**PATHOPHYSIOLOGY OF NARCOLEPSY AND IDIOPATHIC HYPERSOMNIA:
A MULTIMODAL NEUROIMAGING STUDY**

I. CONCEPT, OBJECTIVES AND IMPACT

CENTRAL HYPERSOMNIAS: CLINICAL SUBTYPES

Narcolepsy and idiopathic hypersomnia (IH) are central disorders of hypersomnolence (or central hypersomnias), characterized by severe daytime sleepiness. They are **frequent** neurological disorders of sleep, with a prevalence of 0.05% (for narcolepsy)[1] to 0.3% (for IH),[2] which is 15 to 100 times more than multiple sclerosis[3] and Parkinson's disease.[4] They are also **severe** conditions, due to the dramatic consequences of daytime sleepiness, ranging from increased risk of accidents to cognitive impairment and depression, leading to a poor quality of life.[1]

These conditions display a few *distinctive* clinical features. Narcolepsy is characterized by rapid entries into rapid-eye-movement (REM) sleep (i.e. the so-called sleep-onset REM periods or SOREMPs). A majority of narcolepsy patients also present *cataplexy*, i.e. episodes of muscle atonia triggered by emotional stimulation.[1] However approximately 30% of narcolepsy patients never experience cataplectic episodes,[5] which led to the clinical categorization of narcolepsy in two subtypes: narcolepsy with cataplexy (or Type 1; NT1), and narcolepsy without cataplexy (or Type 2; NT2).[6] In contrast distinctive features in IH include the unrefreshing nature of sleep periods with difficulty waking up (i.e., 'sleep drunkenness'), a fewer number of SOREMPs and the absence of cataplexy.[7] In addition, as a proportion of IH display a long sleep time (often > 11h), IH patients are sometimes sub-categorized between short and long sleepers.[8] Despite these differences, there is also a *clinical overlap* between these conditions. Daytime sleepiness is common to NT1, NT2 and IH, as confirmed by short latencies to sleep during naps. All three conditions have similar age of onset, during adolescence or young adulthood.[1, 9, 10] In addition, 18% of narcolepsy patients have a long sleep time (>11h), along with more unrefreshing sleep periods and less or no cataplexy,[11] which questions the pertinence of the current distinction between NT2 and IH.[9, 12]

In addition to sleep disruptions, the inability to stay vigilant during the day in all these central hypersomnias is associated with reduced quality of life, cognitive alterations and emotional disturbances. NT1 has been shown to affect a large variety of physiological processes, including the mesolimbic pathways regulating emotion, motivation and reward processing.[13-15] More specifically, NT1 patients showed abnormal emotional processing under unpleasant[16] as well as pleasant conditions, such as humorous pictures[17] and when winning on a rewarding game.[15] Such studies of emotional processing have not been investigated in IH and NT2 so far. Nevertheless it is important to note that, similarly to NT1 patients who often report psychological co-morbidities (e.g., depressive and anxiety symptoms[18, 19]), more than 20% of NT2 and IH patients report mood changes and depressive symptoms.[13, 19]

Additionally, patients with central hypersomnias often report cognitive impairment but the objective findings on cognitive function in those conditions are sparse and incomplete.[20] So far, it appears that attention is the most impaired cognitive domain, especially simple attentional functions in NT1,[21, 22] but some studies also reported that complex functions, involving executive control and sustained attention were impaired in NT1, NT2 and IH.[21, 23, 24] More specifically, each of these conditions showed slower reaction time and more errors in all attention tasks (e.g., selective, divided, sustained attention[25]) compared to healthy controls, but NT1 patients performed the worst on each of these attention tasks. In contrast, IH and NT2 patients showed normal results in the early part of the sustained attention task but performed worse during the later portion of the task,[25] suggesting an inability to maintain stable alertness for an extended time. These attentional difficulties have been thought to mediate the memory complaints self-reported by many patients with central

hypersomnias.[20, 23] However, objective memory assessments have not consistently found significant memory impairment in these conditions. Neuroimaging studies of NT1 (see below) reported structural alteration in the hippocampus[26] and abnormal hippocampal and frontal activities during memory encoding,[27] without reduced memory performance.[26] One study, using self-reported questionnaires, reported memory complaints in IH,[23] but there was no further investigation of cognition in this population. Therefore a systematic characterization and comparison of cognitive performances in NT1, NT2 and IH is still needed, in order to delineate the disturbances that are specific to each condition.

