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Circadian rhythms and attentional dysfunction in type 1 Narcolepsy

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Chapter 1

General introduction

Central Disorders of Hypersomnolence

Sleepiness is a common experience, with estimated prevalence of excessive daytime sleepiness (EDS) in general population ranging from 4% to 21%.[1]

For many of these individuals, such sleepiness may be simply caused by poor sleep habits, illicit and prescribed substances, work and family demands (including shift work), or self-imposed sleep times that are not sufficient to maintain alertness throughout the day. For others, daytime sleepiness may be related to a more serious medical condition.

Central disorders of hypersomnolence are a group of neurological characterized by EDS in the absence of disrupted nocturnal sleep or circadian rhythm disorders.[2]

According the most recent version of the International Classification of Sleep Disorders, Third Edition (ICSD-3), three persistent hypersomnolence disorders are not associated with another medical conditions or substance abuse: narcolepsy type 1 (NT1), narcolepsy type 2 (NT2), and Idiopathic Hypersomnia (IH).[2]

NT1 was the first of these disorders to be comprehensively described, dating back to a case published in 1880 by Jean Baptiste Gélineau.[3]

The classic symptoms of NT1 are EDS, cataplexy (sudden and transient loss of muscular tone usually evoked by emotions), dissociated rapid eye movement (REM) sleep manifestations such as sleep paralysis (temporary inability to move voluntary muscles) and/or hallucinations at the wake-sleep transition, and nocturnal sleep disruption.[2]

EDS is the cardinal symptom, and often the most disabling. Patients with narcolepsy type 1 experience repeatedly episodes of an irrepressible need to sleep or lapses into sleep. Most patients awaken refreshed after a sleep episode but begin to feel sleepy again after variable times.[2]

NT1 is also characterized by disordered regulation of rapid-eye-movement (REM) sleep. REM sleep normally occurs only during the usual sleep period and includes vivid, story-like dreams, rapid (saccadic) eye movements, and paralysis of nearly all skeletal muscles, except the muscle of

respiration. REM sleep can occur in persons with NT1at any time of day, and the classic elements of REM sleep often intrude into wakefulness, creating peculiar intermediate states.[4]

Cataplexy is the most dramatic of the dissociated REM sleep–like states presented by NT1 patients and is defined as the sudden loss of muscle tone in response to a strong emotion, usually laughing.[5] Typical cataplexy is present in 65% to 75% of individuals with NT1 and is quite specific, only rarely cataplexy or cataplexy-like episodes will occur in other disorders (including Coffin-Lowry syndrome, Norrie disease, and Niemann-Pick disease type C).[6] The presence or absence of cataplexy is a key distinguishing feature between the two types of narcolepsy, which are now recognized to be quite different entities despite their similar nomenclature.[2,7]

NT1 is caused by the loss of hypothalamic neurons that produces hypocretin, a wakefulness-associated neuropeptide.NT1 patients have reduced or absent levels of hypocretin-1 (hcrt-1) in their cerebrospinal fluid (CSF), accordingly the disease is further classified into NT1 (with hypocretin-1 deficiency and cataplexy) and Narcolepsy Type 2 (NT2), where cataplexy is absent and hypocretin levels are usually in the normal range.[8,9]

Idiopathic hypersomnia (IH) was detailed by Bedrich Roth in a series of 642 patients seen over 30 years.[10] IH is characterized by EDS in absence of dissociated REM sleep manifestation and cataplexy, and have a clinical presentation more similar to narcolepsy type 2 than type 1.[11]

Most patients with IH feel unrefreshed after naps, which are usually long and in contrast to NT1 usually present high sleep efficiency (≥90%).[12] IH patients report great difficulty with awakening, experiencing a prolonged state in which motor functions return before full awareness or there is partial return of both after awakening. CSF hypocretin-1 concentrations in patients with IH are normal.[2,8]

Epidemiology

The prevalence of narcolepsy type 1 is 0.025% to 0.05%.[13] Globally, the prevalence varies from highest in Japan (0.16%) to lowest in Israel (0.0002%).[14] The age of onset in clinical populations appears to be bimodal, with the first peak at 15 years and the second at 35 years.[15,16]

The exact prevalence of narcolepsy type 2 is uncertain.[2] Cases of narcolepsy without cataplexy represent 15% to 25% of the clinic narcoleptic population. The age of onset mirrors that of narcolepsy type 1.Prevalence and incidence of IH are not known. Some studies have suggested a higher prevalence in women. The age of onset of IH symptoms ranges from the late teens to 35 years of age.[2]

Pathophysiology

The neuropeptide hypocretin (also called orexin) was first identified in 1998.[17,18]

Hypocretin is produced in the lateral hypothalamus and is involved in the regulation of feeding, stress response, reward, and the autonomic nervous system.

Cerebrospinal fluid (CFS) hypocretin-1 levels are reduced in the majority (90%-95%) of subjects with narcolepsy and typical cataplexy.[19] While loss of hypocretin neurons is also seen in 10% to 30% of cases of narcolepsy type 2, most patients with NT2 have normal hypocretin levels.[8]

The loss of hypocretin and development of narcolepsy type 1 involves both genetic and environmental factors, likely resulting from an autoimmune attack on hypocretin neurons in genetically susceptible individuals. The clear genetic predisposition is seen in the 10 to 40 times higher risk of narcolepsy in first-degree relatives of patients.[20]

Human leukocyte antigen (HLA) DQB1*06:02 is present in > 85% to 95% of patients with typical cataplexy but is not specific, since it is also present in 40% of cases of type 2 narcolepsy and 24% of healthy control subjects.[21]

Despite this apparent genetic predisposition to narcolepsy, concordance rates in identical twins are only 25% to 31%, implicating substantial environmental or stochastic factors.[19]

The combination of HLA association, genetic polymorphisms in immune genes, and apparent triggering of disease by infection or vaccination all suggest an autoimmune basis for hypocretin-deficient narcolepsy, that however has yet to be conclusively demonstrated.

The pathophysiology of narcolepsy type 2 and IH are not yet known. A familial component has been proposed in IH, as a family history of EDS is commonly reported by patients.[22]

The HLA DQB1*0602 allele implicated in narcolepsy has been shown to be increased in IH patients in some, but not all studies. IH is rarely caused by hypocretin deficiency, supporting the concept of different pathogeneses of central disorders of hypersomnolence.[18]

Differential Diagnosis

Given these overlapping clinical features, the diagnosis of central disorder of hypersomnolence requires attention to both clinical presentation and sleep laboratory test, especially the multiple sleep latency test (MSLT).[23]

The MSLT consists of five 20-min nap opportunities at 2-h intervals. A nocturnal polysomnography (PSG) immediately prior the MSLT is mandatory to ensure a sufficient amount of sleep (> 6 h) and to rule out other sleep disorders, and sleep logs and/or actigraphy in the two week before to document regular sleep timing and duration and to rule out insufficient sleep.

Two parameters of most interest are the mean sleep latency (MSLT-sl) and the number of sleep-onset REM periods (SOREMPs). The sleep latency is the first epoch of sleep (any stage), and the MSLT-sl is the mean across all naps. A SOREMP is the presence of at least one epoch of REM during a nap opportunity. The MSLT is a major factor in current classification of patients with central disorders of hypersomnolence: the number of SOREMPs determines whether a patient with clinical complaints of hypersomnolence is classified as having narcolepsy (if they have two or more SOREMPs) or if they have IH (if they have fewer than two SOREMPs).[24]

Despite being the gold standard for the diagnosis of narcolepsy, like most diagnostic modalities, it is not without flaws. First, MSLT-sl and SOREMPs are specific since up to 30% of the normal population may have a MSLT-sl ≤8 min, the current cut-off for the hypersomnolence disorders. Second, the MSLT may not be adequately sensitive for IH; the 8-min cutoff was determined for patients with narcolepsy and extended to IH for "simplicity," without independent determination.[25] Third, the subjective experience of sleepiness (indexed by the Epworth Sleepiness Scale - ESS) correlates only modestly with MSLT-sl. Optimal diagnostic methods for IH require further study.

Diagnostic delay

Despite being one of the most common causes of chronic sleepiness, affecting about 1 in 2000 people, narcolepsy may remain undiagnosed in as many as half of all affected people with narcolepsy, since many clinicians are unfamiliar with this disorder.[26,27]

Usually there is a long interval from the onset of symptoms before a diagnosis is made. The symptoms often start in the second or third decades of life and new symptoms can develop over many years. Delays between the first symptom and diagnosis have been reported to range from 1 to 60 years, with a mean delay of between 16 and 22 years.[28] Patients with a more recent onset of symptoms have been reported to have a shorter interval before they were diagnosed compared to those whose symptoms started further in the past.

A number of factors to see if they were associated with a delay in diagnosis, among all the lack of any reliable screening tool.[28,29]

Several tool has been proposed ranging from questionnaire, to biological marker, however none of them seem suitable for screening large-population. To date this research question still remains open and is crucially important for the quality of life of this clinical population.

Cognitive impairments

Narcolepsy significantly interferes with several aspects of daily life, wielding negative social and professional impacts that may considerably affect the quality of life.[30]

A great portion of patients complain about attention and memory problems, and those problems are presumably responsible for a wide variety of difficulties in everyday life, such as problems at school and home and difficulties to obtain and maintain employment.[31]

These have led several investigators to question whether an underlying cognitive impairment might accompany the classical symptoms of NT1.[32]

Evidence for genuine cognitive impairment in narcolepsy is scarce and sometimes contradictory. Standardized empirical investigations of memory abilities yielded intact or only modestly impaired short- and long-term memory in narcoleptics patients.[33,34]

In the area of attention, different studies showed that patients with narcolepsy have slower reaction times (RTs) than controls, even in relatively simple tasks.[35] It is also frequently reported, that performance in patients with narcolepsy is more variable than in controls.[36]

Nonetheless, in spite of the relevance of the dual disturbance of vigilance and sleep, the most commonly used tests to measure the burden of narcolepsy focus solely on the tendency to fall asleep (MSLT) and the ability of stay awake (Maintenance of Wakefulness Test-MWT).[23]

Identifying and implement objective neuropsychological tests, which may show results deviating from the patient's individual impression, should be implemented in the routine diagnostic evaluation.

Aims

In this dissertation, we aimed to:

A) Identify behavioral biomarker for Type 1 Narcolepsy, able to screen large at risk-population.

B) Evaluate attentional performances of patients with Type 1 and Type 2 Narcolepsy, compared to healthy controls.

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Chapter 2

Actigraphic assessment of sleep/wake behavior in central disorders of hypersomnolence

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ABSTRACT

Objective. To evaluate the reliability of actigraphy to distinguish the features of daytime and nighttime sleep between patients with central disorders of hypersomnolence and healthy controls.

Methods. Thirty-nine drug-naïve patients with Narcolepsy Type 1, twenty-four drug-naïve patients with Idiopathic Hypersomnia and thirty age- and sex- matched healthy controls underwent to seven days of actigraphic and self-report monitoring of sleep/wake behavior.

The following variables were examined: estimated time in bed (TIB), estimated total sleep time, estimated sleep latency (SOL), estimated sleep efficiency, estimated wake after sleep onset, number of estimated awakenings (Awk), number of estimated awakenings longer than 5 minutes, estimated sleep motor activity (SMA), number of estimated naps, mean duration of the longest estimated nap (NapD) and daytime motor activity.

Results. All actigraphic parameters significantly differentiated the three groups, except estimated TIB and estimated SOL. A discriminant score computed combining actigraphic parameters from nighttime (estimated SMA, estimated Awk) and daytime (estimated NapD) periods showed a wide area under the curve (0.935) and a good balance between positive (95%) and negative predictive (87%) values in Narcolepsy Type 1cases.

Conclusion. Actigraphy provided a reliable objective measurement of sleep quality and daytime napping behavior able to distinguish central disorders of hypersomnolence and in particular Narcolepsy Type 1. The nycthemeral profile, combined with a careful clinical evaluation, may be an ecological information, useful to track disease course.

Keyword: Actigraphy, Narcolepsy Type 1, Idiopathic Hypersomnia, nycthemeral profile, motor activity

INTRODUCTION

Narcolepsy Type 1 (NT1) and Idiopathic Hypersomnia (IH) are two central disorders of hypersomnolence characterized by chronic sleepiness not explained by altered nocturnal sleep or sleep deprivation.[1] Although hypersomnolence features may overlap in IH and NT1, these disorders display distinct clinical, neurophysiological and biochemical presentations.[2]

NT1 is characterized by daytime and nighttime symptoms: daytime sleepiness (hypersomnolence), with sleep attacks characterized by direct transitions into rapid eye movement (REM) sleep (sleep-onset REM periods - SOREMPs); cataplexy (sudden loss of muscle tone triggered by emotions), sleep paralyses and hallucinations, and disrupted nighttime sleep.[1,3] Patients with NT1, indeed, present a nighttime sleep interrupted by numerous and prolonged awakenings, and also abnormal simple movements (e.g. nocturnal myoclonus) or complex behaviors (e.g. parasomnias) during both REM and non-REM sleep.[4] Conversely, IH is characterized by hypersomnolence and nocturnal sleep with normal features and, possibly, long duration.[1,5]

Nocturnal sleep and hypersomnolence are usually explored through nocturnal polysomnography (PSG) and multiple sleep latency test (MSLT) respectively. MSLT is the gold standard laboratory-based measure of daytime sleep propensity, currently used worldwide as main diagnostic tool for the differential diagnosis among central disorders of hypersomnolence.[6,7]

Recently, several studies attempted a more naturalistic approach by comparing the features of spontaneous daytime sleep at 24-hour continuous polysomnography versus MSLT results, in the context of central disorders of hypersomnolence.[8,9] These studies emphasized the utility of prolonged recordings performed under conditions more similar to usual life habits of patients. Nevertheless, 24-hour recordings are difficult to apply to clinical practice.

Actigraphic monitoring has become, over the last two decades, a widely used assessment tool in sleep medicine to continuously document several nycthemeral cycles, barely interfering with a subject's daily routine. [10,11]

According to the current International Classification of Sleep Disorders 3nd edition (ICSD-3), actigraphy plays a major role for the objective monitoring of sleep schedule and duration in the weeks prior to MSLT in central disorders of hypersomnolence, in order to objectively rule out insufficient sleep or circadian rhythms misalignment.[1] Actigraphic studies on central disorders of hypersomnolence are rare, and the above ICSD-3 recommendations were mostly due to consensus,[12] although preliminary data are of interest: Middelkoop and co-authors compared the circadian pattern of motor activity of 14 drug-free NT1 patients versus age- and sex- matched controls, and demonstrated that diurnal and nocturnal measures of "uninterrupted immobility" (defined as mean duration of periods without activity) were able to discriminate between groups, with NT1 patients showing a higher nocturnal motor activity profile.[13] More recently, Bruck and co-authors confirmed those findings, even though in a small cohort of NT1 patients (n = 9). Additionally, the authors showed that the actigraphic measurement of "immobility" could be successfully used to differentiate between medicated and unmedicated NT1 patients treated with wake-promoting medication.[14] Finally, Poryazova and co-authors showed that actigraphic estimated sleep quality significantly improved after treatment with sodium oxybate, suggesting that actigraphy could offer a cheaper and simpler alternative to PSG for assessing sodium oxybate treatment effects.[15]

The purpose of this study was to determine if actigraphy can reliably characterize the circadian profile of sleep and wakefulness of different central disorders of hypersomnolence and distinguish NT1 from IH patients and healthy controls by combining diurnal hypo-activity (as index of hypersomnolence) and nocturnal hyper-activity (as index of disrupted sleep quality) measures.

