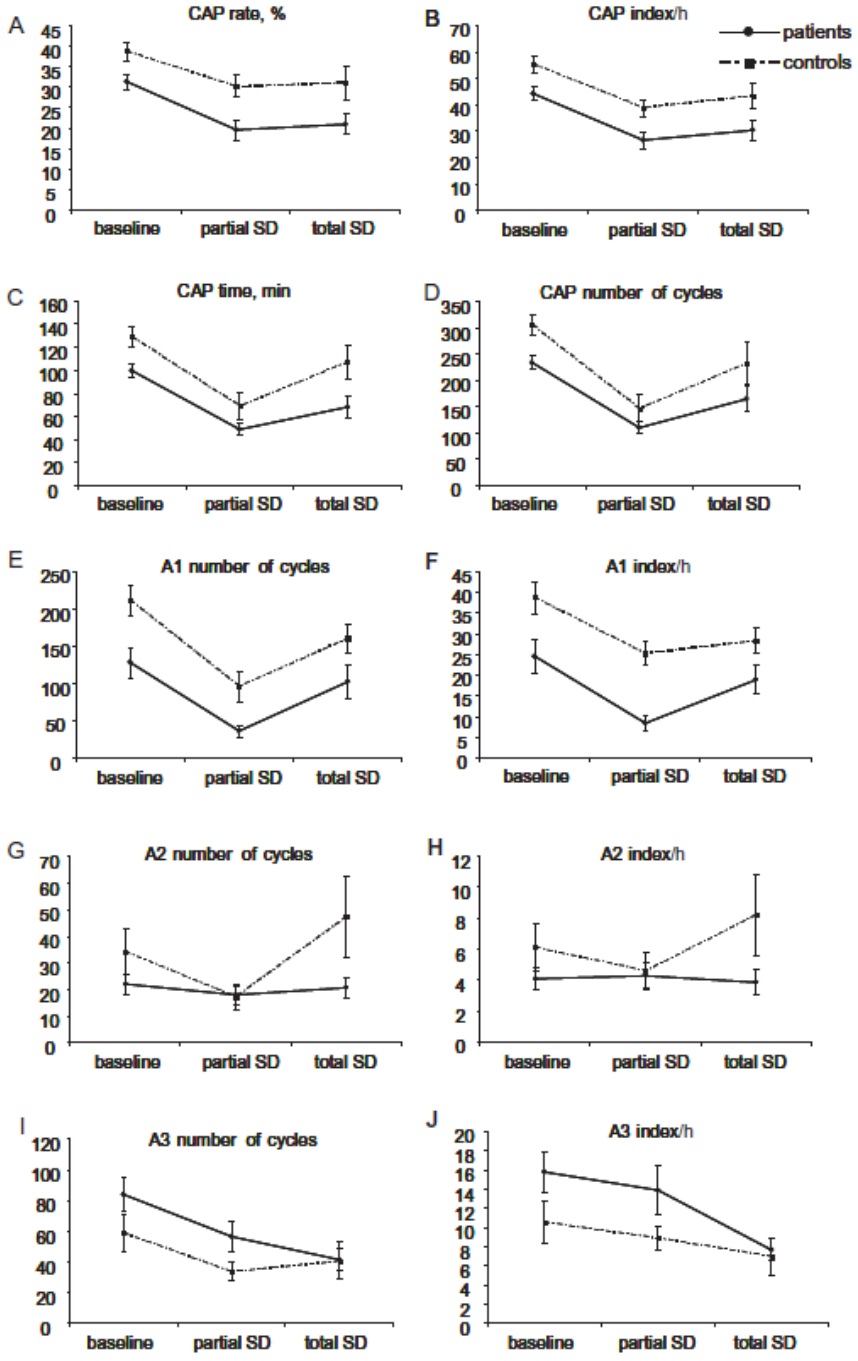
Center for Biomedical Imaging, Massachusetts General Hospital, Boston, MA, USA). Regional cortical volumes, cortical thickness and gyrification were computed.

4. RESULTS

In *study 1* we found a much higher frequency of TBI prior to the occurrence of NC (19%) in comparison to the general population (about 1%)[42].

Study 2 showed significantly lower cyclic alternating pattern (CAP) rate, CAP index, CAP time, number of CAP cycles, A1 index and number of A1 cycles in comparison to healthy controls. A2 and A3 subtypes were not significantly different between patients and controls. CAP parameters decreased significantly after partial and total sleep deprivation. Most of these changes followed a similar pattern after partial and total SD in patients and controls (Fig. 5).