The blurred boundaries between hypersomnia subtypes based on clinical phenotypes illustrate the importance of reassessing the categorization of these conditions according to objective biomarkers reflecting the underlying pathophysiological mechanisms. To date only NT1 has been thoroughly investigated, with the demonstration of a deficiency in orexin-A, also called hypocretin-1, a neuropeptide involved in the stabilization of sleep-wake states.[28] This deficit, which can be measured invasively from cerebrospinal fluid (CSF) samples, is believed to be responsible for the abrupt sleep episodes during the day experienced by NT1.[1] In contrast, the mechanisms of NT2 and IH seem clearly distinct from those of NT1, as no consistent decrease in hypocretin-1 level has been observed in either NT2[29] or IH.[29, 30] In these two conditions, it has been suggested that an endogenous GABAergic mechanism might promote daytime sleepiness by enhancing GABA-A receptor signalling, but this hypothesis was mainly based on a pilot study of a small and heterogeneous sample of 7 individuals with various hypersomnia phenotypes (including some NT2 and IH) using CSF samples.[31] Therefore, *while key biomarkers and pathophysiological mechanisms have been uncovered in NT1, both NT2 and IH remain poorly understood conditions with unclear neural biomarkers and mechanisms.* The **important gap in the pathophysiology of narcolepsy and IH** limits the development of effective treatments targeting the specific mechanisms of these disorders.
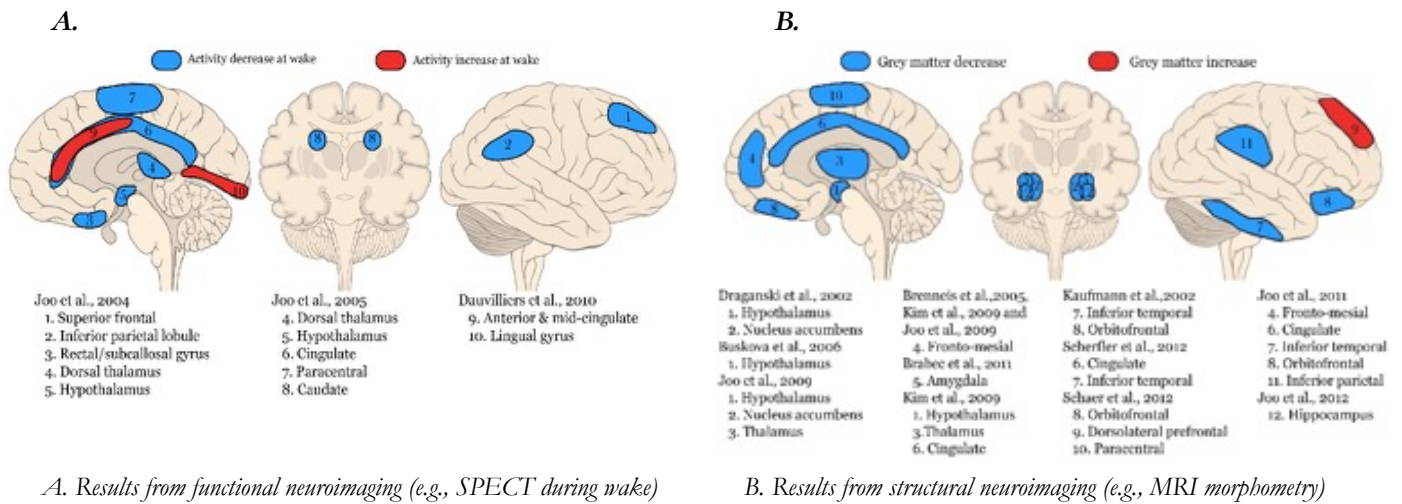
NEUROIMAGING OF NARCOLEPSY

Neuroimaging has played a key role in recent advances on the **mechanisms** of sleep disorders. In chronic insomnia, the reported lack of decrease in brain metabolism during the descent to sleep supports the concept of a hyperarousal state[32] (see our review[33]). In REM sleep behavior disorder (RBD), we previously identified neuroimaging patterns predicting the evolution of RBD towards neurodegenerative disorders.[34] In both examples, neuroimaging had **clinical impact**: the concept of hyperarousal fueled the principles of cognitive-behavioral therapy for insomnia, and imaging biomarkers of RBD identified patients with higher neurodegenerative risk for whom disease-modifying therapies will be indicated.

In several review articles, we discussed the contribution of **neuroimaging** to the understanding of NT1,[35-39] with the demonstration of **functional** (e.g., with Single Photon Emission Computed Tomography [SPECT] during rested wakefulness)[40, 41] and **anatomical** (e.g., with Magnetic Resonance Imaging [MRI] morphometry)[42-48] alterations in the *hypothalamus*, which constitute non-invasive biomarkers of NT1 in line with a deficiency of the hypocretin system. Changes were also observed in other subcortical (thalamus, caudate) and cortical regions (superior frontal, inferior parietal, cingulate), possibly reflecting projection sites of hypocretinergic neurons (see fig. 1). In addition, several fMRI studies in NT1 demonstrated altered responses in the hypothalamus and amygdala during emotional stimulation tasks[17, 49, 50], and in the amygdala, striatum, nucleus accumbens and ventromedial prefrontal cortex during a reward task.[15] While a widespread disruption of white matter integrity was found in NT1,[45, 51] the only neuroimaging study of NT2 found no significant white matter changes, but did not assess grey matter or functional changes.[52] Besides our pilot study presented below, there has been no neuroimaging study, either anatomical or functional, in *IH*.

**Fig. 1. Neuroimaging of Narcolepsy with Cataplexy (NT1)**.

This figure summarizes findings from previous neuroimaging studies conducted in NT1. Color codes are indicated on the top of each panel. Numbers refer to brain regions and corresponding articles indicated on the bottom of each panel. From O'Byrne, Salimi & Dang-Vu 2016.[38]

A.

B.

A. Results from functional neuroimaging (e.g., SPECT during wake)

B. Results from structural neuroimaging (e.g., MRI morphometry)

PILOT DATA: NEUROIMAGING OF IDIOPATHIC HYPERSOMNIA (IH)

In order to inform our concept, we performed a pilot study on IH participants (age 21-57 years) and good sleepers (GS, controls) matched for age and sex. They completed *neuroimaging during resting wakefulness*, with high-resolution SPECT using ^{99m}Tc-ECD to evaluate changes in regional cerebral blood flow (*rCBF*), as well as MRI (3-Tesla) to identify anatomical changes (*morphometry*) and resting-state *functional connectivity* (fMRI). In IH, psychotropic medications such as psychostimulants were withdrawn 2 weeks before the start of the protocol and during the whole study procedure.