MATERIAL E METHODS

Subjects

Subjects were patients evaluated for complaints of hypersomnolence, from March 2010 to January 2014, at the outpatient clinic for Narcolepsy of the Department of Biomedical and Neuromotor Sciences, University of Bologna, and who received a final diagnosis of central disorders of hypersomnolence according to the ICSD-3 criteria.[1]

All patients underwent the following diagnostic protocol: (a) clinical evaluation performed by the same sleep specialist (G.P.); (b) assessment of subjective sleepiness by means of Italian version of the Epworth Sleepiness Scale (ESS);[16] (c) seven days of actigraphic and self-report monitoring of sleep-wake behavior; (d) 48-hour continuous polysomnographic recording followed by (e) a MSLT with five nap opportunities; and (f) lumbar puncture to assay hypocretin-1 levels, where possible.

The final study sample included 63 patients, consisting of thirty-nine NT1 patients (22 males, mean age 34 ± 16 years) and twenty-four IH patients (11 males, mean age 32 ± 15 years). All patients were drug-naïve at the time of actigraphic recording.

NT1 patients fulfilled the ICSD-3 criteria presenting: persistent daytime sleepiness (ESS score 16.41 ± 3.42), a clear-cut history of cataplexy (*n* = 39/39), and mean sleep latency <8 min (mean 3.14 ± 1.77) with at least two sleep-onset REM periods (mean 3.92 ± 1.10) at the MSLT. All patients were HLA DQB1*0602 positive and had reduced (i.e. < 110 pg/ml) or undetectable hypocretin-1 levels (mean 26.70 ± 27.72) when tested (*n* = 24/39).

IH diagnosis required the following criteria according to current ICSD-3: presence of persistent daytime sleepiness (ESS score 14.50 ± 3.57), absence of cataplexy, mean sleep latency <8 min (mean 6.14 ± 1.09) with fewer than two SOREM at MSLT (mean 0.38 ± 0.49) and nocturnal PSG, adequate schedule and duration of the main sleep period documented by actigraphy (estimated total sleep time = 417 ± 75 min.), and no evidence of concurrent sleep or medical disorder as stated by nocturnal PSG and clinical evaluation respectively. CSF hypocretin-1 concentration was in the normal range (i.e. > 200 pg/ml) in all patients tested (*n* = 11, mean 335.55 ± 138.03); four patients were HLA DQB1*0602 positive (16.6 %).

Thirty healthy controls (15 males, mean age 29 ± 9 years) were recruited from the local community. Participants were clinically screened to rule out sleep or medical disorders; only subjects with regular sleep schedule and without complaints of sleep disturbance or daytime sleepiness (mean ESS score 4.47 ± 2.63) were included.

The study was approved by the local review board and all participants signed a written informed consent.

Actigraphic assessment

Actigraphy is based on small wrist-watch like devices that monitor movements for extended periods of time. Actigraphy is a semi quantitative method, provides an indirect assessment of sleep through the use of computerized scoring algorithms applied to the raw activity data.

The Micro Motionlogger® Watch actigraph (Ambulatory Monitoring, Inc., Adrsley, NY) was used in the present study. The hardware consists of a triaxial accelerometer; overall sensitivity is 0.01g at the midpoint of bandpass filter which is set at 2-3 Hz, and sampling frequency is set at 32 Hz. This device has also a temperature and an ambient light sensor. Actigraphs were initialized in zero crossing mode to collect data in 1-min epochs. The raw activity data were analyzed through ActionW-2 version 2.7.1 software using the mathematical model validated by Cole and co-authors.[17]. The algorithm computes a weighted sum of the activity in the current epoch, the preceding 4 epochs, and the following two epochs as follows: $S = 0.0033\ (1.06_{a-4} + 0.54_{a-3} + 0.58_{a-2} + 0.76_{a-1} + 2.3_{a0} + 0.74_{a1} + 0.67_{a2})$; where from an4 to an1 are the activity counts from the preceding 4 minutes, a1 and a2 are activity counts from the following 2 minutes, and a0 is the current epoch that is scored as sleep when $S < 1$.

Participants were asked to wear the actigraph on the non-dominant arm over seven consecutive days, starting at the clinic visit, which are sufficient to obtain a meaningful description of the rest-activity behavior.[18] Subjects were instructed to maintain their habitual sleep/wake schedule during

the recording period. Parallel to actigraphic assessment, subjects were asked to fill out a daily sleep log in which they would report: (i) what time they went to bed at night and their last awakening in the morning, and (ii) frequency and duration of diurnal naps. Moreover, subjects were instructed to push a button located on the side of the watch ("event marker") to mark occurrences such as time in and out of bed and periods when the actigraph was not worn. Using both event-marked points and sleep log information, scoring was checked in order to identify sleep-wake periods and eliminate periods when actigraph was removed.

Nighttime and daytime measures

For *nighttime period*, corresponding to the time between when the subject went to bed and switched off the light and final self-reported awakening in the morning, we considered the following actigraphic measures: estimated time in bed (TIB – time in minutes, between reported light off and light on), estimated total sleep time (TST – sum, in minutes, of all sleep epochs between light off and light on); estimated sleep onset latency (SOL – interval in minutes, between light off and sleep onset); estimated sleep motor activity (SMA – mean number of movements within one minute, during sleep epochs); estimated wake after sleep onset (WASO – sum, in minutes, of all wake epochs between sleep onset and sleep end); estimated sleep efficiency (SE%, the ratio of TST to TIB multiplied by 100); estimated wake episodes (Awk – number of epochs scored as wake between sleep onset and sleep end) and number of estimated wake episodes lasting more than 5 consecutive epochs (Awk>5).

For the *daytime period*, corresponding to the time between the final self-reported awakening in the morning and the beginning of a new major sleep period, we considered the following actigraphic measures: daytime motor activity (DMA – mean number of movements within one minute, during estimated wake period); number of estimated sleep episodes lasting more than 5 consecutive epochs

(Nap); and mean duration of longest estimated sleep episodes (NapD – mean duration, in minutes, of the longest estimated sleep episodes).

Statistical analyses

Data for each group were explored using descriptive statistics (mean ± SD). Group differences in demographic and clinical data were analyzed with Pearson's chi-square test for categorical variables, Mann-Whitney test for ordinal data, and one-way between-group analysis of variance (ANOVA) for continuous variables.

Comparisons of actigraphic variables were performed by means of ANOVA, post-hoc comparisons were performed using Bonferroni test.

A stepwise multiple discriminant analysis with Wilks' s Lambda method was carried out to explore whether a set of variables is more effective in predicting group membership; F values of 3.84 for entry into, and 2.71 for removal from, the discriminant analysis were used. The first discriminant function selected was applied to the data and a receiver operating characteristic (ROC) curve was generated.[19]

Values of the Area under ROC curves were used to select cut-off values; the Youden Index (i.e. the higher value obtained calculating sensitivity+specificity-1) was used to determine optimal cut-off values, finally positive and negative predictive values were computed.[20]

All statistical analyses were performed using SPSS 19.0 (SPSS, Inc. Chicago, Ill). *P*-value <0.05 was considered statistically significant.

RESULTS

Chi-square and one-way ANOVA analyses revealed no differences among groups in either gender or age, respectively. One-way ANOVA showed no difference between NT1 and IH regarding levels

of subjective sleepiness, while both clinical groups displayed higher ESS score than controls, as expected for inclusion criteria ($p < 0.0001$). Demographics, clinical, MSLT and biochemical data for all subjects are shown in Table 1. Overall the wearing time of the actigraphic device exceed 90% of the total recording time (mean values 95.40 ± 2.73%, range 90.21-99.13%).

Actigraphic nighttime and daytime data for the three groups are reported in Table 2, together with significance values of the ANOVA and post-hoc results. Results showed a main group effect for all actigraphic parameters considered except estimated TIB and estimated SOL. Post hoc contrast disclosed that: (a) NT1, IH and controls spent in bed the same amount of time, but NT1 patients slept significantly less than both IH and controls; (b) NT1 patients had the lowest estimated SE% with more time spent in estimated WASO than the other two groups; (c) NT1 patients showed the highest frequency of estimated nocturnal awakenings, followed by IH and controls respectively (NT1 > IH > Controls); (d) estimated nighttime motor activity levels of NT1 patients were significantly higher than those of IH and controls; (e) daytime motor activity levels of NT1 and IH patients were significantly reduced than those of controls; and (f) NT1 patients showed the highest frequency of daytime estimated naps, followed by IH and controls respectively. The same trend of differences was observed regarding average duration of the estimated longest nap (NT1 > IH > Controls). Stepwise discriminant analysis was performed including all variables that significantly differed among groups in the ANOVA.

The first function selected included three predictors estimated SMA, estimated Awk and estimated NapD that accounted for 97% of the explained between-group variance (Wilks Lambda = 0.292, p = 0.0001, Eigenvalue = 2.228) and was computed as Discriminant Score (DS): SMA*0.049+Awk*0.095+NapD*0.04-2.934. Overall, this linear function correctly classified 81.7% of the cases. More in detail, classification accuracy was 87.2% for NT1 (n = 34/39), 58.3% for IH (n = 14/24), and 93.3% for healthy controls (n = 28/30) respectively. At ROC curve analysis, DS showed an area under the curve of 0.935 (Figure 1); using a balanced approach, a cut-off of

mean DS equal to -1.05 produced a good balance between positive (0.95) and negative (0.87) predictive values.

DISCUSSION

This study was the first to investigate the actigraphic estimated daytime and nighttime sleep in a group of drug-naïve patients with different central disorders of hypersomnolence (namely NT1 and IH) and in healthy controls. Altogether, our findings showed that NT1 patients displayed a 24-hour actigraphic profile characterized by nighttime and daytime impairment, while IH patients displayed a daytime impairment without differences in overall estimated sleep quality when compared to healthy controls. The discrete daytime and nighttime actigraphic profile of patients suffering from central disorders of hypersomnolence suggests that actigraphy may provide useful information when combined with a careful clinical examination, and can also possibly distinguish among groups. Analyzing nighttime period we found that NT1 patients presented a marked decrease in estimated sleep quality, which was characterized by reduced total sleep time with numerous estimated awakenings, extended time spent in estimated WASO and high representation of motor events, when compared with IH patients and controls. Conversely, patients with IH showed higher frequency of estimated nocturnal awakenings when compared with controls, without any other between-group difference. These results are in line with PSG studies in documenting the features of disrupted nighttime sleep in NT1.[4,21] Noteworthy, levels of nocturnal motor activity rendered a different pattern in NT1, further confirming that an increased motor activity during nightsleep is an intrinsic feature of this disease.[22]

Loss or impaired hypothalamic hypocretin (HCRT) signaling may, at least in part, explain the nighttime motor dysfunction of NT1 patients.[23] Hypocretin axons are found throughout the brain with dense projections to brainstem nuclei and to basal forebrain regions; under typical conditions hypocretinergic neurons promote motor activity during wakefulness and inhibit motor activity

during REM sleep.[24] In patients with NT1 the opposite seems to take place, with motor inhibition and sleep occurring during the major wakefulness period, as well as enhanced muscle tone and motor activity during sleep.[25]

During daytime patients with NT1 and IH present a more scattered distribution of estimated naps and a reduction in mean motor activity levels when compared to healthy controls. The nature and severity of diurnal impairment, however, differed among IH and NT1, with NT1 patients displaying highest estimated nap frequency and lowest motor activity level.

Our results confirm earlier studies that, however, considered different actigraphic variables (i.e. immobility) and tested smaller groups of narcoleptic patients, reporting a trend in difference between NT1 and controls regarding the diurnal period.[13,14] In addition, by comparing patients with NT1 and IH, we extended these findings to other central disorders of hypersomnolence with comparable levels of subjective sleepiness, pinpointing that actigraphy may contribute to reliably render the features of daytime behavior in different central disorders of hypersomnolence. Overall, we found that the actigraphic nycthemeral profile is able to reliably differentiate among groups. Moreover, it can be useful, in combination with PSG and MSLT, in both the diagnostic work-up of central disorders of hypersomnolence and in the follow-up as objective measures of disease course and treatments efficacy.

Noteworthy, we found that the combined use of both nocturnal (estimated SMA, estimated Awk) and diurnal (estimated NapD) parameters performed better in NT1 cases than any single actigraphic measure. Indeed, these parameters reflect two intrinsic features of NT1, namely disrupted nocturnal sleep (estimated Awk, estimated SMA) and hypersomnolence (estimated NapD).

Some limitations of the present study should be acknowledged. First, although reporting on the largest actigraphic evaluation on NT1 patients in the literature, we are still underpowered to stratify the findings by different age groups. Second, some cautions need to be used in interpreting the diurnal motor activity data since DMA levels are clearly influenced by the scattered distribution and duration of sleep episodes during daytime. Future studies using chronobiological approach may

help to establish whether the decrease in DMA levels still persists despite the elevated frequency of diurnal sleep episodes.

Overall, the present study shows that actigraphic monitoring is a useful technique to objectively assess the features of sleep-wake profile of central disorders of hypersomnolence, with the main advantage of providing more naturalistic information. Further studies are needed to explore whether the actigraphic measurements considered are sensitive enough to detect treatment effects on both nighttime and daytime sleep.

Acknowledgment

None

Table 1 – Demographic characteristics, scale scores and MSLT data of patients with narcolepsy type 1, idiopathic hypersomnia, and healthy controls.

Demographic and clinical data	Narcolepsy Type 1 *n* = 39 mean ± SD (%)	Idiopathic Hypersomnia *n* = 24 mean ± SD (%)	Healthy Controls *n* = 30 mean ± SD	*P*-value[a]
Age, years	34.21 ± 15.58	31.96 ± 15.20	29.37 ± 9.47	0.38
Male gender	23	11	15	0.56
BMI	27.20 ± 5.62	24.78 ± 4.43	22.79 ± 2.81	<0.001
ESS score	16.41 ± 3.42	14.50 ± 3.57	4.47 ± 2.63	<0.0001
CSF hypocretin-1	26.70 ± 27.72	335.55 ± 138.03		<0.0001
HLA DQB1*0602 positive	39 (100)	4 (16.6)		<0.0001
MSLT data				
MSLT sleep latency, minutes	3.14 ± 1.77	6.14 ± 1.09		<0.0001
SOREMs, numbers	3.92 ± 1.10	0.38 ± 0.49		<0.0001

BMI = body mass index; ESS = Epworth sleepiness scale; MSLT = multiple sleep latency test; SOREMP = sleep-onset REM period. Data are presented as mean ± SD or number (percentage). Data were based on total number of subjects in each group, except CFS hypocretin-1, which included data from 24 (NT1) and 11 (IH) subjects, respectively.