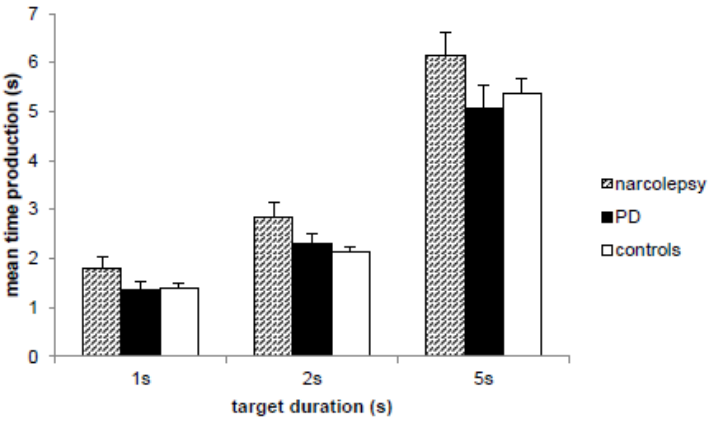
Fig. 5: Graphic representation of the dynamics in CAP parameters at baseline and after partial and total sleep deprivation during morning recovery sleep.



In *study 3,* orgasmolepsy was found in 10% of our NC patients, yet one patient with excessive daytime sleepiness due to behaviourally induced insufficient sleep syndrome also reported the symptom. All NC patients who experienced orgasmolepsy had frequent partial cataplexy and severe daytime sleepiness. In the female NC patients, orgasmolepsy occurred by each sexual intercourse, and the male patient reported orgasmolepsy only when in a relationship involving emotional commitment and trust. In the patient with behaviourally induced insufficient sleep syndrome and orgasmolepsy, cataplexy-like symptoms involved unilaterally upper or lower limbs in association with negative emotions or sports activities.

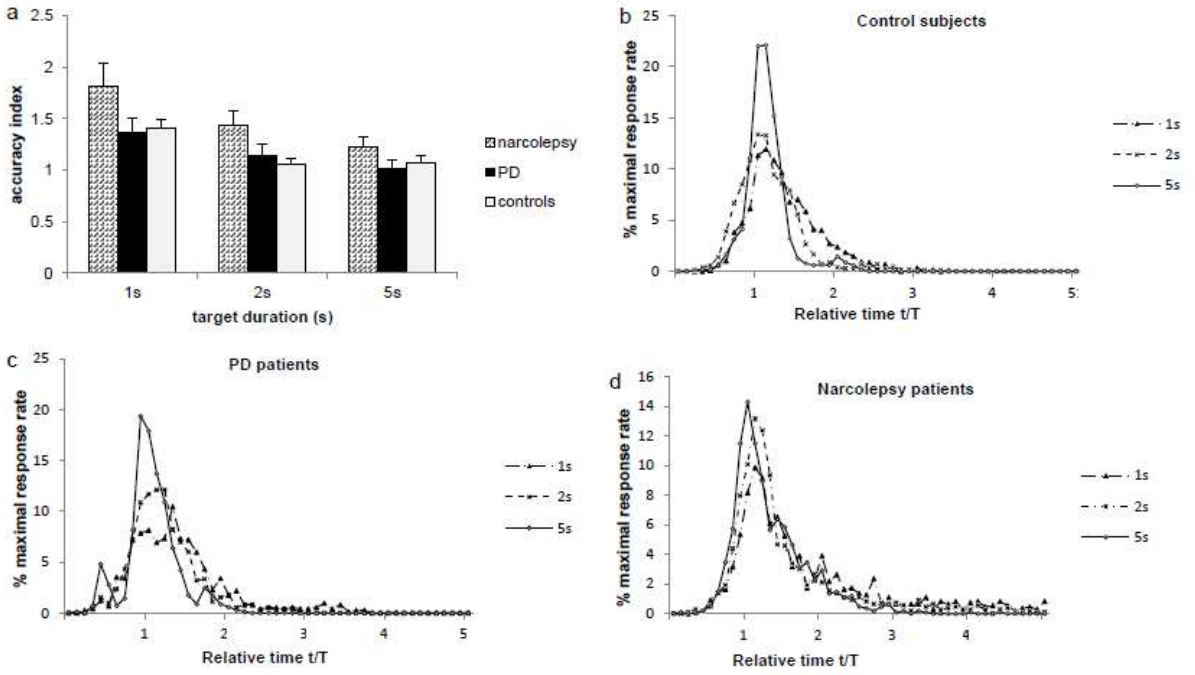
In *study 4,* NC patients tended to overproduce all time target durations, compared to patients with Parkinson's disease (PD) and healthy control subjects (Fig. 6).