SPECT data showed decreased rCBF in 13 IH compared to 16 GS in regions encompassing the *default-mode network* (DMN: medial prefrontal cortex, posterior cingulate cortex) (see fig. 2A) (Boucetta… Dang-Vu, Sleep 2017).[53] Interestingly, in both medial prefrontal and posterior cingulate cortices, rCBF was correlated with self-reported (Epworth sleepiness scale, ESS) and objective (mean sleep latency during naps) daytime sleepiness, suggesting that these cortical changes contributed to IH clinical severity. When comparing these pilot findings to published data in NT1 (fig. 1A), a distinct distribution of deficits seems to emerge between NT1 and IH, where NT1 has a broader extent of rCBF decreases including the hypothalamus, while IH deficits are mainly concentrated on the DMN with a preservation of the hypothalamus, in line with the absence of hypocretin-1 loss in IH. Interestingly, this *rCBF distribution during wakefulness in IH was strikingly similar to the rCBF patterns associated with non-rapid-eye-movement (NREM) sleep in GS,* as shown by our previous findings of rCBF decreases in the DMN of 23 GS in proportion of slow wave activity during NREM sleep (see fig. 2B) (Dang-Vu et al., Neuroimage 2005).[54] Importantly rCBF changes during wakefulness in IH were different from those observed during a non-specific state of sleepiness such as sleep deprivation. Using data from a separate study (Dang-Vu et al., PLoS One 2015),[55] we assessed rCBF in GS during wakefulness in two sessions, i.e. after a normal night of sleep and after a night of sleep deprivation, and found that rCBF decreases after sleep deprivation (compared to after sleep) were located in lateral fronto-temporal cortices, in a distribution strikingly distinct from IH (see fig. 2C). Thus *inducing a state of sleepiness in GS did not reproduce the rCBF changes observed in IH*, which are thus likely to reflect a specific *trait* of IH.

Fig. 2. Regional cerebral blood flow (rCBF) distribution in IH and good sleepers (GS).
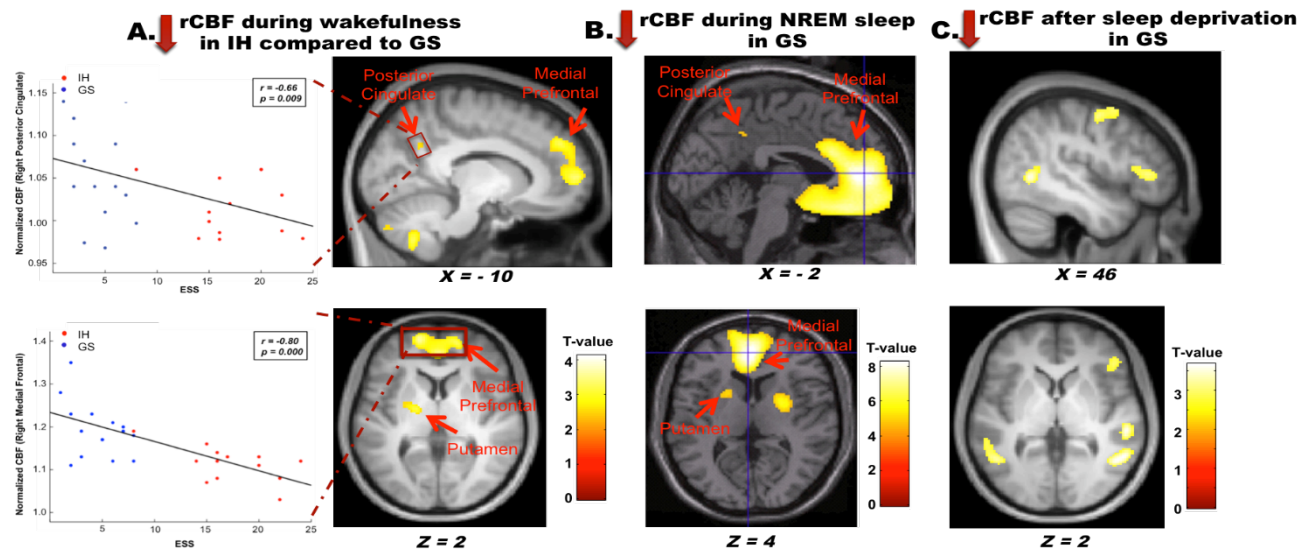

A. SPECT with ^{99m}Tc-ECD during rested wakefulness in 13 IH compared to 16 GS (p<0.01): Right panels, distribution of rCBF decreases in IH; Left panels, scatter plots showing correlations between rCBF decrease in each region and higher self-reported daytime sleepiness (Epworth Sleepiness Scale, ESS).

B. PET with $H_2^{15}O$ during NREM sleep in 23 GS (p<0.05corr.): rCBF decreases correlated with slow wave activity; note the __similarity__ of distribution with IH (A). From Dang-Vu et al. 2005.[54]

C. SPECT with ^{99m}Tc-ECD during wakefulness in 9 GS after sleep deprivation compared to after normal sleep (p<0.01): rCBF decreases after sleep deprivation; note the __difference__ of distribution with IH (A).