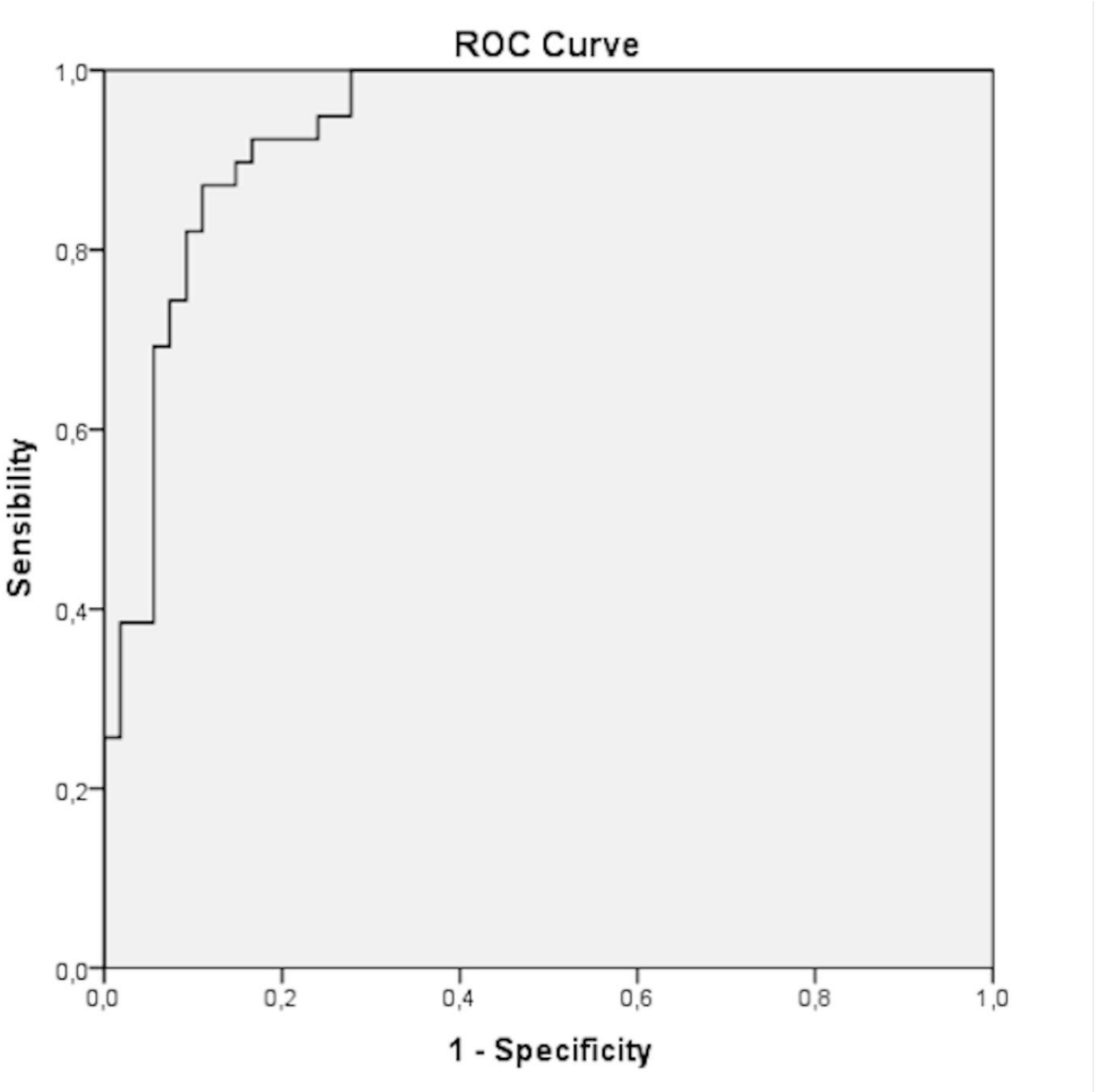
[a]*P*-values were derived from One-way ANOVA, Chi-square test or Mann-Whitney U test, as appropriate.

Table 2 – Actigraphic measures (Means and SD) and post-hoc results for NT1, IH and Control group.

	NT1 Group (*n*=39)	IH Group (*n*=24)	Control Group (*n*=30)		NT1 vs Control	NT1 vs IH	IH vs Control
	Mean ± SD	Mean ± SD	Mean ± SD	*P*-value	post-hoc/ *t*-test	post-hoc/ *t*-test	post-hoc/ *t*-test
Nighttime period							
TIB (min.)	471.04 ± 65.13	465.07 ± 85.59	478.82 ± 59.69	ns			
TST (min.)	362.57 ± 83.49	417.41 ± 74.73	457.97 ± 53.04	<0.00001	0.0001	0.01	ns
SOL (min.)	13.29 ± 13.34	11.53 ± 9.73	8.32 ± 4.68	ns			
SE (%)	76.97 ± 13.97	89.65 ± 5.34	95.70 ± 1.99	<0.00001	0.0001	0.0001	ns
WASO (min.)	92.50 ± 55.17	36.46 ± 26.68	12.49 ± 8.80	<0.00001	0.0001	0.0001	ns
Awk (*n*°)	17.13 ± 6.90	11.95 ± 6.63	3.47 ± 2.13	<0.00001	0.0001	0.005	0.0001
Awk>5 (*n*°)	5.18 ± 2.49	2.54 ± 2.10	1.20 ± 0.89	<0.00001	0.0001	0.0001	0.05
SMA (counts)	29.80 ± 14.25	16.12 ± 6.21	9.98 ± 3.27	<0.00001	0.0001	0.0001	ns
Daytime period							
DMA (counts)	192.41 ± 30.26	199.14 ± 45.11	222.88 ± 15.94	<0.0005	0.0001	ns	0.05
Nap (*n*°)	3.51 ± 1.67	2 ± 2.36	0.5 ± 0.90	<0.00001	0.0001	0.005	0.005
NapD (min.)	35.46 ± 15.50	19.83 ± 17.38	6.40 ± 11.46	<0.00001	0.0001	0.0001	0.005
DS	1.57 ± 1.17	-0.22 ± 1.17	-1.86 ± 0.46	<0.00001	0.0001	0.0001	0.0001

SD – standard deviation; TIB – estimated time in bed (min.); TST – estimated total sleep time (min.); SOL – estimated sleep onset latency (min.); SE% – estimated sleep efficiency; WASO – estimated wake after sleep onset (min.); Awk – number of estimated awakenings; Awk>5 – number of estimated awakenings longer than 5 consecutive minutes; SMA – mean estimated sleep motor activity (number of movements in one minute); DMA – mean daytime motor activity (number of movements in one minute); Nap – number of daytime estimated sleep episodes longer than 5 consecutive minutes; NapD – mean duration of longest estimated sleep episode (min.); DS – discriminant score.

Figure 1 – Receiver operating characteristic curve for the combination (DS) of Awk (number of estimated awakenings), SMA (estimated sleep motor activity) and NapD (mean duration of longest daytime estimated sleep episode)



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Chapter 3

Circadian Rest-Activity Rhythm in Pediatric Type 1 Narcolepsy

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ABSTRACT

Study Objectives: Pediatric type 1 narcolepsy is often challenging to diagnose and remains largely undiagnosed. Excessive daytime sleepiness, disrupted nocturnal sleep, and a peculiar phenotype of cataplexy are the prominent features. Scarce knowledge are available about the regulation of circadian rhythms in affected children. This study compared circadian rest-activity rhythm and actigraphic estimated sleep measures of type 1 narcolepsy children versus healthy controls.

Design: Case-control, rest-activity rhythm was quantified over seven days by actigraphy.

Setting: Children studied during the school week.

Patients or Participants: Twenty-two drug-naïve type 1 narcolepsy children and twenty-one age- and sex- matched controls.

Interventions: N/A.

Measurements and Results: Circadian activity rhythms were analyzed through functional linear modeling; nocturnal and diurnal sleep measures were estimated from activity using a validated algorithm. Type 1 narcolepsy children presented an altered rest-activity rhythm characterized by enhanced motor activity throughout the night and blunted activity in the first afternoon. No difference was found between type 1 narcolepsy children and controls in the timing of circadian phase. Actigraphic sleep measures showed good discriminant capabilities in assessing type 1 narcolepsy nycthemeral disruption.

Conclusions: Circadian rest-activity rhythm is altered in pediatric type 1 narcolepsy. Recording motor activity by actigraphy promises to be a reliable objective marker in the complex diagnostic pathway of type 1 narcolepsy in children. This simple and cheap screening could help to improve diagnosis, and may prove useful to assess disease severity and reduce diagnostic delay.

Keywords: Narcolepsy, Pediatrics, Circadian Rhythms, Motor Activity, Actigraphy.

INTRODUCTION

Type 1 narcolepsy (NT1) is a lifelong central nervous system disorder characterized by chronic hypersomnolence with multiple sleep attacks during daytime, cataplexy (sudden and transient loss of muscular tone usually evoked by emotions), dissociated rapid eye movement (REM) sleep manifestations such as sleep paralysis (temporary inability to move voluntary muscles) and/or hallucinations at the wake-sleep transition, and nocturnal sleep disruption.[1,2] The disease is linked to the loss of hypothalamic hypocretin-producing neurons, which leads to cerebrospinal fluid (CSF) hypocretin-1 (hcrt-1) deficiency.[1,3]

NT1 is regarded as rare with estimated prevalence between 25 and 50 per 100.000 in the general population,[4] however a large proportion of the expected cases are undiagnosed or remain misdiagnosed in both adults and children.[5,6] Until recently, NT1 has been poorly recognized in children although the majority of the patients report the onset of symptoms in childhood and adolescence.[5] Indeed, scarce epidemiological data on pediatric NT1 are available,[7] nevertheless the improving disease awareness and the recent peak of incidence after H1N1 influenza pandemic and vaccine has led to a relevant increase in NT1 diagnoses in children and adolescents.[8,9]

Diagnosis of childhood NT1 remains often challenging, especially close to symptom onset, given the frequent paradoxical presentation of hypersomnolence as hyperactivity and the peculiar cataplexy phenotype with persistent and spontaneous hypotonic features and falls, intermingled with active movements and further enhanced by emotions.[10-12] This variable clinical presentation, and the still scarce awareness about this peculiar features, has frequently led to misdiagnosis with behavioral, psychiatric or neurological disorders, further delaying proper diagnosis and treatment.[6,13]

Recently, several studies have highlighted that actigraphy, an objective method for quantitative assessment of motor activity and indirect assessment of sleep based on wearable technology, offers unique potentialities in the clinical work-up of adult NT1 cases.[14,15] Actigraphic monitoring

discriminated NT1 from other central hypersomnias in patients and healthy controls,[14] and quantified treatment response to wake-promoting drugs and sodium oxybate.[16,17]

Besides sleep assessment,[18] prolonged actigraphic monitoring also offers a way to evaluate the robustness of the endogenous rhythms driven and synchronized by the master circadian pacemaker through direct evaluation of rest-activity rhythm.[19] To date only a limited number of studies has investigated circadian rhythms in NT1, describing increased daytime secretion of melatonin,[20,21] while circadian rhythms of core body temperature and cortisol were essentially preserved.[22] However, these studies examined exclusively adult patients with a long disease history.

The main purpose of the current study is to investigate the rest-activity rhythm in pediatric NT1 patients versus healthy children by means of actigraphy. Actigraphy is a non-invasive method that provides circadian measures collected in the subject's natural environment, thus representing a particularly valuable approach to assess rest\activity behavior among pediatric populations.[23,24] Furthermore, prolonged actigraphic monitoring allows the extraction of densely recorded time series of motor activity suitable to be analyzed with advanced techniques for data modeling.[25] The secondary aim of the study is to compare actigraphic estimated nocturnal and diurnal sleep measures of NT1 children with age- and sex- matched controls to explore whether actigraphic assessment shows good discriminant capability in pediatric NT1 cases, together with the analyses of correlates between clinical (BMI and hcrt-1), neurophysiological and actigraphic-derived sleep measures.

METHOD

Subjects

The study included 22 patients, drug-naïve children and adolescents (10 males, mean age 12.09±2.37 years, range 7-15 years), with a final diagnosis of NT1 evaluated at the Outpatient

Clinic for Narcolepsy of the Department of Biomedical and Neuromotor Sciences, University of Bologna from January 2012 to September 2013.

Patients underwent the following diagnostic procedures: clinical assessment, at home actigraphic monitoring, cerebral magnetic resonance imaging (to rule out secondary cases), and then hospitalization with 48-hr continuous polysomnographic (PSG) recording, multiple sleep latency test (MSLT), cataplexy video-documentation, human leukocyte antigen (HLA) typing to confirm DQB1*06:02 haplotype and, whenever possible, CSF hcrt-1 assay.[26]

Clinical evaluation was systematically conducted by the same sleep specialist (G.P.), it included the assessment of subjective sleepiness with the Epworth Sleepiness Scale adapted for children and adolescents (aESS),[27]and of circadian preferences by means of the Italian version of the reduced Morningness-Eveningness questionnaire for Children and Adolescents (rMEQ-CA).[28]

All patients fulfilled the current ICSD-3 clinical criteria for NT1 presenting severe cataplexy (*n* = 22/22) and daytime sleepiness (aESS score: 13.86 ± 3.37). Twenty out of 22 cases had mean MSLT sleep latency (MSLT-sl) < 8 min with multiple sleep-onset REM periods (SOREMPs). Seventeen patients (including the 2 cases with MSLT-sl > 8 min) underwent lumbar puncture and all had low (≤ 110 pg/ml) or undetectable CSF hcrt-1 levels; all patients carried the HLA DQB1*06:02 allele.

Twenty-one age- and sex-matched healthy children (13 males, mean age 10.95±2.25 years, range 7-16 years), recruited at a school in Rome, were selected from the anonymous database of the Pediatric Sleep University Center, Sapienza University, Rome. This series of children belong to the same group of controls used in a previously published study.[29]

The study was approved by the internal review board and written informed consent was signed by parents of children.

Procedure

Rest-activity rhythm was monitored during the school week, outside of holidays and vacation. Participants were required to wear the actigraph on the non-dominant wrist for one week (before hospitalization for NT1 patients), providing five complete nycthemeral cycles, which are necessary to obtain a reliable description of sleep and rest-activity rhythm in children.[30]

The Micro Motionlogger® Watch actigraph (Ambulatory Monitoring, Inc., Ardsley, NY), consisting of a triaxial accelerometer with case temperature and ambient light sensors, was used in the present study. Actigraphs quantify motor activity exceeding 0.01*g* at a sampling frequency of 32 Hz, the values for each sample are used to compute the average activity counts within the chosen time window (epoch). Devices were initialized for zero-crossing mode to collect data in one-minute epochs in accordance with the practice parameters for the use of actigraphy.[23]

Participants were asked to maintain their usual sleep/wake schedule during the recording period. Children wore the device continuously throughout the 24 hours, except when bathing/showering, and were instructed to push the event-marker button on the device to mark time in and out of bed. In addition, a sleep diary was used to obtain subjective information from the children, for children under 11 years of age (i.e. 5 NT1 and 9 control children) we asked parents/caregivers to fill in daily the sleep diary and help them with the event-marker procedure, if necessary.

The information contained in the sleep diary included the bedtime, the wake time, and the rise time, additionally we asked to record events that might bias the actigraphic recording such as periods of device removal.

Actigraphic recording was visually edited by an experienced scorer who used the information provided by event-marker points and sleep dairy to indentify the major nocturnal sleep period. Periods of device removal detailed in the diary were further verified from the case temperature channel and excluded from analysis.

Circadian rest–activity rhythm and actigraphic sleep assessment

For circadian motor activity analysis we extracted raw activity data per minute (time series) using Action 4 software version 1.16 (Ambulatory Monitoring, Inc., Ardsley, NY) and processed them with R statistical software to apply Functional Linear Modeling (FLM) according to the model put forward by Wang and co-authors.[31] FLM belongs to a broader family of statistical techniques (Functional Data Analysis) that represent observations arising from time-series in the form of functions.[25,32] This approach allows the analysis of the circadian features of motor activity through direct analysis of raw activity data. FLM replaces the motor activity counts with a function that models the data, reduces variability, and compares sets of functions to explore whether and when they statistically differ between groups.