Fig. 6: Mean time productions for each target duration in each group, mean ± SEM



The temporal accuracy index was computed by taking the ratio of the duration produced to the target duration (1, 2 or 5 s). Because target duration is considered as the reference, a ratio <1 corresponds to shorter productions and a ratio >1 corresponds to longer productions than the target duration. Narcolepsy patients tended to have higher accuracy index (pointing to time overproduction) in comparison to controls and PD patients (Fig.7).

Fig. 7: Accuracy of time production/estimation



t-real time production/estimation, T-target time; a – mean accuracy of time production/estimation for each group and each target duration, mean ± SEM; b – distribution of accuracy in control subjects for each target duration; c - distribution of accuracy in PD patients for each target duration; d - distribution of accuracy in narcolepsy patients for each target duration

NC patients also showed higher absolute variability than controls and PD patients (Fig. 7 and Fig. 8).

Fig. 7: Absolute variability of time production/estimation for each group and each target duration, mean ± SEM.

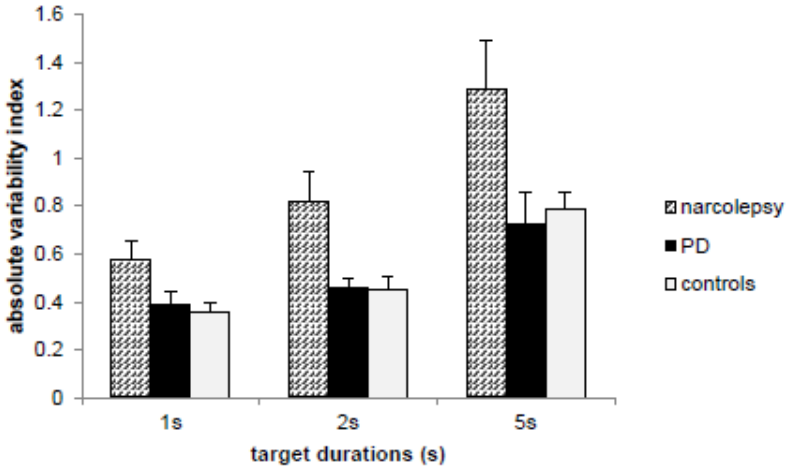
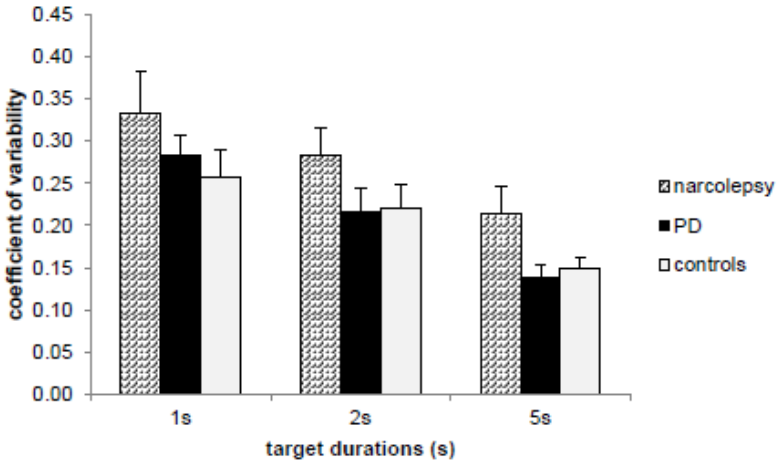
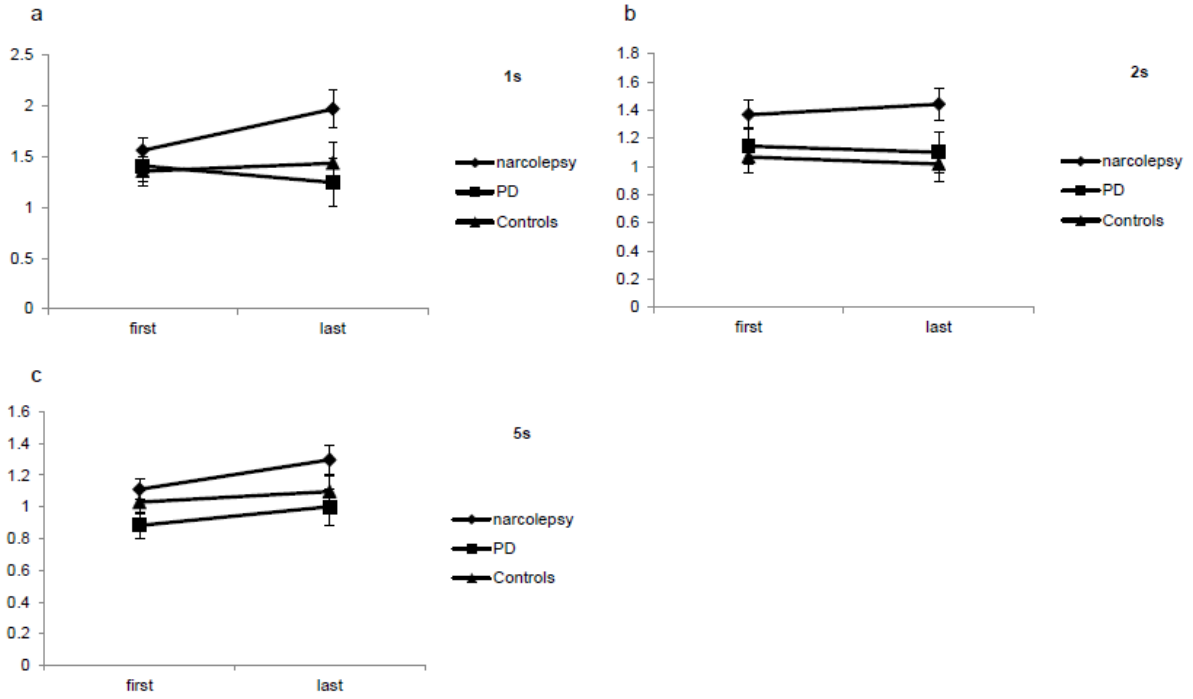