MRI pilot data included structural and functional (fMRI) sequences. **Structural** MRI was analyzed using **cortical thickness** assessment in 12 IH compared to 16 GS, and showed *increased cortical thickness in the sensorimotor cortex and DMN*, which was correlated with higher self-reported (ESS) sleepiness (see fig. 3A). These cortical thickness increases contrast with the decreases in rCBF shown above, and might constitute compensatory changes to the state of chronic sleepiness presented by IH patients. Beyond the study of specific brain regions, an increasing number of reports have investigated connectivity between brain areas in the search for the mechanisms of sleep disorders such as RBD[56] and insomnia (e.g., see Suh, Kim, Dang-Vu et al., Sleep 2016). While there has been no connectivity study published yet in NT2 we conducted a pilot **structural covariance** and **resting-state fMRI connectivity** analysis during wakefulness in 12 IH participants compared to 16 GS controls. Given the SPECT pilot findings, we focused our preliminary analysis on the structural and functional connectivity within the DMN, by identifying brain voxels showing correlations with cortical thickness (structural covariance) or spontaneous fluctuations of the fMRI signal (functional connectivity) within the DMN. We found that these correlations were found between a larger number of brain areas within the DMN in IH compared to GS. In particular, the posterior cingulate cortex in IH showed cortical thickness correlations with a larger number of DMN areas and demonstrated increased functional connectivity (with the lateral parietal cortex) than GS (see fig. 3B). This pattern can be interpreted as a relative compensatory *increase in structural and functional connectivity within the DMN during wakefulness in IH*. Interestingly, the transition from wakefulness to NREM sleep in good sleepers has been characterized by a breakdown of functional connectivity between the anterior and posterior nodes of the DMN,[57] which suggests that the increased connectivity found in IH at wake might constitute a compensatory response to sustained breakdowns in connectivity associated with prolonged periods of sleep.

Fig. 3. MRI: cortical thickness and connectivity in IH and GS.
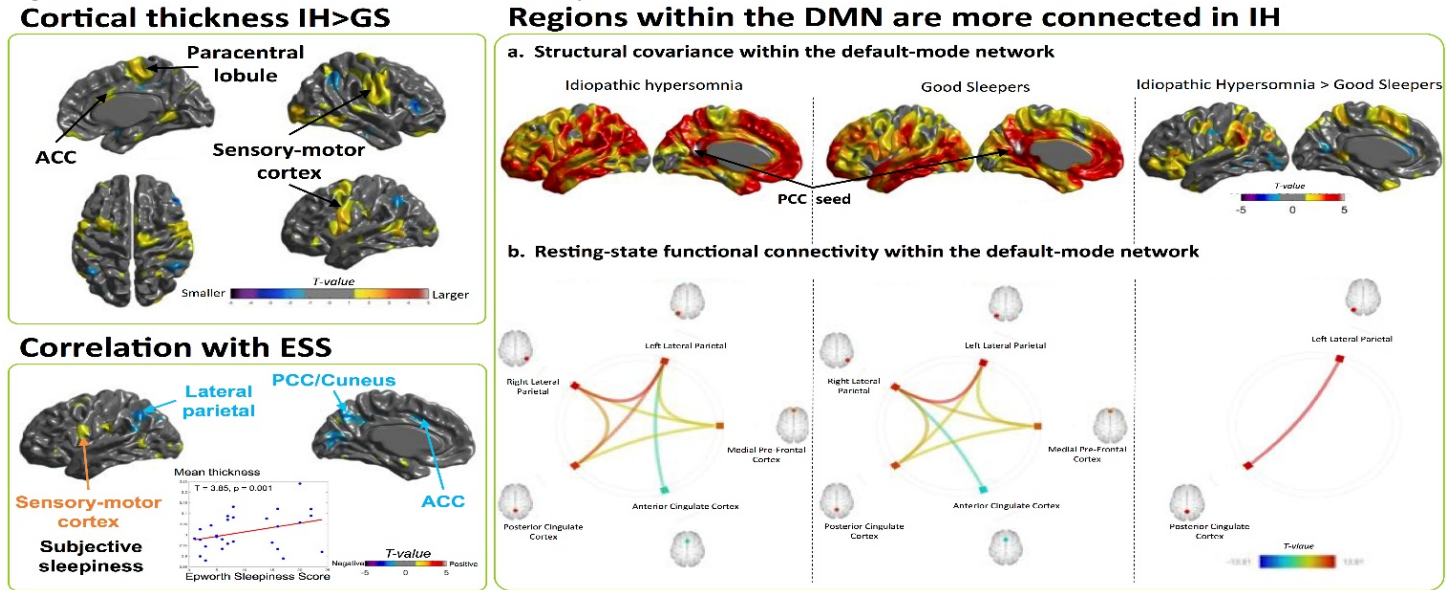
A. Cortical thickness analysis showing group comparison between 12 IH and 16 GS. Upper panel: cortical thickness in the sensorimotor cortex and parts of the DMN was increased in IH (p < 0.01). Lower panel: scatter plot showing correlation between mean cortical thickness increase and higher sleepiness (ESS).

B. Structural covariance and resting-state fMRI functional connectivity of the posterior cingulate in 12 IH and 16 GS. (a) Maps of cortical covariance in IH (left panel) and GS (middle panel). The group comparison (threshold T > 2.5, equivalent to uncorrected p < 0.01; right panel) shows that cortical thickness correlations with the posterior cingulate cortex (PCC, seed) were larger in IH, extending to the lateral parietal cortex. (b) Group difference functional connectivity (threshold at p < 0.05 after correction for multiple comparisons using false discovery rate) showed a larger functional connectivity between the posterior cingulate cortex and left lateral parietal cortex in IH compared to GS.