We considered only data from 20:00 Sunday to 20:00 Friday, in order to avoid possible variations related to weekend days. Activity gaps during daytime due to device removal were filled up with average activity values from the same time-period of the remaining days; days containing gaps longer than one hour were excluded from analysis.

The five continuous nycthemeral cycles of actigraphic data were averaged into a single 24-hour motor activity pattern and converted into a function adopting a Fourier expansion model with $n = 9$ basis permutation fitted at a 24-hr periodicity.

Actigraphic estimated sleep measures were computed with the Action W-2® software version 2.7.1 (Ambulatory Monitoring, Inc., Ardsley, NY), this software identified each epoch as sleep or wake using the mathematical model validated by Sadeh.[33]

Actigraphic recordings were divided into nighttime and daytime periods according to individual bedtime and wake-up time; mean daytime and nighttime parameters were computed across the school week for each subject.

We considered the following actigraphic measures for sleep timing: bed time (BT – clock time, in hours and minutes, when subject goes to bed and turns off the light), get up time (GUT – clock time, in hours and minutes, when subject gets out of bed in the morning), time in bed (TIB – time, in minutes, from BT to GUT), and midpoint of sleep (MS – clock time, in hours and minutes, that

split in half the TIB). For the nighttime period the following measures were considered: estimated sleep onset latency (*e*SOL – interval, in minutes, between BT and sleep onset, the latter determined as the first epoch of a block of 20 consecutive min after BT with no more than one epoch scored as wake); estimated total sleep time (*e*TST – sum, in minutes, of all sleep epochs between sleep onset and GUT); estimated wake after sleep onset (*e*WASO – sum of minutes scored as wake between sleep onset and GUT); estimated sleep efficiency (*e*SE% – the ratio of TST to TIB multiplied by 100); number of estimated awakenings (*e*Awk – number of wake episodes between sleep onset and GUT); estimated awakenings lasting more than five consecutive minutes (*e*Awk>5); and sleep motor activity (SMA – sum of all activity counts in 1-minute epochs during TIB divided by TIB duration in minutes). From the daytime period we considered the following measures: daytime motor activity (DMA – sum of all activity counts in 1-minute epochs for the time period between GUT and BT divided by its duration in minutes); daytime estimated total sleep time (*e*DTST – sum of minutes scored as sleep between GUT and BT); estimated nap frequency (*e*Nap – number of sleep episodes between GUT and BT, where nap is defined as an interval of at least 10 min up to 3 hours scored as sleep, preceded and followed by a period of at least 30 continuous minutes scored as wake); and mean duration of longest estimated nap (*e*NapD – mean duration, in minutes, of the longest daytime sleep episode).

Statistical Analysis

All continuous and categorical data were explored with descriptive (mean ± standard deviation) and frequency statistics for each group. Differences between groups in demographical data, BMI and scale scores were analyzed with chi-square and independent samples *t*-test.

Circadian activity patterns analysis was undertaken with R and the *Actigraphy* library in R; FLM was used to test differences in the time-course of motor activity between groups.

For each actigraphic measure independent sample *t*-tests were performed to compare NT1 and control children, followed by effect size (Cohen's *d*) computation.[34]

Finally, the relationship between clinical data (BMI and Hcrt-1 levels), questionnaire scores (aESS and rMEQ-CA), neurophysiological measures (MSLT-sl and number of SOREMPs), and actigraphic-derived sleep measures were explored, separately for each group, with Pearson's correlation coefficient analyses.

Statistical analyses were conducted using SPSS 19.0 (SPSS, Inc. Chicago, Ill). Results with *p* values <0.05 were considered statistically significant.

RESULTS

Table 1 shows demographic, clinical and neurophysiological characteristics of the sample and questionnaire scores. Detailed nocturnal PSG features of the NT1 sample are reported in the supplementary table. NT1 patients displayed higher aESS scores and BMI than controls without differing in the distribution of circadian typologies or rMEQ-CA scores.

Circadian mean motor activity profiles of each group resulting from Fourier expansion are shown in Figure 1 (upper panel) together with F-statistics results (lower panel). Where the observed statistic (i.e., the red solid line) is above the blue dashed line (i.e. global critical test of significance with $\alpha = 0.05$) the groups have statistically different activity counts at that specific time point (1 min time window). Both groups showed a preserved overall structure, with lower activity during night hours and higher activity during daytime, and comparable TIB. NT1 patients had significantly higher motor activity throughout nighttime (from 23:00 till 6:00), similar motor activity during morning between 7:00 and 12:00, and a significantly marked decrease of motor activity in the afternoon starting from 12:00 till about 18:00 with no further differences from the latter time to 23:00 compared to controls.

Actigraphic sleep measures are reported in Table 2, together with significance values at *t*-test, Cohen's *d* and 95% confidence intervals (CI). NT1 patients and controls went to bed (BT) and woke up (GUT) at similar time, thus displaying comparable sleep phase. All actigraphic nighttime measures but TIB, *e*SOL, and sleep timing differed between the two groups: NT1 patients slept less during nighttime (*e*TST), displayed lower *e*SE% with increased frequency of sleep interruptions (*e*Awk) and prolonged awakenings (*e*Awk>5), higher amount of *e*WASO and enhanced SMA than controls. During daytime NT1 children had more *e*DTST with more frequent *e*Nap occurrence, longer nap duration (*e*NapD), and lower motor activity during wakefulness (DMA) than controls. Pearson's correlation analyses are reported in Table 3 for NT1 patients and controls respectively. In the NT1 group we found that: (a) BMI was positively correlated with aESS and SMA, and negatively with TIB, *e*TST, *e*SE%, *e*Awk and DMA; (b) aESS was directly related to SMA and *e*WASO, and inversely related to *e*TST, and *e*SE%; (c) rMEQ-CA was positively correlated with TIB and *e*TST, and negatively correlated with SMA; and (d) CSF hcrt-1 level was positively correlated with *e*TST, and MSLT-sl, and negatively with *e*Nap and aESS. In the control group, only a negative correlation between aESS and TIB reached statistical significance.

DISCUSSION

Our study was the first specifically aimed at analyzing rest-activity rhythm in a sizeable group of drug-naïve NT1 children, monitored in real-life setting during the school week. We found that, despite a comparable sleep phase, NT1 children showed an altered circadian rest-activity rhythm compared to age- and sex- matched healthy children.

Circadian analyses revealed that the most striking differences between our cohort of NT1 versus control children were time-locked to nighttime, when NT1 children presented higher motor activity levels maintained throughout the nocturnal period, and to the early afternoon, when NT1 children displayed lower motor activity. Conversely, NT1 and control children showed similar activity

levels during morning and evening hours. To our knowledge, the present report is the first investigation on circadian rhythms in pediatric NT1 patients, nonetheless the circadian rhythm abnormalities highlighted herewith are remarkably similar to those reported on adult NT1 patients. In line with our findings, a recent study compared circadian pattern of melatonin secretion (through assay of plasma melatonin concentration) of adult NT1 patients and controls and showed that, although average hormone concentrations across the 24-hr did not differ between groups, the circadian pattern of melatonin release was altered in NT1, with patients presenting a higher proportion of melatonin secreted during daytime and a major peak of secretion in the early afternoon between 14:00 and 16:00.[21]

Further evidences for altered circadian rhythmicity in NT1 is supported also by cognitive studies: Schneider and co-authors compared daytime variations of performances in cognitive task assessing alertness and selective attention in four groups of adults with sleep disorders (NT1, psychophysiological insomnia, and treated or untreated obstructive sleep apnea syndrome) and controls, highlighting a peculiar pattern of daytime fluctuations in NT1 patients, along with the lowest mean performance in all cognitive functions.[35] Performance of NT1 patients was higher in the early morning (08:00), thereafter it quickly deteriorates until reaching a nadir in the early afternoon (14:00) before rebounding again to levels similar to those of morning session around 18:00. On the contrary, healthy controls and patients with other sleep disorders showed an initial increase in performance and did not presented such major fluctuations during daytime.

The above findings, along with our observations, suggest that the loss of hypocretinergic neurons may lead to an imbalance between the sleep-wake regulating homeostatic and circadian processes, [36,37] weakening the circadian waking drive and its ability to oppose the homeostatic sleep pressure.[38] As a result the ultradian and semi-circadian fluctuation of sleep propensity may became predominant with untimely intrusion of sleep, regardless of circadian phase. [39,40]

Analyzing actigraphic derived nocturnal and diurnal sleep measures we highlighted the good discriminant capability of actigraphic monitoring in depicting the marked impairment of both

nocturnal sleep and daytime wakefulness in a young cohort of drug-naïve NT1 patients. Indeed, our cohort of drug-naïve NT1 children presented numerous sleep episodes and lower motor activity counts during daytime, associated with major nocturnal sleep fragmentation and enhanced motor activity during nighttime, as previously reported in adult NT1 drug-naïve cases.[14] This peculiar nycthemeral disruption, already detectable by means of actigraphy in NT1 children close to disease onset, should be regarded as an intrinsic disease hallmark.

Although actigraphy recommendations in the diagnostic work-up of NT1 are confined to rule out sleep deprivation and circadian rhythm disorders prior to MSLT,[1] we showed the good discriminant capabilities of actigraphic assessment, both in adult and pediatric NT1 cases.[14] Actigraphy also offers the possibility to monitor patients in their own environment allowing us to document long-lasting diurnal sleep episodes that are often reported in childhood NT1.[41] The standard diagnostic approach, based on the nocturnal polysomnography followed by the MSLT, allows a proper neurophysiological diagnosis with high sensitivity and specificity, but does not give insight into this very common aspect of NT1 hypersomnolence.[1]

Finally, we reported that increased BMI, high subjective sleepiness and low CSF hcrt-1 levels were associated with the severity of nycthemeral disruption in childhood NT1 pointing to the possibility to further objectively stratify disease severity.

Some limitations of the present study need to be acknowledged. First, although this cohort represents the largest actigraphic study on pediatric NT1, the sample is still relatively small and prevented us from categorizing children according to pubertal maturation. Second, we did not evaluate other markers of the circadian clock (e.g. melatonin or cortisol) besides the rest-activity rhythm to test whether the blunted motor activity pattern during daytime was coupled with altered endocrine secretions.

Although actigraphy remains a screening method that cannot substitute the gold standard diagnostic protocol for NT1 (namely nocturnal polysomnography followed by MSLT and CSF hcrt-1 measurement), this monitoring may offer a complementary measure to support the diagnosis of

pediatric NT1 especially enhancing the diagnostic probability of questionable cases, to track disease course over time, and to tailor supportive strategies. First, we showed that actigraphy can document different daytime and nighttime impairments in a more ecological and cost-effective way compared to laboratory procedures, and could represent an objective tool to assess disease burden in real-life settings. Second, given that behavioral treatment (i.e. regularly scheduled naps) is a major management strategy for NT1,[42] actigraphy can be used to objectively assess, and possibly adjust, the napping schedule. Third, different studies have shown the ability of actigraphy in assessing wake-promoting drugs and sodium oxybate effects, highlighting that actigraphy could represent a very less expensive and ecological approach to assess treatment outcome and prospectively track disease course.[16,17] Fourth, the observation of a discrete circadian profile of blunted motor activity in NT1 children provided additional insight into the nature of diurnal variation and suggested that the quantitative assessment of motor activity is a promising behavioral biomarker of NT1 in young patients. Further studies are needed to test the reliability of actigraphy for wide-scale epidemiological studies as a screening tool to steer towards proper diagnosis of NT1 and hopefully reduce diagnostic delay and disease burden.

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Table 1. Demographics, scale scores, neurophysiological data and hypocretin-1 levels of children with type 1 narcolepsy and healthy controls.

Variable	Mean ± SD	Mean ± SD	p[a]	ES	95% CI	
	NT1 (*n*=22)	Controls (*n*=21)			lower	upper
Demographic and clinical data						
Male/female	(10/12)	(13/8)	0.28			
Age, years	12.09 ± 2.37	10.95 ± 2.25	0.11		-0.28	2.56
Age at NT1 onset, years	9.66 ± 2.21					
Diagnostic delay, years	3.09 ± 2.16 (range 0.2-8)					
BMI	24.53 ± 6.83	19.83 ± 2.68	<0.0005	0.9	1.48	7.93
aESS	13.86 ± 3.37	3.14 ± 3.17	<0.0001	3.27	8.7	12.74
rMEQ-CA	15.32 ± 3.66	15.14 ± 3.07	0.87		-1.91	2.26
Chronotype (*m**i**e*)	(7:13:2)	(2:18:1)	0.14			
MSLT and Hypocretin-1 data						
MSLT-sl, min	4.15 ± 3.02 (range 1-13*)					
SOREMPs, number	4.36 ± 0.85 (range 2 - 5)					
CSF hcrt-1, pg/ml	18.64 ± 28.98 (*n* = 17; range 0-109.30)					

NT1= type 1 narcolepsy; ES= Cohen's *d*; CI = confidence interval of difference between means; BMI = body mass index; aESS = adapted Epworth sleepiness scale; rMEQ-CA = reduced Morningness-Eveningness questionnaire for Children and Adolescent; chronotype: *m* = morning type, *i* = intermediate type, *e* = evening type; MSLT = multiple sleep latency test; MLST-sl = mean sleep latency at MSLT; SOREMPs = sleep-onset REM periods; CSF = cerebrospinal fluid; hctr-1 = hypocretin-1.

*2 cases with MSLT-sl > 8 min

[a] p-values derived from Chi-square test or Student's t-test as appropriate.

Table 2. Actigraphic nighttime, daytime and sleep timing measures (Means and SD) of children with type 1 narcolepsy and healthy controls.

Variable	Mean ± SD	Mean ± SD	*t*-test$_{(41)}$	p	Cohen's *d*	95 % CI	
	NT1 (*n* = 22)	Control (*n* = 21)				lower	upper
Sleep Timing							
BT	22:37 ± 00:51	22:22 ± 00:40	1.07	*ns*		-0.22	0.72
GUT	07:06 ± 00:37	07:12 ± 00:24	-0.65	*ns*		-0.42	0.22
MS	02:51 ± 00:33	02:47 ± 00:27	0.52	*ns*		-0.23	0.39
TIB (min)	510.34 ± 59.12	532.10 ± 35.98	-1.45	*ns*		-52.08	8.56
Nighttime period							
*e*SOL (min)	10.97 ± 3.70	11.31 ± 6.11	-0.22	*ns*		-3.44	2.75
*e*TST (min)	326.84 ± 77.90	479.60 ± 39.53	-8.05	<0.0001	-2.46	-191.09	-114.43
*e*SE%	63.89 ± 12.95	90.19 ± 5.20	-8.66	<0.0001	-2.64	-32.44	-20.17
*e*WASO (min)	167.42 ± 65.34	39.05 ± 27.79	8.31	<0.0001	2.54	97.17	159.56
*e*Awk (n°)	32.10 ± 7.60	15.10 ± 6.04	8.1	<0.0001	2.47	12.76	21.24
*e*Awk>5 (n°)	10.09 ± 2.63	3.07 ± 2.64	8.74	<0.0001	2.66	5.4	8.64
SMA (counts)	30.54 ± 12.37	11.64 ± 4.18	6.64	<0.0001	2.03	13.15	24.64
Daytime period							
DMA (counts)	197.66 ± 23.20	219.29 ± 17.69	-3.42	<0.001	-1.05	-34.37	-8.87
*e*DTST (min)	66.93 ± 26.47	5.33 ± 7.79	10.24	<0.0001	3.13	49.45	73.74
*e*Nap (n°)	4.81 ± 1.76	0.29 ± 0.46	11.41	<0.0001	3.48	3.72	5.32
*e*NapD (min)	38.53 ± 15.21	4.48 ± 7.36	9.27	<0.0001	2.83	26.64	41.47

BT, clock time (in hours and minutes) when subject goes to bed and turns off the light; GUT, clock time (in hours and minutes) when subject gets out of bed in the morning; MS, clock time (in hours and minutes) that split in half the TIB; TIB, time in bed (min); *e*SOL, estimated sleep onset latency (min); *e*TST, estimated total sleep time (min); *e*SE%, estimated sleep efficiency (%); *e*WASO estimated wake after sleep onset (min); *e*Awk, number of estimated awakenings; *e*Awk >5, number of estimated awakenings longer than 5 consecutive minutes; SMA, mean activity counts during TIB; DMA, mean activity counts during daytime; *e*DTST estimated total sleep time during daytime (min); *e*Nap, number of daytime estimated sleep episodes longer than 5 consecutive minutes; *e*NapD, mean duration of longest estimated sleep episode (min)

Table 3 Pearson's correlations between clinical, neurophysiological data, scale scores and actigraphic-derived measures.