Fig. 8: Relative variability (coefficient of variability) of time production/estimation for each group and each target duration, mean ± SEM.



The analysis of the temporal course of time estimation showed more pronounced overproduction of each target duration at the end of each trial in NC patients, whereas performance was more or less stable in controls and PD patients (Fig. 9).

Fig. 9: Dynamics of accuracy of time estimation, mean ± SEM in the first and last five trials in each group of each target duration: a – for 1s, b – for 2s and c – for 5s.



Studies 5-7 assessed different aspects of emotional processing.

Humorous pictures in study 5 elicited reduced hypothalamic response (Fig. 10) together with enhanced amygdala (Fig. 10), left insula and nucleus accumbens (Fig. 12) response in NC.

Fig. 10: Increased response to humour in right hypothalamus in controls compared to NC patients:

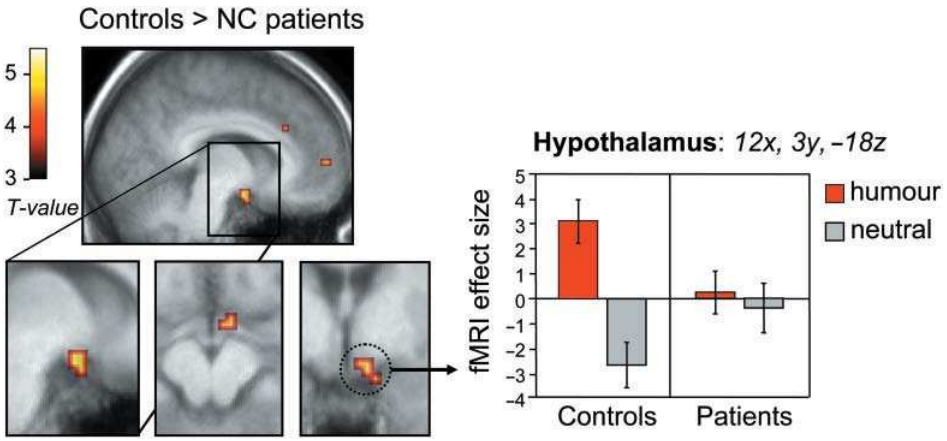


Fig. 11: Increased amygdala response to humour in NC patients compared to controls.