*In sum, our pilot data indicate that IH is characterized by specific anatomical and functional changes during rested wakefulness, involving regions belonging to the DMN, which showed lower rCBF along with increased thickness and connectivity. While strikingly distinct from those induced by sleep deprivation, the functional changes are reminiscent of the patterns observed during normal NREM sleep, which might indicate the **persistence of NREM sleep-like features during wakefulness in IH**. These preliminary data constitute the first neuroimaging study of IH and suggest for the first time the involvement of **incomplete sleep-wake transitions** in the pathophysiology of IH. Such hypothesis requires further support from the assessment of brain activity across the sleep-wake cycle in hypersomnia subtypes and controls. In line with earlier preliminary data suggesting a GABAergic mechanism,[31] the sleep-like DMN alterations during wakefulness in IH might be due to a **local increase of GABA** neurotransmission. Indeed, previous neuroimaging studies in healthy individuals had shown that modulating GABA levels affect brain activation in the DMN.[58, 59] To support this hypothesis, a systematic investigation of GABA levels across hypersomnia phenotypes is needed.*

OBJECTIVES AND HYPOTHESES

Given the lack of knowledge on the pathophysiology of central hypersomnias and the potential shown by neuroimaging to address this issue, our **objective** is to extend our pilot work to larger samples, further clinical groups and complementary **neuroimaging** methods in order to identify the **non-invasive neural biomarkers** and **mechanisms** characterizing each phenotype of **narcolepsy and IH**.

We will aim at assessing differences between NT1, NT2, IH and partially sleep deprived GS in:

a. *Brain responses across the sleep-wake cycle using simultaneous EEG-fMRI*

b. *Brain responses during wakefulness using fMRI, especially in response to attentional and rewarded-associative memory tasks*
c. *Regional brain volumes and cortical thickness using MRI morphometry,*
d. *Brain connectivity using structural covariance and resting-state fMRI functional connectivity,*
e. *And regional GABA levels using MR spectroscopy*

Based on the existing literature reviewed above and our pilot data, we **hypothesize** that:

- NT1 would display decreases in *volume or thickness* in the hypothalamus and fronto-parietal cortical areas in line with the deficits in hypocretin-1 neurons and their projection sites, whereas IH would show a preservation of the hypothalamus and an increase in thickness limited to cortical areas involved in the modulation of sleep-wake transitions, such as the DMN;

- IH would show *smaller increases in brain responses in the DMN from NREM sleep to wakefulness* compared to NT1 and GS, reflecting the persistence of NREM sleep-like features during wakefulness in IH;

- NT1, NT2, IH would all display slower reaction time in both attentional and rewarded-associative memory tasks, along with more errors compared to controls. IH and NT2 would show larger fluctuation in performance, especially toward the end of the tasks, while NT1 would show more stable performance throughout the tasks.).

- IH should feel less refreshed (and in a state of sleep inertia) after the nap compared to GS and probably NT1 and NT2..

- NT1, NT2 and IH would display altered activation of the sustained attentional circuits (especially medial, dorsolateral prefrontal and parietal cortices) compared to controls. IH and NT2 would display a greater decline in fronto-parietal activity over the course of the task compared to controls.

- NT1 would display *altered responses in reward brain circuits* (amygdala, striatum, nucleus accumbens) during wakefulness rewarded-associative memory tasks, while these circuits would be preserved in IH; all these disorders would show abnormal frontal and hippocampal activity during memory encoding.

- IH (but not NT1) would show increases in *structural and functional connectivity* within the DMN during wakefulness, in line with the concept of compensatory increases to sustained periods of connectivity breakdowns during sleep; NT1 would instead show a decreased connectivity between the hypothalamus and fronto-parietal cortices, in line with hypocretin-1 system alteration;

- IH would show *increased GABA levels in the DMN* (particularly in medial prefrontal and posterior cingulate) compared to NT1 and GS, in line with the involvement of an endogenous GABAergic process in IH;

- Given the similarities with IH in terms of clinical presentation and hypocretin-1 preservation, NT2 would display brain responses, volume/thickness, connectivity and GABA patterns overlapping with those of IH. There have indeed been recent articles questioning the distinction between NT2 and IH,[9] and providing some evidence that NT2 and IH belong to the same disease cluster.[12]

II. APPROACHES AND METHODS

GENERAL RESEARCH PLAN (see fig. 4)

Four groups of participants will be studied: NT1, NT2, IH, and partially sleep deprived controls (GS). They will undergo:

(i)   **Sleep recordings** (polysomnography [PSG]; multiple sleep latency test [MSLT]) to confirm eligibility;

(ii)   **MRI** including **functional** (fMRI) sequences for assessments of *resting-state functional connectivity,* brain responses during *cognitive tasks* (attention, rewarded-associative memory) and across *sleep-wake* transitions,