	NT1 (n = 22)				Controls (n = 21)		
	BMI	aESS	rMEQ-CA	hcrt-1 (n=17)	BMI	aESS	rMEQ-CA
TIB	-0.50*	-0.20	0.43*	0.09	0.16	-0.51*	0.43
*e*SOL	0.33	0.18	-0.22	-0.01	-0.16	-0.07	0.24
*e*TST	-0.67**	-0.59**	0.56**	0.49*	0.12	-0.42	0.23
*e*SE%	-0.57**	-0.60**	0.41	0.47	0	-0.04	-0.13
*e*WASO	0.32	0.50*	-0.27	-0.42	0.06	-0.03	0.15
*e*Awk	-0.53*	-0.32	0.23	-0.10	-0.17	0.09	0.10
*e*Awk>5	0.07	0.36	-0.14	-0.40	0.22	-0.21	0.25
SMA	0.63**	0.59**	-0.51*	-0.28	0.12	0.03	0.16
DMA	-0.11	0.02	0.08	0.29	0.11	0.17	-0.07
*e*DTST	-0.01	-0.03	-0.22	-0.31	0.25	-0.29	0.29
*e*Nap	-0.04	-0.05	-0.06	-0.58*	-0.13	-0.20	-0.07
*e*NapD	-0.28	-0.14	-0.25	-0.31	0.24	-0.29	0.21
MSLT-sl	-0.13	-0.18	0.24	0.61**			
SOREMPs	0.06	-0.07	-0.10	-0.21			
BMI	–	0.58**	-0.29	-0.30	–	0.03	-0.09
aESS		–	-0.24	-0.59*		–	-0.37
rMEQ-CA			–	-0.40			–

NT1= type 1 narcolepsy; aESS = adapted Epworth sleepiness scale; rMEQ-CA = reduced Morningness-Eveningness questionnaire for Children and Adolescent; hcrt-1 = hypocretin-1; TIB = time in bed; *e*SOL = sleep onset latency; *e*TST = total sleep time; *e*SE% = sleep efficiency; *e*WASO = wake after sleep onset; *e*Awk = number of awakenings; *e*Awk>5 = number of awakenings longer than 5 minutes; SMA = mean motor activity counts during nighttime; DMA = mean motor activity counts during daytime; *e*DTST = daytime total sleep time; *e*Nap = number of diurnal sleep episodes; *e*NapD = mean duration of longest diurnal sleep episode; MSLT-sl = mean sleep latency at multiple sleep latency test; SOREMPs = sleep onset REM periods. *$p<0.05$; **$p<0.01$

Legend

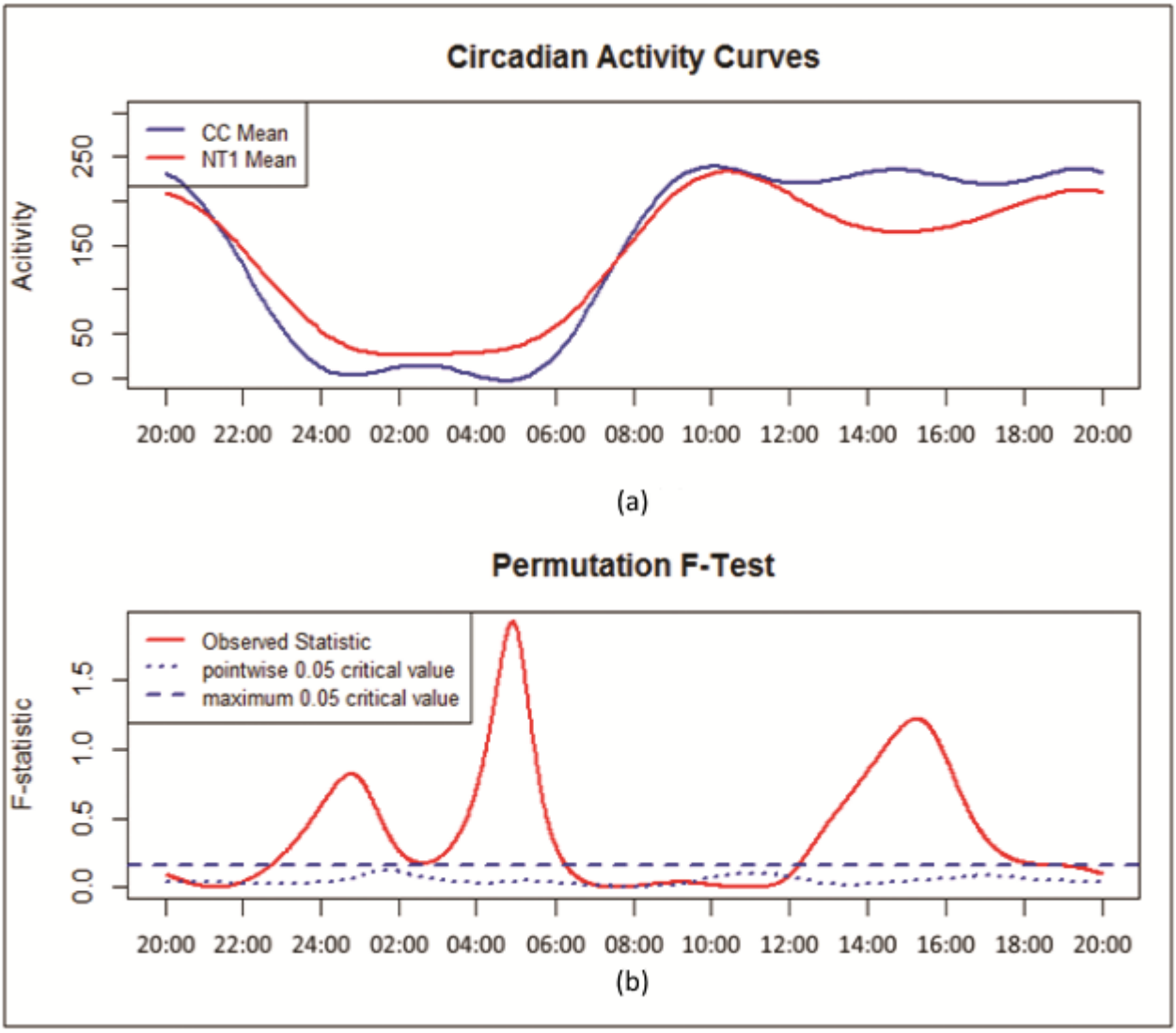

Figure 1. Functional linear modeling for NT1 and controls. Plot (a) shows estimated activity patterns for the two groups. Plot (b) shows F-test result, the red curve represents the observed statistic, the blue dashed and dotted lines correspond to a global and point-wise test of significance at $\alpha = 0.05$.

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Chapter 4

Functioning of attentional networks and ADHD symptoms in narcolepsy with or without hypocretin deficiency.

In Submission as:

Filardi M, Pizza F, Tonetti, Plazzi G, Natale V. Functioning of attentional networks and ADHD symptoms in narcolepsy with or without hypocretin deficiency.

ABSTRACT

Objectives: Attentional impairments are a common symptom in type 1 narcolepsy (NT1) that adversely interferes with several aspects of everyday life. Nevertheless, only few study attempted to characterize attentional deficits in NT1 and results are still elusive. This study examined whether NT1 patients would exhibit deficits in alerting, orienting, and executive control of attention relative to patients with type 2 narcolepsy (NT2) and healthy controls.

Methods: Twenty-one NT1 patients compared to Fifteen NT2 patients and twenty-two healthy controls matched for age and gender. All participants completed the attentional network test (ANT) and responded to questionnaires regarding frequency of ADHD symptoms, sleepiness, anxiety and depression.

Results: NT1 display a deficit in alerting network relative to NT2 and controls, while orienting and executive network resulted preserved. Moreover, alerting network efficiency significantly correlate with levels subjective sleepiness in NT1 patients. NT1 and NT2 patients reported an increased frequency of ADHD symptoms relative to controls, NT1 patients also present higher intensity of depressive symptom. The severity of ADHD symptoms significantly correlated with subjective sleepiness and depression.

Conclusions: This study show that NT1 patients have a selective deficit in alerting network functioning, and that NT1 and NT2 patients report an increased frequency of ADHD symptoms. Take account of attentional deficits and ADHD comorbidity in clinical assessment of narcolepsy may be of great beneficial for patients.

INTRODUCTION

Type 1 Narcolepsy (NT1) is a life-long neurological disorder characterized by chronic hypersomnolence with multiple sleep attacks, often characterized by direct transitions into rapid eye movement (REM) sleep (sleep-onset REM periods, SOREMPs), untimely manifestations of dissociated REM sleep, the most specific of which is cataplexy (sudden loss of muscle tone triggered by emotions),[1] and nocturnal sleep disruption.[2,3]

It is now recognized that NT1 is caused by a loss of hypothalamic neurons that produces hypocretin, a wakefulness-associated neuropeptide.[4] NT1 patients have reduced or absent levels of hypocretin-1 (hcrt-1) in their cerebrospinal fluid (CSF), accordingly the disease is further classified into NT1 (with hypocretin-1 deficiency and cataplexy) and Narcolepsy Type 2 (NT2), where cataplexy is absent and hypocretin levels are usually in the normal range.[1]

Although initially described as specifically involved in regulating arousal, wakefulness, and feeding behavior, is now clear that hypocretin influence and participate in a wide range of behavioral and physiological processes.[5,6] Hypocretinergic neurons have a key role in facilitating cognitive processes, particularly in regulating attention and arousal, via strong interaction with monoaminergic and cholinergic systems in the basal forebrain regions.[7]

These findings have led several investigators to question whether an underlying cognitive impairment might accompany the classical symptoms of NT1. A considerable number of clinical reports support this hypothesis: concentration and memory problems are reported by a significant portion of NT1 patients,[8] and represent a major concern in this clinical population.[9]

Nevertheless, has been unexpectedly difficult to document and characterize such impairments in experimental studies.[10] Memory assessments with standardized neuropsychological batteries showed intact performance of NT1 patients in short and long-term memory task.[11]

Several reports have pointed to deficit in attentional functioning. In the majority of studies NT1 patients present markedly slower and more variable reaction times (RTs) relative to controls, while performance accuracy resulted mainly preserved.[12-14]

Consistent empirical evidences showed that NT1 patients display impairments in tonic alertness and sustained attention, observable during long and monotonous tasks.[15-17]

Evidences of impairments in a specific attentional subcomponent are less consistent: in a extensive investigation on attentional domain in NT1, Rieger and co-authors reported specific deficits in divided and flexible attention, with preserved phasic alertness and focused attention.[12] Similarly, Naumann and co-authors reported intact tonic alertness and arousability, while deficits emerged in more complex tasks that requires a higher cognitive load.[13]

Despite the available evidence seems to point at a selective deficit in attentional subcomponents rather than to a global impairment, the extremely limited number of studies and the discrepancy between results does not allow to state meaningful conclusions. One of the major difficulties in comparing studies' outcomes is due the great heterogeneity of neuropsychological tests chosen for evaluate the different aspects of attentional process. A possible approach to obviate this issue is select cognitive tasks designed according to proposed neurocognitive models of attentional functioning. According to an influential neurocognitive model, attention is a complex and multidimensional system defined as the activity of a network of separate anatomical areas, each dedicated to a discrete aspect of attentional processes.[18] Three separate subsystems were postulated: the alerting, orienting, and executive control networks.[19]

Alerting network encompasses the ability to achieve and maintain a state of high sensitivity to incoming stimuli; orienting network encompasses the ability to selectively drive attention to task-informative sensory stimuli; the executive control network encompasses the ability to focus attention on task-relevant stimuli and ignore distracting information. Neuroimaging studies have demonstrated that each network is associated with the activation of specific, although partially overlapping, set of brain areas.[20,21]

Within this theoretical framework, the attention network test (ANT) was designed in order to assess the functioning of each network during a single testing session.[22]

The ANT has quickly become a popular tool in neurocognitive research, and has been extensively applied to characterize attentional impairments in several clinical populations, including patients with stroke, schizophrenia, and attention-deficit/hyperactivity disorder (ADHD).[23-,25] However, to the best of our knowledge, it has never been used in patients with central disorders of hypersomnolence.

The main aim of present study is to investigate whether specific changes in attentional networks functioning could be observed in narcoleptic patients with hypocretin deficiency (NT1) compared to narcoleptic patients without hypocretin deficiency (NT2) and healthy controls matched for age and gender. A secondary aim of the study is to evaluate frequency and severity of ADHD and obsessive-compulsive disorder (OCD) symptoms in adult NT1 and NT2 patients versus healthy controls.

Recently, two studies showed that NT1 children present an increased frequency of ADHD symptoms compared to controls;[26,27] however, to the best of our knowledge, no attempt was conducted to determine whether higher prevalence of ADHD symptoms persist in adult NT1 patients. Given that deficit in alertness and disrupted sleep are considered to be involved in the pathogenesis of ADHD, we expect to find higher prevalence of ADHD symptoms in adult NT1 cases.[28] On the other hand, evidences of an association between NT1 and OCD are limited to few case reports,[29] nonetheless a shared trigger for an autoimmune process (i.e. streptococcal infections), is supposed to be involved in the pathogenesis of both diseases.[30]

Finally, we are interested in exploring the relationship between clinical, biochemical, self-report measures and performances at attentional task.

METHOD

Subjects

Subjects were consecutive adult patients evaluated for complaints of chronic hypersomnolence at the outpatient clinic for Narcolepsy of the Department of Biomedical and Neuromotor Sciences, University of Bologna, and who received a final diagnosis of narcolepsy (either type 1 and type 2) according to current International Classification of Sleep Disorders 3rd edition (ICSD-3) criteria.[1] All patients underwent a standardized diagnostic protocol encompassing clinical evaluation with assessment of subjective sleepiness with the Epworth Sleepiness Scale (ESS),[31] at-home actigraphic monitoring and cerebral magnetic resonance imaging (to rule out secondary cases), and hospitalization with 48-hr continuous polysomnographic recording, Multiple Sleep Latency Test (MSLT), cataplexy video-documentation, human leukocyte antigen (HLA) typing and, whenever possible, lumbar puncture for CSF hypocretin-1 (Hcrt-1) assay.

The final study sample included 36 patients, consisting of twenty-one NT1 patients (9 males, mean age 36.19 ± 11.94 years) and fifteen NT2 patients (6 males, mean age 35.47 ± 13.05 years).