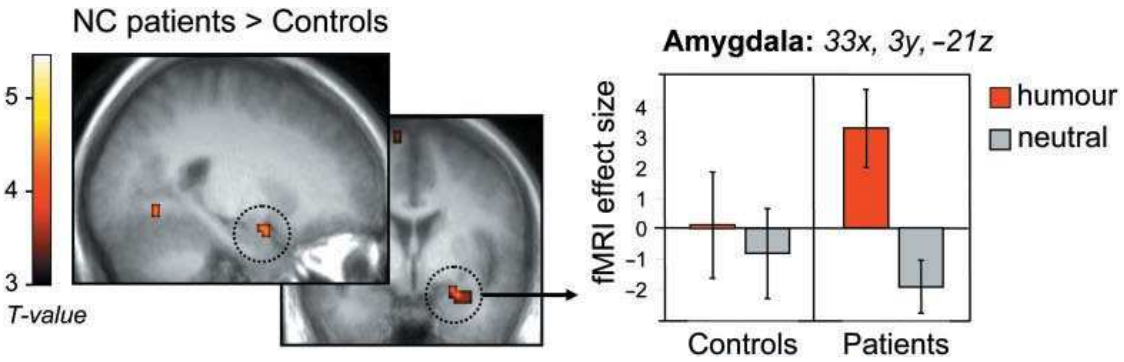
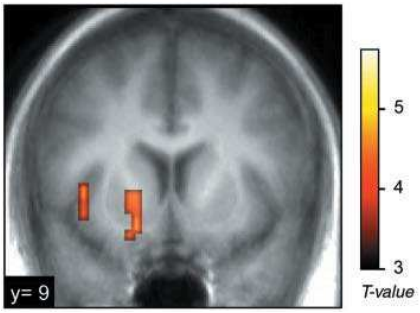
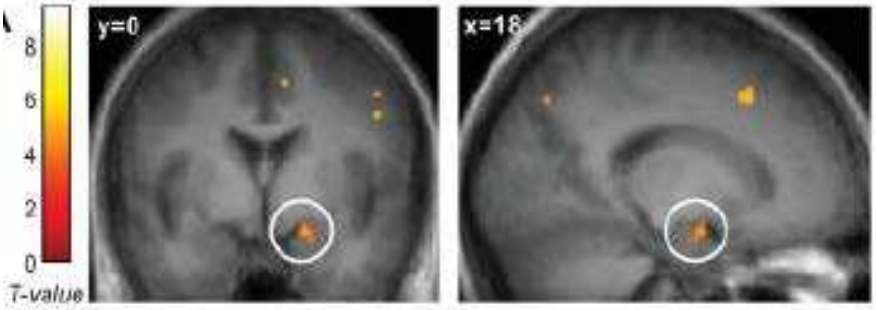


Fig 12: Increased fMRI signal in left insula and nucleus accumbens in NC patients.



In *study 6,* NC patients had no enhancement of amygdala response to conditioned stimuli and no increase in functional coupling between the amygdala and medial prefrontal cortex in comparison to control subjects (Fig. 13).

Fig. 13: Activation of amygdala in control subjects.



Study 7 showed that activity in the dopaminergic VTA was not modulated in NC patients during high reward expectancy (Fig. 14), and that ventral striatum activity was reduced during winning. The patients showed abnormal activity increases in the amygdala and in dorsal striatum for positive outcomes (Fig. 16). In addition, activity in the nucleus accumbens and the ventral-medial prefrontal cortex correlated with disease duration.

Fig. 14: VTA activation during the presentation of high-incentive cues in controls compared to NC patients.