anatomical sequences to assess changes in *regional volumes and cortical thickness*, and **spectroscopy** to measure regional *GABA* levels. *All steps will be conducted at the PERFORM Center*, which includes all platforms required to conduct this multimodal approach.
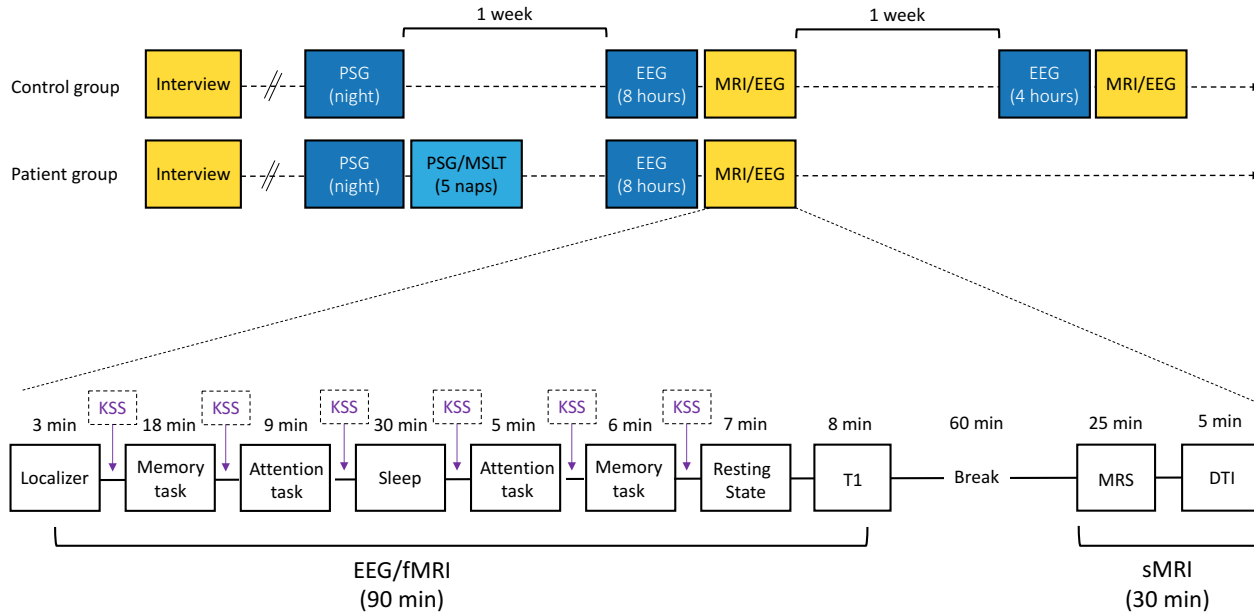

Fig. 4. General research plan: *Sequence and timing of the main measurements during data collection*

PARTICIPANTS

Patients (≈90) will be recruited from sleep clinics and through advertisements in patients' associations such as 'Fondation Sommeil', while controls (≈30) will be recruited through advertisements posted in local newspapers, social media and other communication outlets (e.g. emails). Interested participants will first be screened using a telephone-based questionnaire and then invited to our laboratory for a semi-structured diagnostic interview to confirm they meet the inclusion criteria.

Inclusion criteria for *NT1, NT2 and IH* will be determined based on standard criteria:[6]
All 3 conditions require: 1) excessive daytime sleepiness for ≥ 3 months, 2) daytime mean sleep latency ≤ 8 min, 3) no other causes of hypersomnia (e.g., other sleep or neurological disorders).
Specific criteria:
   - NT1: ≥ 2 SOREMPs *and* definite history of cataplexy.
   - NT2: ≥ 2 SOREMPs *and* absence of cataplexy.
   - IH: < 2 SOREMPs *and* absence of cataplexy; if total sleep time is ≥ 11h (as assessed by 1 week of actigraphy), then criterion 2 (sleep latency ≤ 8 min) not required. This total sleep time criterion will also be used to subdivide the IH group into IH with (≥ 11h) or without (<11h) long sleep time, given data suggesting that these 2 subgroups represent different clinical entities with distinct pathophysiology.[8] Exploratory analyses will look at differences between these 2 subgroups across the different neuroimaging modalities used in the current study.
Good sleepers (GS, controls) will be defined by the absence of sleep disorders (including NT1, NT2 and IH) and medical conditions (see exclusion criteria below), as determined by the semi-structured interview and

PSG. In addition, the absence of insomnia symptoms, short sleep (i.e., sleep duration < 6h.), and irregular sleep schedules in controls will be assessed by actigraphy during 1 week prior to each visit. In order to rule out the possibility that differences between GS and NT1, NT2 and IH might be due to the sleepiness state rather than trait differences, GS will be sleep restricted the night before the imaging acquisitions, i.e. they will be requested to limit their sleep to a maximum of 4 hours (partial sleep deprivation [SD] as checked by actigraphy). This will ensure they are in a state of sleepiness during the imaging protocol, and will also facilitate their sleep during the EEG/fMRI session.

The following <u>exclusion criteria</u> will apply to all groups (NT1, NT2, IH and GS):
 - Systemic or neurological diseases (e.g., infections, inflammatory disorders, dementia, epilepsy);
 - Other sleep disorders[6] (e.g., apnea-hypopnea index > 5/h, REM sleep behaviour disorder as screened by the Single-Question Screen for REM Sleep Behavior Disorder,[60] restless legs syndrome with score > 10 at the Restless Legs Syndrome Rating Scale,[61] insomnia disorder, circadian rhythm disorder);
 - Having worked on night shifts during the last month;
 - < 18 or > 65 years of age;
 - Major psychiatric disorder[62] (e.g., major depression, psychotic or bipolar disorder) including a depressive mood score and/or an anxiety mood score (HADS)[63] >10;
 - History of head injury, encephalopathy, former intracranial surgery, alcoholism or substance abuse;
 - Contraindications for MRI exam (e.g., claustrophobia, metallic implants).

Groups will be matched for age and sex (narcolepsy and IH have similar age and sex distributions[10]). Psychotropic medications that can influence sleep and alertness (i.e., psychostimulants, antidepressants, neuroleptics, hypnotics and anxiolytics) will be withdrawn one week before the start of the protocol and during the whole study procedure. Participants will not be allowed to drink alcoholic or caffeinated beverages from the night prior to each visit at the lab.