All NT1 patients fulfilled current ICSD-3 clinical criteria for type 1 narcolepsy presenting severe daytime sleepiness (mean ESS = 15.90 ± 5.30) and clear-cut cataplexy (n = 21/21).[1]

Nineteen out of 21 cases had mean sleep latency < 8 min (mean 4.41 ± 4.80) with at least two SOREMPs (mean 3.71 ± 1.49) at the MSLT. Nineteen patients (including the 2 cases with MSLT sleep latency > 8 min.) underwent lumbar puncture and all had low (≤ 110 pg/ml) or undetectable CSF Hcrt-1 levels; all patients were drug-naïve at time of evaluation.

NT2 diagnosis was made according to current ICSD-3 criteria with all patients presenting persistent daytime sleepiness (mean ESS= 15.53 ± 5.45), absence of cataplexy, and mean sleep latency < 8 min (mean 7.31 ± 2.98) with at least two SOREMP (mean 1.4 ± 1.72) at MSLT or nocturnal polysomnography.[1] Hcrt-1 concentration was higher than >110 pg/ml in all patients tested (n = 11/14),[1] thirteen patients were drug-naïve, two patients had discontinued treatment with psychostimulants three week prior to hospitalization.

Twenty-two age- and sex- matched controls (11 males, mean age 34.95 ± 11.52 years) were recruited from the local community. Participants were screened to rule out sleep or medical disorders; more in detail they filled in the ESS, the Beck Depression Inventory (BDI),[32] the State-Trait Anxiety Inventory (STAI-Y),[33] and underwent to seven day of actigraphic monitoring. Subjects with regular sleep schedule and without complaints of daytime sleepiness (mean ESS = 7.05 ± 3.34), depression (BDI < 21), or anxiety (STAI < 40) were included. The study was approved by the internal review board and all participants provided written informed consent.

Attention Network Test

The original version of the ANT, was used in this study.[22] The ANT is a combination of the cued reaction time[34] and the flanker task,[35] by manipulating stimulus property the task assess the efficiency of each network measuring subjects' RTs. The task was presented via E-Prime 2.0 software (Psychology Software Tools, Inc., Sharpsburg, PA), stimuli were displayed on a 15-in monitor and responses collected via "Q" and " P" keys on a standard keyboard. Participants were seated approximately at 60 cm from the monitor, in a sound-attenuated room with controlled luminance levels, and were instructed to focus on a fixation cross that appears in the center of the monitor and remains on screen until the end of the test.

The sequence of events of the task is reported in Figure 1. In each trial, after a variable fixation period (range 400–1600 ms), a warning cue lasting 100 ms is presented (four cue conditions: no-cue, double-cue, spatial-cue, or center-cue), subsequent to a constant fixation period (400 ms) the target and flankers were presented until the participant responds or the maximum duration interval (1700 ms) is expired.

The target, a central arrow in a row of five arrows, can appear 1.06° above or below the fixation point and is flanked by arrows (2 on each side) pointing in the same direction (congruent condition), or in the opposite direction (incongruent condition), or is not flanked by additional stimuli (neutral

condition). Participant have to indicate, as quickly and accurately possible, in which direction the central arrow is pointing.

An index for the alerting network is derived subtracting mean RTs of trials with double-cue from mean RTs of trials without warning cue ($RT_{no\ cue} - RT_{double\ cue}$); both no-cue and double-cue conditions do not provide information on the spatial location of incoming target, but the double-cue alerts participant of the imminent appearance of the target.

An index for the orienting network is computed subtracting mean RTs of trials with spatial-cue from mean RTs of trials with center-cue ($RT_{center\ cue} - RT_{spatial\ cue}$); both cues warn subject of the imminent appearance of the target, but only spatial-cue provides meaningful information able to enhance the orientation of attention toward the appropriate location.

Finally, an index for the executive control network is calculated by subtracting mean RTs of trials with congruent flanker from mean RTs of trials with incongruent flanker ($RT_{incongruent} - RT_{congruent}$); the two conditions differ in the type of information provided by flankers that facilitate the discrimination of stimuli when congruent, while distract subject when incongruent.

Self-Report Measures

Upon completing the ANT, participants completed questionnaire assessing presence and severity of symptoms of ADHD and OCD.

The Adult ADHD Self-Report Scale Symptom Checklist (ASRS) is a questionnaire designed to assess the severity of ADHD symptoms in adulthood, according to DSM-IV criteria.[36]

The ASRS encloses two subscales, for a total of 18 items: one subscale assess inattentive symptoms (i.e. difficulties to pay attention, excessive distractibility, difficulties organizing tasks) the second assess hyperactive/impulsive symptoms (i.e. fidget or restless behavior, excessive activity, difficulty in remaining seated).

Participants were asked to rate each item on a 5-point Likert scale (ranging from 0 = never to 4 = very often): the sum of nine item assessing inattentive symptoms determine the ASRS inattentive score ($ASRS_{In}$), the sum of nine item assessing hyperactive/impulsive symptoms determine the ASRS hyperactive score ($ASRS_{Hy}$), a score computed on six items (ASRS Screener, $ASRS_{Scr}$) provides clinical cut-off value.[37]

The reduced version of the Obsessive Compulsive Inventory (OCIr) is a 18-item questionnaire assessing the degree of distress associated with obsessions and compulsions.

Participants were asked to express, on a 5 point Likert scale (ranging from 0 = not at all to 4 = extremely) the distress associated with the proposed statements.[38]

Beck Depression Inventory (BDI) and State-Trait Anxiety Inventory (STAI) were used to assess participants' levels of depression and anxiety, respectively.[32,33] Circadian typology was evaluated with the reduced version of morningness/eveningness questionnaire (rMEQ).[39]

Procedure

NT1 and NT2 patients were evaluated during the hospitalization finalized to the diagnostic protocol. The experimental evaluation took place over two days: in the first day participants were instructed on study protocol and provided written informed consent, then practiced with a full version of the ANT, in order to minimize possible learning effect;[40] in the second day, after an overnight polysomnography, the ANT was administered at a fixed hour (after the mid-afternoon peak of sleepiness,[41] around 17:30), given that performances can vary as a function of time-of-day.[42]

The experimenter was present at the beginning of the testing session to start the task and answer participants' questions, the instructions (verbal and written) describes the procedures and emphasize the importance of a quick and accurate response.

Participants completed 1 practice block of 26 trials with feedback, followed by three experimental block without feedback consisting of 96 trials each (4 cue conditions × 2 target locations × 2 target directions × 3 flanker conditions × 2 repetitions); trials were presented in random order.

Healthy controls underwent the same experimental protocol; however, in order to ensure adequate sleep duration (at least 6 hr) and regular sleep schedule prior to testing session, they were monitored throughout 7 days by means of actigraphy.

Controls were evaluated at the Laboratory of Applied Chronopsychology, Department of Psychology, University of Bologna, at the same hour of day (i.e. 17:30) of the clinical groups.

Statistical Analyses

Data were explored with descriptive statistics (mean ± SD). Group differences in demographical data, BMI and questionnaire scores were analyzed with chi-square test, Mann-Whitney U test, and one-way analysis of variance (ANOVA).

For the attentional task we computed the mean overall RTs, accuracy rate, and the efficiency of the three attentional networks; moreover we also computed mean RTs for each participant in the 12 different test conditions (4 cue types X 3 flanker types). Trials in which participants made errors or trials with RTs ± 3 SD from the mean were excluded from analysis. RTs are generally slower in patients, consequently we calculated a proportional transformation (Pro) on network score to examine specific effects in a manner that is independent from global slowing; proportional scores were computed dividing networks score by a measure of information processing speed (RT_{All} = mean RTs for all 12 warning Cue X flanker conditions).[43]

For the ANT networks score, we first conducted a correlation analyses to assess the independence of the three attentional component. Subsequently, we carried out a MANOVA considering the following variables: overall RTs, accuracy, Alerting, Orienting and Executive network score and the proportional scores of these latter.

In the event of a significant omnibus effect at MANOVA, univariate effects for dependent variables were explored, where these were significant Bonferroni post-hoc were used to determine the nature of the between groups effects, partial-Eta² (np2) was computed as a measure of effect size. Furthermore for each attentional network, we carried a repeated measures ANOVA with group as between subject factor and the type of cue or flanker that defined the specific network as within-subject factor (2 levels) (i.e., no-cue and double-cue for Alerting, central-cue and spatial-cue for Orienting, incongruent and congruent flanker for Executive).

Finally, the relationship between clinical, self-reported measures and performance at cognitive task was explored, separately for each group, with correlation coefficient analysis. Statistical analyses were conducted using SPSS 19.0 (SPSS, Inc. Chicago, Ill). Results with p values <0.05 were considered statistically significant.

RESULTS

Demographic, clinical, neurophysiological characteristics and questionnaires scores are reported in Table 1, together with ANOVA and post-hoc results. Chi-square and one-way ANOVA analyses showed no group differences in either gender and age, as well as in circadian typology distribution. NT1 patients display higher BMI than NT2 and controls, without any significant difference between these latter. One-way ANOVA showed a main group effect for $ASRS_{In}$ and ESS, with both NT1 and NT2 patients reporting higher frequency of ADHD inattentive symptoms and higher level of subjective sleepiness than controls. For the NT1 group, difference also emerged in the $ASRS_{Hy}$ and BDI score, with patients reporting higher frequency of ADHD hyperactive symptoms and higher intensity of depressive symptoms than controls, without any other between group difference. No differences were observed in state and trait anxiety level as well as in OCIr score.

The first set of correlations, aimed to explore the independence of attentional networks score, highlighted very feeble associations between alerting and orienting, alerting and executive, and

orienting and executive score (r = 0.14, r = 0.01, and r = -0.12 respectively, all $p \geq 0.38$). Mean and standard deviation of ANT network scores, proportional score, overall RTs, and accuracy data for the three groups are shown in Table 2, together with significance values at ANOVA, partial-Eta², and Post-Hoc results.

NT1 and NT2 patients display slower overall RTs than the controls, without differ in performance accuracy. Analyses shows that NT1 patients present higher alerting score than NT2 and controls, this trend in difference reach statistical significance even when network score is computed in a manner that is independent from a global slowing of processing speed. Contrarily, no differences emerged between patients and controls on orienting and executive networks score, nor in the proportional scores of these latter. Box plot for attentional network scores of the three groups are reported in Figure 2.

Results of the three repeated measures ANOVA performed on mean RTs for the clue and flanker conditions that define the attentional processes assessed by the ANT are reported in Figure 3.

The alerting effect was explored with a 3 (Group: NT1, NT2, controls) X 2 (Cue condition: no-cue, double-cue) repeated-measure ANOVA; analysis showed a main effect of Group ($F_{2,55} = 8.935$; $p = 0.0004$; $n_p^2 = 0.245$) with both NT1 and NT2 patients responded more slowly than controls, and a main effect of Cue ($F_{1,55} = 143.626$; $p < 0.0001$; $n_p^2 = 0.723$) with faster RTs in the double-cue than in the no-cue condition.

Most important, a two-way interaction "Cue by Group" resulted statistically significant ($F_{2,55} = 7.394$; $p < 0.001$; $n_p^2 = 0.212$); this interaction was due to larger differences between RTs in no-cue (666.86 ± 21.93 ms) and double-cue (601.57 ± 21.05 ms) condition of NT1 patients relative to NT2 (no-cue 636.73 ± 25.95 ms, double clue 602.73 ± 24.91 ms) and controls (no-cue 535.58 ± 21.43 ms, double clue 498.96 ± 20.57 ms).

The orienting effect was explored with a 3 (Group: NT1, NT2, controls) X 2 (Cue condition: center-cue, spatial-cue) repeated-measure ANOVA, results show a main effect of Group ($F_{2,55} = 9.727$; $p = 0.0002$; $n_p^2 = 0.261$) with both clinical groups were more slower than controls, and a main effect of

Cue ($F_{1,55}$ = 114.074; p < 0.0001; n_p^2 = 0.675) with faster RTs in the spatial-cue compared to center-cue condition. The interaction between factors did not reach statistical significance ($F_{2,55}$ = 0.971; p = 0.385; n_p^2 = 0.034).

The flanker effect was explored with carrying a 3 (Group: NT1, NT2, controls) X 2 (Flanker type: congruent, incongruent) repeated-measure ANOVA; results highlighted a main effect of Group ($F_{2,55}$ = 9.232; p = 0.0003; n_p^2 = 0.251), and a main affect of Flanker ($F_{1,55}$ = 127.648; p < 0.0001; n_p^2 = 0.699) with faster RTs in the congruent-flanker condition compared to incongruent-flanker condition. The interaction between factors ($F_{2,55}$ = 0.767; p = 0.469; n_p^2 = 0.027) did not reach statistical significance.

Pearson's correlation analyses are reported, separately for each group, in Table 3. In the NT1 group BDI was positively correlated with ESS, $ASRS_{In}$ and $ASRS_{Hy}$, the ESS was directly related to ASRSIn and display a negative relationship with alerting network efficiency (Figure 4). In the NT2 group, only a positive correlation between $ASRS_{In}$ and $ASRS_{Hy}$ reached statistical significance, no significant correlation were observed for the control group.

DISCUSSION

The present study is the first aimed at investigating the functioning of the three attentional networks postulates by Posner and co-authors, in a sizable cohort of drug-naive patients with NT1 and NT2 versus healthy controls.[19] In accordance with previous studies , NT1 and NT2 patients resulted markedly more slow than controls, without differ in performance accuracy.[12-14] The most striking finding is a specific alteration in the alerting network of NT1, while no difference emerged respect to control regarding the orienting and executive control network. The aberrant functioning of alerting network showed in NT1 patients, result statistically significant even when network score is computed with a proportional score transformation, that is a robust way to unveil group difference in attentional processes independently from global slowing. The larger alerting effect

displayed by NT1 patients, relative to NT2 patients and controls, may indicate either an inability to benefit from warning-cue to speed-up RTs, or a deficit in maintaining adequate alertness in absence of warning-cues.

Bearing in mind that ANT network scores are computed with a subtractive logic, larger alerting scores do not necessarily indicate less efficient performance, but may reflect that one group need to compensate the difficulties aroused by the task increasing the effort.[44] In this scenario, for a proper interpretation of ANT outcome, is crucial to consider the distinction between tonic and phasic alertness processes: in trials without warning-cue participants must rely on their own internal arousal system, hence RTs in no-cue trials reflect the more tonic aspects of alertness; conversely when a warning-cue precede the event of interest participants may use the information provided to enhance RTs, hence RTs in the double-cue trials reflect the phasic component of alertness (i.e. arousability).

Considering that in trials with double-cue NT1 and NT2 display similar mean RTs (601.57 ± 21.05 ms *vs* 602.73 ± 24.91 ms) compared to RTs in trials without warning cue (666.86 ± 21.93 ms *vs* 636.73 ± 25.95 ms), we can conclude that in NT1 patients the tonic component of alertness is markedly impaired while the ability to use warning-cue to improve RTs is essentially preserved. This profile of impaired tonic alertness with preserved arousability presented by NT1 patients is consistent with previous studies, that however assessed attentional functioning by means of separate and independent cognitive tasks, and highlights the sensitivity of the ANT in rendering this peculiar attentional functioning within a single, short, testing session.[12,13]

In contrast with previous report,[14, 17,45] no evidence of impaired executive control of attention was observed in our sample of narcoleptic; we interpreted this ambiguous result as a clue that the ANT is not sensitive enough for detect an impairment in executive functions for this clinical population. The peculiar impairment of alerting processes of NT1 patients, might offer new insight on the nature of cognitive deficit affecting this clinical population, and on the impact of the latter on complains of memory deterioration, often reported by NT1 patients.