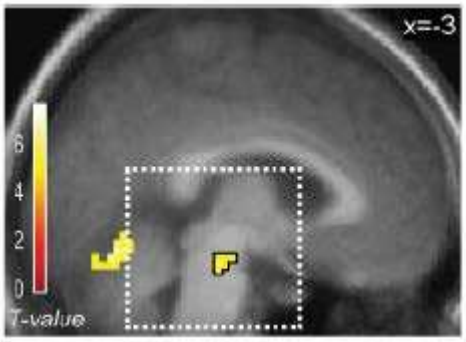
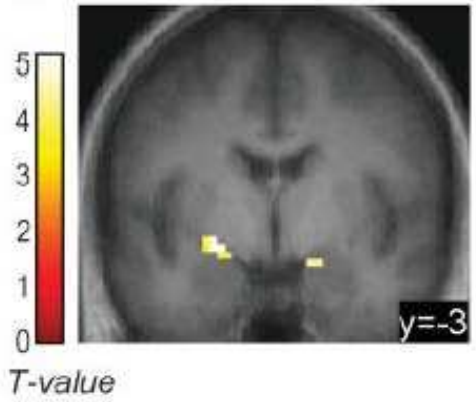
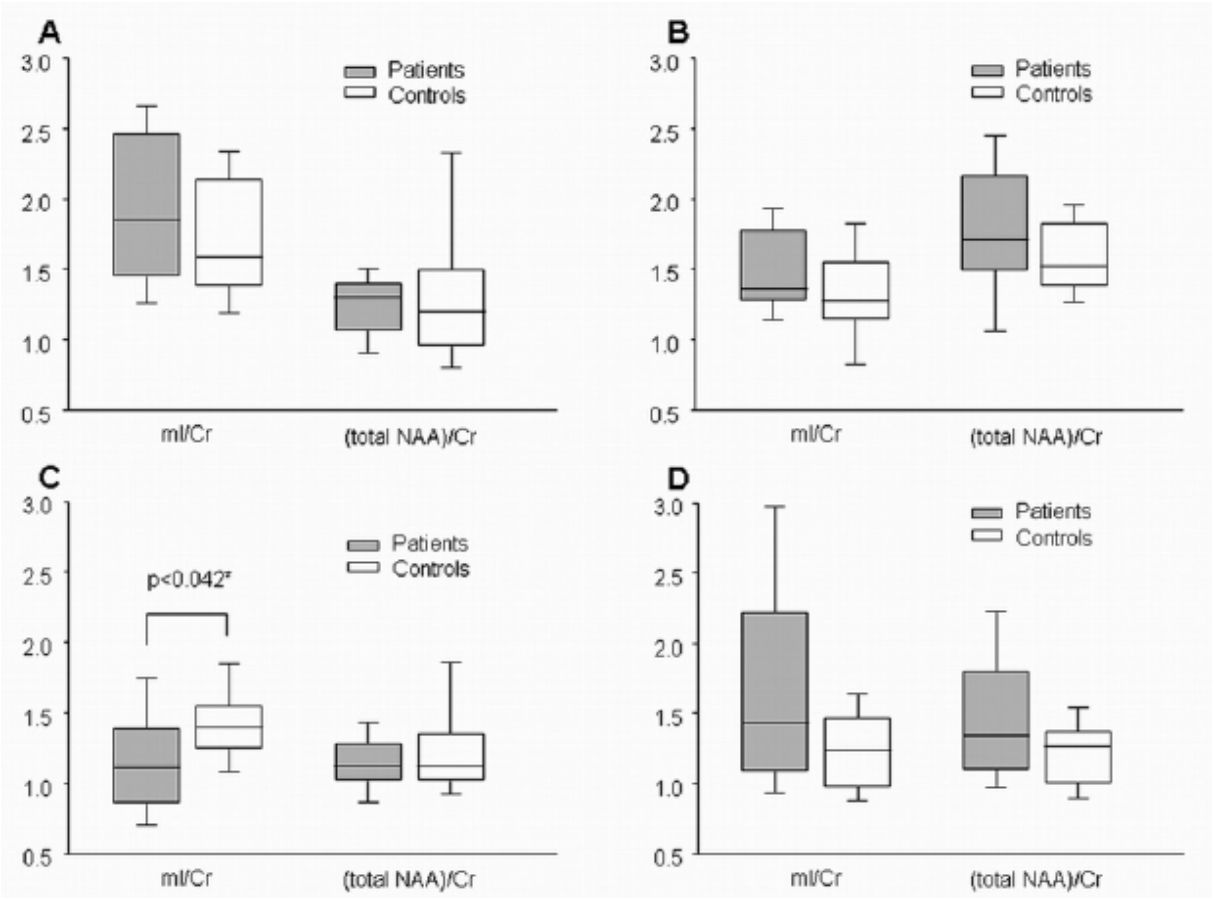


Fig. 15: Increased amygdala activity for positive outcomes in NC patients in comparison to controls.



Having demonstrated abnormal activation in hypothalamus and amygdala in S*tudies 5-7,* we also showed metabolic changes in these regions in *study 8,* namely myoInositol (mI)/Cr was significantly lower in the right amygdala of NC patients, compared to controls (Fig. 16).