SLEEP RECORDINGS: are required to confirm eligibility and determine groups. They will consist of an **overnight PSG** to screen out other sleep disorders (e.g., sleep apnea), followed by **MSLT** (five 20-min. nap recordings at 8AM, 10 AM, noon, 2PM, and 4PM[64]) to assess daytime mean sleep latency and SOREMPs for NT1, NT2 and IH only. Sleep recordings will include a 32-channel electroencephalography (EEG; sampling rate = 512 Hz), electrooculography, electromyography, and cardiorespiratory sensors (for PSG only). Participants will also have 1 week of *actigraphy* prior to each lab visit, to assess total sleep time over several days and ensure participants follow regular sleep-wake schedules before each visit. It will also allow us to support the classification of participants into the IH group and its subtypes. Actigraphy consists of a wrist-worn accelerometer that estimates sleep-wake times based on assessment of motor activity. Following PSG and MSLT, eligible participants will proceed to neuroimaging. Since a majority of hypersomnia participants will already have had sleep apnea ruled out from their clinical evaluation at a sleep clinic, we expect few participants to be excluded at this stage (in our pilot study, no participant was excluded for sleep apnea).

QUESTIONNAIRES

Several standardized questionnaires will be administered before the inclusion to the protocol and will be used as covariate factors in our analyses. Questionnaires on general sleep include the Pittsburgh Sleep Quality Index (PSQI), a subjective measure of general sleep quality over the past month;[65] the Epworth Sleepiness Scale (ESS) that measures for a person's average sleep propensity during daily life activities;[66] and the Sleep Inertia Questionnaire (SIQ) that evaluate the presence of sleep inertia symptoms after sleep.[67] After the sleep

(nap) session during the MRI visit, the Karolinska Sleepiness Scale (KSS) will be administered to evaluate the subjective level of sleepiness at this particular time.[68]

COGNITIVE TASKS

The attentional task and a memory task will be performed during the fMRI section. Sustained attention to response task (SART) is a GO/NO GO task, which provides indications about sustained attention and motor inhibition.[69, 70] The participants will perform the task twice: before and after the nap. The task will test the level of attention pre- and post-sleep and will give an objective indication on the refreshing aspect of the nap across the different groups. Participants will be shown digits from 0 to 9 presented one at a time and they will have to press a key (on an MRI compatible response box) as quickly as possible when a digit appears (GO) except if it is the digit "3" (NO GO). The test consists of 4 cycles, each with a baseline level where no response is needed and two difficulty levels, differing in time the digit is being shown. The duration of the task will be 9 min. We will analyze the number of commission errors (GO when the "3" appears), the number of omissions (no response to a "GO" signal), and the sum of both types of errors (i.e., total number of errors).[70] Subjects will be given the instructions to focus on accuracy, while still responding quickly.

The rewarded-associative memory task will be composed of 3 sessions: learning, immediate recall and delayed recall (post-nap). During encoding phase a participant will be presented a picture and corresponding text, and will be required to answer a question pertaining to the association. During the recall phase, participants will be required to correctly match the image to the correct text from a set of options. After the nap, participants will be retested again on a set of the original pairs of pictures and text, in addition to novel images or text to prevent blind responding. The participants will be told that their final monetary gain depends on their performance, which is supposed to ensure that the participants pay attention to the distinct reward levels associated with each sequence and to increase the motivation to obtain the rewards. All participants will receive the same amount of monetary compensation at the end of the study. The memory task will take a total of 24 minutes (12 minutes for the learning phase, and 6 minutes for each recall phase).

MRI: will be performed in a 3-T. scanner (beginning around 2PM) starting with the EEG/fMRI sequences during a first session of wakefulness task (i.e., the SART and the encoding/learning session of the rewarded-associative memory task), then participants will be given the possibility to sleep during 35 min. If they do not succeed in falling asleep or awake before the end of the 35 minutes, they will be able to stop the session whenever they want. After a simple questionnaire on their sleepiness (Karolinska Scale), they will perform the second session of **wakefulness** tasks (i.e., the testing session of the rewarded-associative memory task) as well as the wake resting-session. After the break, participants will undergo the anatomical sequences: T1, spectroscopy (MRS) and then diffusion tensor imaging (DTI).

Functional MRI (fMRI): Multislice T2*-weighted fMRI images will be obtained with a gradient echo-planar sequence using axial slice orientation (38 slices; voxel size = 2.5x2.5x2.5 mm^3; matrix size 80x80x38; TR = 2250 ms; TE = 29.94 ms; flip angle = 80°) acquired during a resting-state session in which participants will be instructed to remain awake with their eyes open and focused on a central fixation cross (duration: 10 minutes) and during the sessions of wakefulness tasks. EEG will be recorded simultaneously during the fMRI session using an MRI-compatible system and processed (e.g., artifact correction) as in our previous studies,[73-75] to monitor vigilance and select the fMRI time series corresponding to wakefulness (for the resting state analysis) or sleep (for the sleep session). The fMRI sequence will include a 35 min sleep opportunity (nap) during which participants will be asked to relax and try to fall asleep. During the EEG-fMRI sessions, a carbon-wire loop system will be used in order to facilitate MR-related artefact correction.[76]