Attention and memory are closely related, in order to memorize something one must attend it: in NT1 the unstable tonic component of alerting process made necessary monitoring and compensation strategies, this can lead to a reduction of the available resources and affect mnemonic processes.

Analyzing self-report measures we showed that in patients with NT1 and NT2, ADHD symptoms were significantly higher relative to controls.

Both NT1 and NT2 patients show an increased frequency of ADHD inattentive symptoms, mirrored by higher mean $ASRS_{In}$ score; NT1 patients presented also an increased frequency of ADHD hyperactive/impulsive symptoms, as well as higher depressive complaints compared to controls.

In contrast with previous reports, narcoleptic patients did not show increased anxiety levels.

Classifying $ASRS_{Scr}$ score into four-stratum (0–24 scoring approach) as proposed by Kessler an co-authors,[37] including low negative (range 0-9), high negative (range 10-13), low positive (range 14-17) and high positive (range 18-24), 90.9 % of healthy controls reported scores in the lower stratum (0-9) and two subjects (9.1%) reported score in the 10-13 interval; on the contrary only the 44% of narcoleptic patients (47.6 % of NT1 and 40 % of NT2, respectively) display score in the first stratum.

To the best of our knowledge, this is the first study that explored the frequency of ADHD symptoms in adult narcoleptic patients; nevertheless, our results are in line with previous investigations that reported an increased ADHD symptomatology in NT1 children,[26,27]and highlight that a higher ADHD symptomatology may persist in a significant portion of adult NT1 cases.

Finally, correlation analysis disclosed that within NT1 patients, higher rating of ADHD symptoms ($ASRS_{In}$ and $ASRS_{Hy}$) is significantly associated with BDI and ESS score, while no significant correlation emerged in NT2 patients and healthy controls. Intriguingly, in NT1 a significant association emerged between ESS and Alerting network score, while no association was observed between subjective measures and performance at cognitive task in NT2 and controls.

A recent study showed that, apart from the classical symptoms, the majority of NT1 patients also suffer from severe fatigue, defined as a subjective experience of mental and/or physical exhaustion that does not disappear after a period of sleep.[46] Although excessive daytime sleepiness and fatigue are distinct symptoms they clearly show overlapping feature, as reflected by the moderate ($r = 0.27$) to high ($r = 0.71$) correlation between measures reported in different studies.[46,47],

Accordingly, we can speculate that the severe fatigue reported by NT1 patients, may be related to the compensatory strategies made necessary by the impaired Alerting network.

Two possible limitations of the present study need to be acknowledged. First, the sample size, especially for the NT2 group, is relatively low this might reduce the generalizability of results. Second, the flanker task consider as a proxy of executive functions the ability to resolve conflicts in the processing of competing stimuli. Although the latter is a key aspect of executive functions, this cognitive domain encompass a wide range of distinct processes, including inhibition, set-shifting, multitasking, planning and working memory; it is therefore possible that there are more group differences in the executive functions that the ANT can assess.

In conclusion our study showed that despite an overall slowing observable in both NT1 and NT1, a specific deficit in alerting network is present in NT1, nonetheless this impairment does not affect the whole attentional domain, since no evidences of impairment in the orienting and executive networks were observed.

Future investigations are required to explore the association between activation of brain areas and performances at the ANT in NT1.

Acknowledgments

We are indebted to all the patients and families participating to this study, most notably the Italian Association of Narcolepsy (AIN onlus) patients. Without their contributions, this study would not have been possible.

Table 1 Demographic, clinical, neurophysiological characteristics and questionnaire score of patients with Narcolepsy Type 1 (NT1), Narcolepsy Type 2 (NT2) and healthy controls.

	NT1 (*n*=21)		NT2 (*n*=15)		CC (*n*=22)		$F_{(2,55)}$	*p*	NT1vs CC post-hoc	NT1vs NT2 post-hoc	NT2 vs CC post-hoc
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range					
Gender (M:F)	9:12		6:9		11:11		0.41 [a]	*ns*			
Age (years)	36.19 ± 11.94	18-66	35.47±13.05	18-67	34.95±11.52	21-58	0.06 [b]	*ns*			
MSLT-sl	4.41 ± 4.80	0.36-20	7.31 ± 2.98	3.20-14.36			57 [c]	0.001			
SOREMPs	3.71 ± 1.49	0-5	1.4 ± 1.72	0-5			55 [c]	0.001			
CSF Hctr-1	24.48 ± 35.79	0-109.10	334.80 ± 99.41	132-513			6.93 [d]	0.0001			
HLA DQB1*0602	18/19		6/12				8.42 [a]	0.005			
Cronotype *m/i/e*	3/13/5		4/10/1		1/18/3		5.56 [a]	*ns*			
BMI	30.41 ± 6.28	17.58-40.40	25.38±2.86	21.77-32.87	21.88±3.13	17.40-26.38	19.48 [b]	0.0001	0.0001	0.005	*ns*
ESS	15.90±5.30	6-24	15.53±5.45	4-24	7.05±3.34	0-13	22.76 [b]	0.0001	0.0001	*ns*	0.0001
BDI	16.86±13.13	0-55	16.27±11.98	3-37	8.14±5.60	1-21	4.32 [b]	0.05	0.05	*ns*	*ns*
STAI-S	46.33±14.01	25-74	44.20±11.92	25-64	38.91±9.55	24-57	1.84 [b]	*ns*			
STAI-T	47.81±14.37	24-80	47.47±14.23	25-68	40.27±9.63	20-57	2.13 [b]	*ns*			
OCIr	18.66±13.25	0-48	15.33±10.33	1-42	11.32±8.36	1-35	1.85 [b]	*ns*			
$ASRS_{In}$	17.61±6.68	5-36	18.47±9.74	0-36	10.64±4.07	1-19	8.06 [b]	0.001	0.005	*ns*	0.005
$ASRS_{Hy}$	14.80±5.51	3-28	13.80±5.21	7-27	10.77±5.02	2-22	3.32 [b]	0.05	0.05	*ns*	*ns*

MSLT-sl = mean sleep latency at multiple sleep latency test; SOREMPs = sleep-onset REM periods; CSF Hctr-1 = cerebrospinal fluid hypocretin-1; Chronotype: *m* = morning type, *i* = intermediate type, *e* = evening type; BMI = body mass index; ESS = Epworth Sleepiness Scale; BDI = Beck Depression Inventory; STAI = State Trait Anxiety Inventory (S = State, T = Trait); OCIr = reduced obsessive-compulsive inventory; ASRS = adult ADHD self-report scale symptom checklist (In = Inattentive, Hy = Hyperactive).

[a] Chi-square test

[b] One-way ANOVA

[c] Mann-Whitney U test

[d] Independent sample T-test

Table 2 Mean, standard deviation (SD), univariate and post-hoc results of attentional network test measures and proportional score (Pro) of Narcolepsy Type 1 (NT1), Narcolepsy Type 2 (NT2) patients and healthy controls.

	NT1 (n = 21)		NT2 (n =15)		CC (n = 22)		Unviariate Results			NT1 vs CC Bonferroni Post-Hoc	NT1 vs NT2 Bonferroni Post-Hoc	NT2 vs CC Bonferroni Post-Hoc
	Mean	SD	Mean	SD	Mean	SD	$F_{(2,55)}$	p	n_p^2			
Alerting	65.30	6.19	34.00	7.33	36.62	6.05	7.40	0.001	.212	0.005	0.006	
Alerting(Pro)	0.11	0.01	0.06	0.01	0.07	0.01	5.35	0.008	.163	0.037	0.013	
Orienting	39.93	5.88	31.57	6.96	44.17	5.74	0.98	*ns*				
Orienting(Pro)	0.06	0.01	0.05	0.01	0.09	0.01	2.76	*ns*				
Executive	42.28	7.18	52.19	8.49	54.07	7.01	0.77	*ns*				
Executive(Pro)	0.07	0.01	0.08	0.01	0.11	0.01	2.31	*ns*				
Overall RTs	621.08	21.15	605.09	25.03	501.60	20.67	2.68	0.0001	.254	0.001		0.007
Accuracy(%)	97.56	0.66	96.52	0.78	98.82	0.64	9.37	*ns*				

Table 3 Pearson's correlations between attentional network score and self-report measures of depression, sleepiness, inattentive and hyperactive complains.

	NT1 (n = 21)				NT2 (n = 15)				CC (n = 22)			
	ESS	BDI	$ASRS_{In}$	$ASRS_{Hy}$	ESS	BDI	$ASRS_{In}$	$ASRS_{Hy}$	ESS	BDI	$ASRS_{In}$	$ASRS_{Hy}$
Alerting	-0.56*	-0.32	-0.16	0.12	0.32	-0.41	-0.28	-0.05	0.01	0.06	0.15	0.11
Orienting	0.14	0.06	0.04	-0.24	0.03	-0.10	-0.29	-0.23	0.05	-0.26	-0.39	-0.18
Executive	-0.03	0.40	-0.04	0.33	0.42	-0.15	-0.26	-0.24	0.05	-0.20	-0.10	0.10
ESS	-	0.55**	0.47*	0.17	-	-0.42	-0.33	-0.09	-	-0.01	-0.07	-0.35
BDI		-	0.67**	0.53*		-	0.30	0.38		-	0.39	0.27
$ASRS_{In}$			-	0.46*			-	0.77**			-	0.26
$ASRS_{Hy}$				-				-				-

ESS = Epworth Sleepiness Scale; BDI = Beck Depression Inventory; ASRS = adult ADHD self-report scale symptom checklist (In = Inattentive, Hy = Hyperactive).
* p <0.05
** p <0.01

Figure legend

Figure 1. Representation of the sequence of events in each trial of the attention network test (ANT)

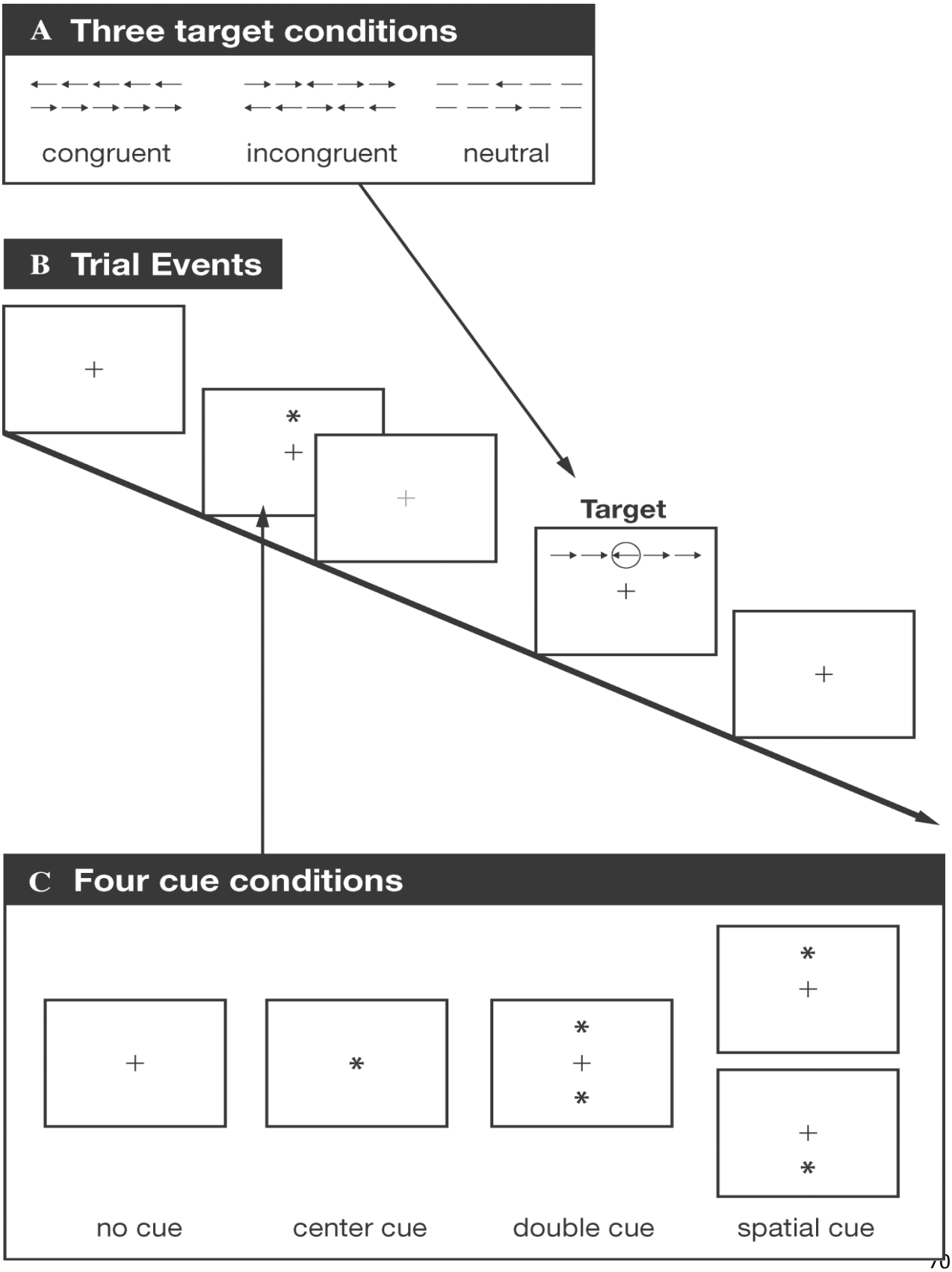
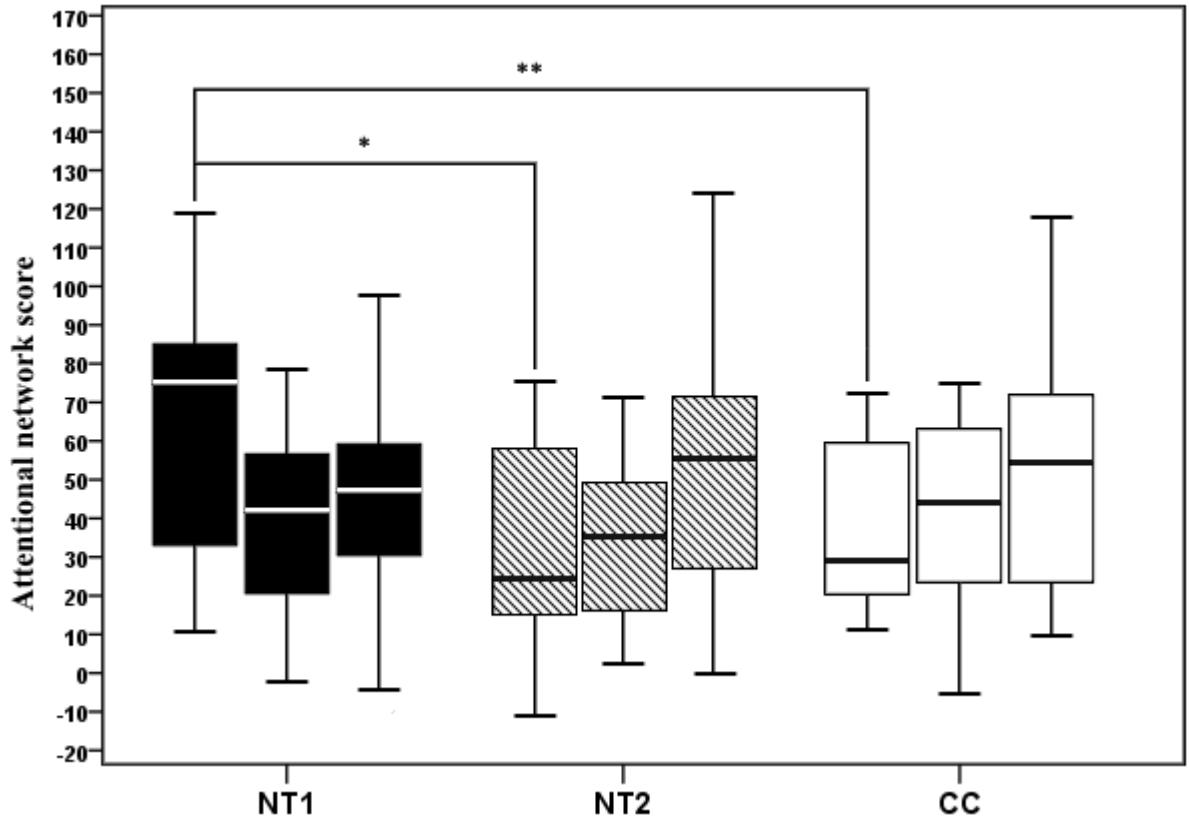
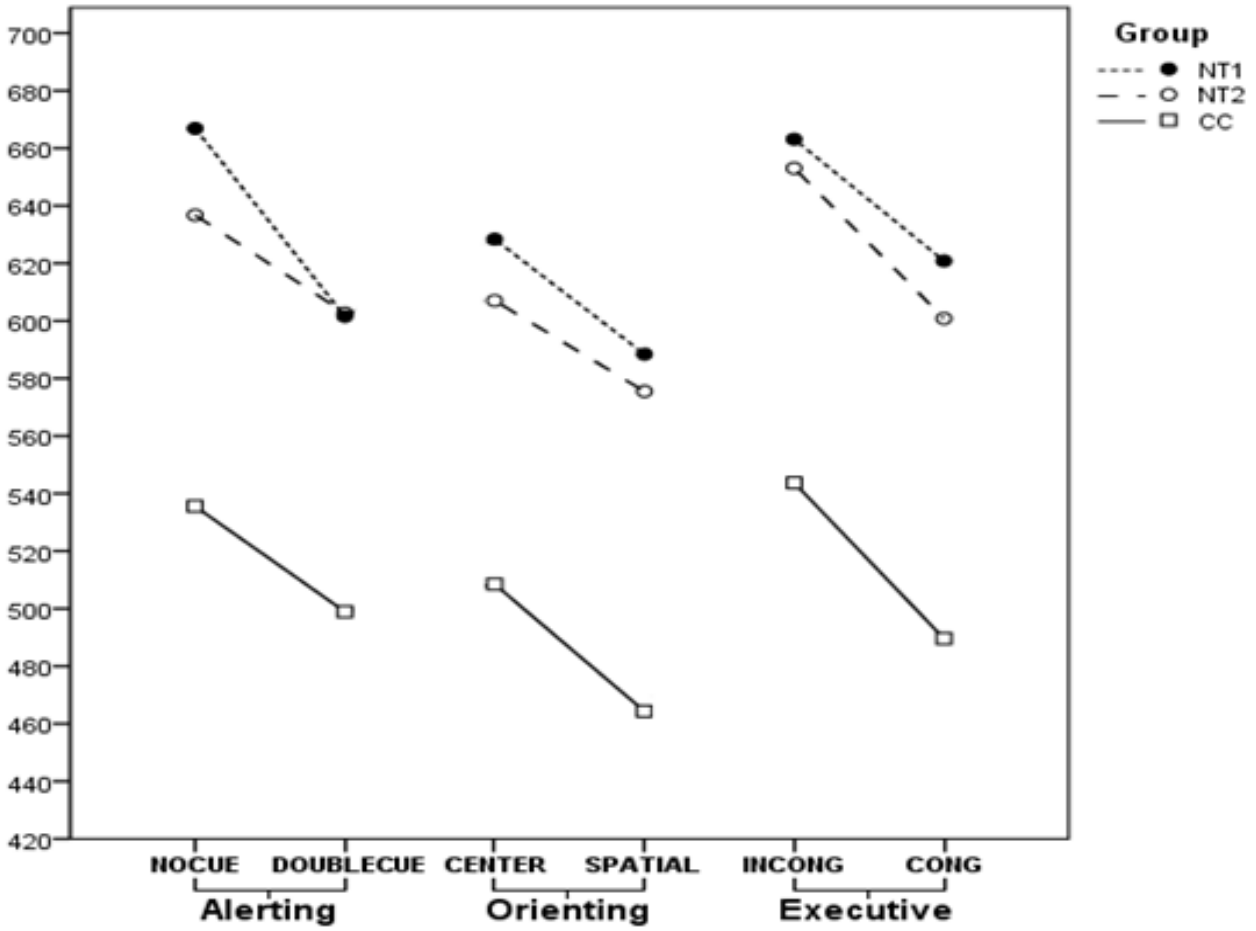