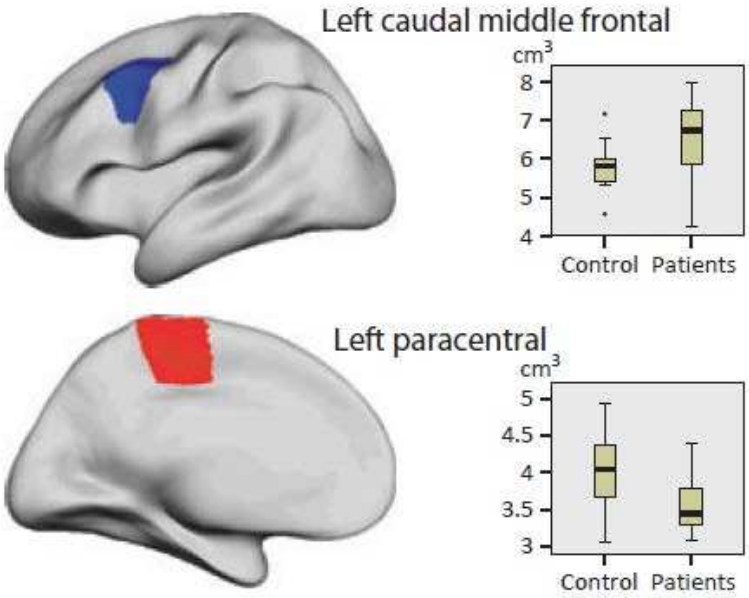
Fig. 16: A: metabolite concentration ratios in hypothalamus; B: metabolite concentration ratios in the pontomesencephalic junction; C: metabolite concentration ratios in the right amygdala; D: metabolite concentration ratios in the left amygdala.



Significant negative correlations were found only in the patients group between (total NAA)/Cr in hypothalamus and ml/Cr in the right amygdala, between ml/Cr in hypothalamus and (total NAA)/Cr in the right amygdala and between ml/Cr in the left amygdala and total NAA)/Cr in the pontomesencephalic junction.

In *study 9*, after showing functional and metabolic brain changes in NC, we demonstrated structural brain changes such as decreased cortical volume in the left paracentral lobule and increased cortical volume in the left caudal part of the middle frontal gyrus (Fig. 17). Cortical thickness in prefrontal areas, especially in the right orbitofrontal cortex, was associated with symptom severity in NC. Further, we observed several clusters of cortical thinning in patients with childhood or adolescent onset of NC compared with patients with adult disease onset.

Fig. 17: Increased volume in the caudal part of the left middle frontal gyrus (in blue) and decreased volume in the left paracentral gyrus (in red) in NC patients in comparison to controls.



5. DISCUSSION AND OUTLOOK

In this work, we attempted to elucidate various etiological, pathophysiological and clinical aspects of NC, using a multimodal approach, comprising of questionnaires, behavioural paradigms, electrophysiological recordings and different neuroimaging methods. We confirmed that NC is more than just a sleep-wake disorder and that its manifestations spread widely beyond the classical sleep-wake complaints.

With *study 1* we attempted to clarify the link between TBI and NC. Our previous findings of deficient hypocretin signalling after TBI[43], the higher prevalence of TBI in our NC population than in the general population[42] and the short intervals between TBI and onset of NC in most of our patients suggest that TBI may cause a symptomatic loss of hypocretin neurons in susceptible patients, which may lead to the clinical picture of NC. Thus external factors together with genetic predisposition seem to be essential for the development of NC.

In *study 2* we examined NC pathophysiology, in particular NREM homeostasis. We used manipulation of sleep pressure and cyclic alternating pattern analysis. The decrease of A1 subtypes in NC during baseline sleep, which are important for the build-up of deep NREM sleep, indicate insufficient NREM sleep intensity in NC. Conversely, A2 and A3 subtypes, which are linked to REM-on mechanisms, did not differ between NC patients and controls, thus suggesting that REM-on mechanisms are operational in NC. The baseline differences between NC patients and controls persisted after total and partial SD and followed a similar pattern, indicating similar homeostatic NREM regulation in NC patients and healthy controls.

Having worked on etiological and pathophysiological mechanisms of NC, in *studies 3-7* we were interested in symptoms and behavioural features of NC which expand beyond the well-known classical clinical characteristics. We also attempted to at least partially link our findings to hypocretin deficiency. In *study 3* we postulated that orgasmolepsy is a distinct

feature of NC, which however, is also reported by patients with other sleep-wake disorders and excessive daytime sleepiness. We hypothesized that insufficient arousal may favour the occurrence of cataplexy and cataplexy-like symptoms, including orgasmolepsy. Considering the dense hypocretinergic projections to areas known to be part of reward networks[20], including the ones involved in sexual arousal and orgasm, we assumed that hypocretin deficiency and reward dysregulation in NC may further facilitate orgasmolepsy.