fMRI analysis: Resting state fMRI time series corresponding to EEG-defined wakefulness will be selected and processed in the Neuroimaging Analysis Kit (NIAK version 1.0.1, http://niak.simexp-lab.org).[77] After standard preprocessing (including de-spiking, motion correction, slice-timing correction, removal of

nuisance signal including signals of local white matter, CSF and physiological noise, spatial normalization, and smoothing), regions of interest (ROI) will be selected based on seeds taken from the literature and will consist of the major nodes of the DMN, including the posterior cingulate and medial prefrontal cortices, as well as the hypothalamus and fronto-parietal cortices (as identified from NT1 neuroimaging studies[40, 41]). *Resting-state functional connectivity* will be assessed by identifying areas (i.e., voxels), whose fMRI time series significantly correlate with average time series of the given ROIs. Corresponding Pearson correlation coefficient r values will then be Fisher-transformed and used for one-way ANOVA to assess group effects for each correlation (p<.05, corr. using a cluster-wise thresholding[78]).

For the wakefulness sessions of *attention and rewarded-associative memory tasks*, after standard preprocessing procedures: realignment, slice timing, normalization and smoothing; ROI related to attention, reward and memory circuitries will be selected, including the hippocampus, the ventral tegmental area., the medial and dorsolateral prefrontal cortex as well as the hypothalamus and the amygdala. General linear model (GLM) approach will be used to compare conditions of interest at the individual level and then for second-level random-effects analyses. For the analyses of the learning session of the rewarded-associative memory task, at individual level, we will include 2 sessions for the 2 learning blocks. Each session will contain 2 regressors for successful trials and 2 regressors for misses according to their reward outcome (high, low). For the SART analysis, we will compare correct GO responses (press key) to correct NO GO responses (motor inhibition). All group comparisons will be performed using ANOVAs. Correction for multiple comparisons will be performed by submitting all reported activations to small-volume correction (SVC) for family-wise error (p<0.05) using ROI.

For the sleep (nap) session, several analyses will be conducted after standard preprocessing and scoring of EEG recordings in order to detect epochs of stable N2-N3 sleep (as well as REM sleep for the NT1 and NT2 groups). A block-design will first compare brain responses during N2-N3 with those during resting-state wakefulness in each subject. An event-related design will assess responses to slow waves and spindles during N2-N3 epochs in each subject as performed in our previous works[74, 75]. A third analysis on N2-N3 functional connectivity will be assessed by identifying areas (i.e., voxels), whose fMRI time series significantly correlate with average time series of the given ROIs (DMN nodes, hypothalamus, fronto-parietal cortices). Corresponding Pearson correlation coefficient r values will then be Fisher-transformed and used for one-way ANOVA to assess group effects for each correlation (p<.05, corr. using a cluster-wise thresholding[78]). Depending on the number of subjects presenting with detectable REM sleep, exploratory analyses will also include block-design and functional connectivity comparisons between REM sleep and wakefulness. All these analyses will be followed by a second-level analysis comparing responses between groups using ANOVA (p<.05, corr.).

Structural MRI: Anatomical neural abnormalities will be assessed with a T1 MP-RAGE sequence (220 slices; voxel size = 1x1x1 mm^3; matrix size = 240x240x220; TR = 8.2 ms; TE = 3.8 ms; duration: 10 min) and DTI twice, with opposite phase-encoding directions (60 slices; voxel size = 2x2x2 mm^3; matrix size = 112x112x60; TR = 6700 ms; TE = 72 ms). Images will be preprocessed (intensity non-uniformity correction, spatial normalization, brain tissue segmentation, Jacobian modulation and smoothing) and analyzed using standard VBM[79] (implemented in SPM) to extract GMV values, and using CIVET to extract cortical thickness as in our previous study.[80] DTI data will be analyzed using FSL and TBSS to extract conventional DTI indices (fractional anisotropy, mean, radial and axial diffusivity). The analysis will use ANOVAs to evaluate group-differences in GMV, cortical thickness and DTI measures while correcting for sex and age (p<.05, corr.).

MR spectroscopy: GABA levels will be assessed using the MEGA-PRESS sequence (TE/TR=68/2000 ms, voxel size = 2.5 x 2.5 x 2.5 cm^3, 352 averages; duration: 24 min). Data will be processed (removal of motion corrupted averages, frequency and phase drift correction using spectral registration,[81] zero-order phase correction) using the FID-A toolkit[82] (https://github.com/cic-methods/fid-a). Spectral quantification will be performed with LCModel[83] (Provencher Inc., Oakville, Canada) using a simulated metabolite basis set. Absolute GABA concentrations will be estimated using the unsuppressed water signal as an internal reference, with appropriate corrections for the effects of the fractional tissue composition within the volume

of interest on the measured water signal. Given our hypotheses, we will assess GABA levels in 2 ROIs of the DMN, i.e. medial prefrontal and posterior cingulate, with seeds taken from the literature.[40, 41, 84] The analysis will use ANOVAs to evaluate group-differences in GABA levels in the medial prefrontal cortex and posterior cingulate ($p<.05$, corr.).

SAMPLE SIZE: We will aim at recruiting approximately 30 participants per group (thus 120 participants in total). This sample size estimation is in line with published studies in NT1 using protocols of structural[26, 44, 85] and functional[40, 41] neuroimaging, in which samples ranging from 24 to 36 subjects per group showed significant differences between NT1 and GS. This sample size estimation is also in agreement with in earlier MR spectroscopy studies reporting significant differences in GABA levels between 16 to 20 controls and similar numbers of sleep disordered participants (e.g., insomnia).[86, 87]

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