Figure 2. Box Plot of the three attentional network score in narcolepsy type 1 (NT1), Narcolepsy type 2 (NT2) and controls.



***** $p = 0.005$
****** $p < 0.01$

Figure 3. Mean RTs (ms) of the cue and flanker conditions relevant for each attentional networks of narcolepsy type 1 (NT1), Narcolepsy type 2 (NT2) and controls.



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Chapter 5

General Discussion

In this dissertation, the three experimental chapters were designed to identify behavioral biomarker of Type 1 Narcolepsy as well as attentional deficits in patients with or without hypocretin-deficiency. In **Chapter 2** we described a new features of the nycthemeral disruption presented by adult type 1 narcolepsy patients.

Our approach focused on prolonged monitoring of motor activity by means of actigraphy, a watch-like device, that allows to record raw activity data for extended time.

Capitalizing on the main advantage of actigraphy i.e. the concurrent documentation of both sleep and wakefulness, we computed a discriminant score that accurately identify NT1 patients, not only relative to controls, but also compared to another central disorder of hypersomnolence (Idiopathic Hypersomia, IH) with an high overlapping of clinical features.

In **Chapter 3** we finely investigated the features of circadian rest-activity rhythm of NT1 children, by applying spectral analysis and permutation test on motor activity data.

We described a new proper pattern of motor activity in NT1 children, with blunted motor activity in the first afternoon and enhanced motor activity throughout the night. We confirmed that actigraphic assessment is highly specific for NT1 also in children close to disease onset. Moreover, we show that actigraphic profile is a promising information able to objectively stratify disease burden.

In **Chapter 4** we described new findings on the attentional deficits of narcoleptic patients (NT1 and NT2) and their relationship with sleepiness, anxiety and depression. Moreover, we firstly reported and increased frequency of ADHD symptoms in adult patients with that NT1 and NT2.

Our results highlighted that both cognitive deficits and psychiatric comorbidity have to be carefully assessed in clinical evaluation of narcolepsy spectrum disorder.

The aims of the current dissertation were as follows and are discussed separately below:

- Identify behavioral biomarker for Type 1 Narcolepsy
- Characterize attentional performances of patients with Type 1 and Type 2 Narcolepsy

Behavioral Biomarker for Type 1 Narcolepsy

Narcolepsy is one of the most common causes of chronic sleepiness, affecting about 1 in 2000 people. Despite the frequency of narcolepsy, the average time from the onset of symptoms to diagnosis is 5 to 15 years, and narcolepsy often remains undiagnosed in as many as half of all affected people. Narcolepsy is often challenging to diagnose and remains and many clinicians are unfamiliar with this disorder. Fortunately, awareness of narcolepsy and other sleep disorders is increasing, and over the past several years researchers have made great progress in understanding narcolepsy. However, to date no screening approach has been identified for this disorder and this is one of the major factor associated with diagnostic delay.

NT1, have a prominent impact on quality of life since its early stages, a delayed diagnosis and consequently a delay in beginning appropriate pharmacological and behavioral treatments markedly heighten disease burden.

In **Chapter 2** we have demonstrated that actigraphy display unique potentialities in the assessment of patients suffering from central disorders of hypersomnolence and in particular NT1

NT1 is indeed a disease affecting the whole 24-hr system, nocturnal sleep cannot be consolidated for long periods and sleep episodes recur several times during the day.

Sleep laboratory assessment usually focus on assessment of nocturnal sleep (by mean of PSG) or diurnal sleepiness (by means of MSLT), however both test can be performed only during hospitalization, moreover they provide a highly-accurate description of sleep but in a very limited time span (maximum 24-hr)

Among all devices used to assess sleep, actigraphy is the only that can concurrently document both sleep wakefulness for extended time period.

Indeed focusing assessment of both diurnal and nocturnal period actigraphy well-render this peculiar nycthemeral rhythm disruption. The actigraphic profile of NT1 patients is very specific and

characterized by a major nocturnal sleep disruption with prolonged awakenings and enhanced motor activity and recurrent daytime naps. Clear-cut differences in actigraphic parameter computed have been found (with CI not overlapping with 0), accordingly we tested the possibility to classify subjects by means of these parameters (one or more, in association).

We showed that the combined use of both nocturnal (estimated SMA, estimated Awk) and diurnal (estimated NapD) parameters performed better in NT1 cases (87% correctly classified) than any single actigraphic measure.

Indeed, these parameters reflect two intrinsic features of NT1, namely disrupted nocturnal sleep (estimated Awk, estimated SMA) and hypersomnolence (estimated NapD). We acknowledge that a classification rule that relies simultaneously on daytime and nighttime measures works well in patients with a severe disruptions of sleep and wakefulness (NT1) otherwise when only diurnal period is disrupted (IH indeed display preserved nocturnal sleep) the discriminant validity of actigraphy did not reach acceptable level and probably should be integrated with other sources of data from the diurnal period.

Upon showing the actigraphic ability of render this peculiar 24-hr disruption (**Chapter 2**), we question whether this peculiar disruption is already noticeable by means of actigraphy in early stages of the disease. We therefore applied actigraphic evaluation to NT1 children as close as possible to disease onset (**Chapter 3**).

We focused more deeply in analyses of circadian pattern of motor activity, applying techniques proper of EEG analyses and physic to raw actigraphic signal. We enrolled NT1 children and healthy controls during the school period, and assess their sleep-wake behavior across seven days. NT1 children systematically display at least one diurnal nap immediately after the end of school time, and present sustained motor activity during sleep that is severely fragmented by numerous and prolonged awakenings. Moreover, we firstly showed evaluation that increased BMI, high subjective sleepiness, and low CSF-hcrt-1 levels were associated with the severity of nycthemeral disruption in NT1 children. Actigraphy allowed us to document long-lasting diurnal sleep episodes that are often

reported in childhood NT1.The standard diagnostic approach, based on the nocturnal polysomnography followed by the MSLT, allows a proper neurophysiological diagnosis with high sensitivity and specificity, but does not give insight into this very common aspect of NT1 hypersomnolence.

This findings have several clinical implication, the ecological description of nycthemeral disruption could be used to plan supportive strategies and tailor personalized treatments. Behavioral treatment (i.e. regularly scheduled naps) is a major management strategy for NT1, actigraphy can be used to objectively assess, and possibly adjust, the napping schedule.

Finally with correlation analyses, we pointed to the possibility of objectively stratify disease severity in the apparently uniform clinical picture of NT1 taking into account clinical, anthropometric, neurophysiological and motor activity features.

Attention deficits and ADHD symptoms in Narcolepsy-spectrum disorder

Attention performances of Narcoleptic patients (either NT1 and NT2) are presence of ADHD and OCD comorbidity are described in **Chapter 4**.

Narcolepsy is a severely disabling disease, which limits patients in many aspects of everyday life As a consequence, health-related quality of life is reported to be poor. In particular, EDS is found to impact on well-being and to impair physical, emotional and social functioning. Depressive symptoms and anxiety disorders have also been described in association with narcolepsy

People with narcolepsy often complain about difficulties with memory, learning and concentration However, research results are heterogeneous as to whether individuals with narcolepsy have objective memory impairments: some studies have pointed to an intact performance of patients with narcolepsy in standard memory tests, others have found only modest memory impairments.

There is more-consistent evidence that patients with narcolepsy have attention and executive function deficits, which could explain their perceptions of memory deficits and, to some extent, their difficulties in some daily life activities (e.g. driving).

We showed that compared to good sleepers and patients with Narcolepsy without hypocretin-deficiency (NT2), hypocretin-deficient Narcoleptic patients (NT1) present a specific profile of attentional impairments.

Our findings for the ANT showed that the overall RTs was longer in Narcoleptic patients (either type 1 and type 2) compared to healthy controls. Results from **Chapter 4** also revealed difference in alerting network functioning of NT1 patients relative to controls, while no differences were observed in the functioning of orienting and executive control network. NT1 patients shows an aberrant functioning of alerting networks, with a greater differences of RTs between trials with and without warning-cue.

The peculiar attentional deficit of NT1 demonstrated herewith, provide new physiological insight on the nature of cognitive in narcolepsy. Our data showed that in NT1 the tonic component of alerting process is highly instable; this made necessary monitoring and compensation strategies and lead to a reduction of free processing resources and affecting thereby the perception of memory deterioration often clinically reported by patients.

Comparing patients with NT1 and NT2, with comparable levels of subjective sleepiness and MSLT sleep latency, but different biochemical profile, we were able to control for the influence of sleepiness and hypocretin-deficiency on attentional performance in the clinical groups.

The clear-cut group differences, evident even in the proportional (Pro) network score, indicate the peculiar impairments of alerting network is attributable to the lack of hypocretinergic neurons.

Analyzing self-reported measure we showed that patients with narcolepsy (either type 1 and type 2) rated their levels of attention in everyday situations to be relatively poor.

Compared with healthy adults, they reported an higher frequency of ADHD Inattentive symptoms; moreover, patients with NT1 reported also and increased frequency of ADHD

hyperactive/impulsive symptoms. To our knowledge this is the first investigation aimed to explore the presence of comorbid ADHD in adult narcoleptic patients, our data disclose that a significant portion of NT1 patients (54%) showed ADHD scores in a standardized questionnaire above the clinical cut-off. In variance to previous investigations only a few patients with narcolepsy showed symptoms of increased anxiety, while compared with adult controls both clinical group reported greater levels of subjective sleepiness. In NT1 patients the severity of depressive complains were positively correlated with levels of subjective sleepiness and severity of ADHD symptoms, moreover a negative relationship between ESS score and alerting network efficiency was observable in NT1. Accordingly, our study showed that not only sleepiness momentary but also ADHD symptoms have an major impact on depressive complains reported by patients with narcolepsy.

This finding is of interest to the clinicians involved in the patients' treatment and counselling. Narcolepsy is a major organic disease with an important impact of increased sleepiness on daily cognition, whether the patients are depressed or not. However, depression may have an additional effect on the patients' subjectively perceived cognitive deficits.

Careful screening for ADHD comorbidity and eventually appropriate treatments may thus alleviate the patients' burden. In some cases, it may be appropriate to discuss with the patients that their subjectively perceived cognitive deficits might be attributed to both sleepiness and depression rather than to objective cognitive decline.

Knowing about the relationship between sleepiness, mood and attention deficits may prevent patients from taking wrong decisions (e.g. about their professional life). The present study also underlines the necessity to perform objective neuropsychological tests, which may also offer the possibility to objectively assess the effectiveness of pharmacological treatments on attentional impairments.