In *study 4* we explored time perception in NC. We expected deficits in accuracy and variability of responses in NC patients as overproduction of target durations in the light of possible prefrontal dysfunction and attention deficits. Indeed we found overproduction and higher variability of all time durations in NC, which indicates impaired short interval timing in the seconds range. The time-course of accuracy and variability of time production within sessions indicate an attention-related mechanism of impaired interval timing. As intact prefrontal cortex is critical for interval timing[44], and the prefrontal cortex receives dense hypocretin projections hypocretin mediated dopaminergic ventral tegmental area projections, a role of hypocretin in time processing can be suggested.

Studies 5-7 focused on different aspects of emotional processing and their link to brain function. The findings of increased amygdala activity together with reduced medial prefrontal and hypothalamic activity during humour processing in *Study 5* suggest that the hypothalamus might have modulatory influence on amygdala activity during positive emotions, possibly via direct projections from hypothalamic hypocretin neurons to the amygdaloid complex[20, 21]. Reduced hypothalamic activation and exaggerated amygdala response to humour could be due to loss of hypothalamic hypocretin neurons in NC. Hypocretin has been shown to influence affective responses through projections to the VTA[22]. In turn, intra-VTA administration of hypocretin increases dopamine levels within the prefrontal cortex[23]. Activity in prefrontal cortex might suppress amygdala responses[45]. The reduced hypothalamic and prefrontal

activity and the increased amygdala activation in NC patients could be due to a dysfunction of hypocretin/dopamine-mediated pathways that usually inhibit amygdala activity. This also could lead to an abnormally high amygdala response to positive emotions in NC. Narcolepsy patients also had elevated fMRI responses to humour in the left nucleus accumbens, a key component of the mesolimbic reward system known to be involved in humour processing[46].

Another aspect of emotional processing in NC was assessed in *study 6*. During aversive conditioning there was no increase in amygdala response to the conditioned stimulus alone, suggesting a selective impairment in emotional learning. Thus *study 6* provided further evidence for amygdala dysfunction in NC.

Study 7 also studied the emotional brain in NC, focusing on reward processing. Hypocretin has been implicated in reward processing in animal studies. This was the first study to assess reward-related brain responses in human NC. It provided proof for the role of hypocretin in reward processing in the human brain. The lack of VTA activation in NC patients might be explained with deficient hypocretinergic stimulation of this region. Additionally brain responses to reward in NC were affected in regions receiving dense hypocretinergic projections and/or those modulated by dopaminergic inputs from the VTA, such as nucleus accumbens and the prefrontal cortex.

In *Studies 8 and 9* we were looking for metabolic and structural correlates of the above demonstrated functional brain dysfunction. Indeed study 8 showed metabolic changes in the right amygdala and also suggested hypothalamo-amygdala dysfunction on metabolic level in NC. *Study 9* then found different volumetric changes, including areas within the prefrontal cortex, rich in hypocretinergic projections, as already mentioned above. Moreover cortical thickness in two prefrontal regions, especially in the right orbitofrontal cortex, was associated with symptom severity in NC. The right orbitofrontal cortex is densely connected to the VTA and amygdala, which in turn were shown to be dysfunctional in *studies 5-7* in the same

sample of patients. The location of the changes in the right orbitofrontal cortex is not a coincidence and can be explained in the context of altered function of the reward circuitry in NC patients. The abnormal activity of the VTA, together with the altered orbitofrontal cortex, could impair the reinforcing effect of positive reward on behaviour in NC patients.

Narcolepsy with cataplexy is a fascinating disease with complex aetiology, pathophysiology and clinics. The clinical manifestations of NC spread beyond sleep-wake disturbances and include impaired time perception, emotional processing, with an emphasis on humour, reward and emotional learning. We characterized some of the functional, metabolic and structural brain substrates of these manifestations.

6. ABBREVIATIONS

CAP	- cyclic alternating pattern
Cr	- creatinephosphocreatine
ECG	- electrocardiography
EDS	- excessive daytime sleepiness
EEG	- electroencephalography
EMG	- electromyography
EOG	- electrooculography
fMRI	- functional magnetic resonance imagimg
HLA	- human leucocyte antigen
mI	- myoInositol
NAA	- N-acetylaspartate
NAAG	- N-acetylaspartateglutamate
NC	- narcolepsy with cataplexy
NREM	- non-rapid eye movement sleep
PD	- Parkinson's disease
REM	- rapid eye movement sleep
SD	- sleep deprivation
SEM	- standard error of the mean
SPM	- statistical parametric mapping
TBI	- traumatic brain injury
total NAA	- NAA+NAAG
VOI	- volume of interest
VTA	- ventral tegmental area

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