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Increased dopaminergic function in the thalamus is associated with excessive daytime sleepiness

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Miss Yousaf - Study concept and design, statistical analysis and interpretation of data and drafting of the manuscript

Dr. Pagano - Study concept and design, study supervision, interpretation of data and drafting of the manuscript

Dr. Niccolini - critical revision of the manuscript for important intellectual content

Dr. Politis - Study concept and design, study supervision, critical revision of the manuscript for important intellectual content and final approval of the manuscript

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Data used in the preparation of this article were obtained from the Parkinson's Progression Markers Initiative (PPMI) database (www.ppmi-info.org/data). For up-to-date information on the study, visit www.ppmi-info.org. PPMI – a public-private partnership - is sponsored by the Michael J. Fox Foundation for Parkinson's Research (MJFF) and is co-funded by MJFF, Abbvie, Avid Radiopharmaceuticals, Biogen Idec, Bristol-Myers Squibb, Covance, Eli Lilly & Co., F. Hoffman-La Roche, Ltd., GE Healthcare, Genentech, GlaxoSmithKline, Lundbeck, Merck, MesoScale, Piramal, Pfizer and UCB.PPMI. Industry partners are contributing to PPMI through financial and in-kind donations and are playing a lead role in providing feedback on study parameters through the Industry Scientific Advisory Board (ISAB). Through close interaction with the study, the ISAB is positioned to inform the selection and review of potential progression markers that could be used in clinical testing.

Miss Yousaf, Dr. Pagano, Dr. Niccolini and Dr. Politis report no disclosures.

Declaration of interests

All authors have no conflict of interest.

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ABSTRACT

Objectives/Background

Excessive daytime sleepiness (EDS) is a common disorder, which can manifest in isolation or in combination with other neurological or psychiatric disorders. We know relatively little about the mechanisms underlying the development of EDS and the clinical management of patients with EDS remains an unmet need. In this study, we hypothesised that thalamic dopaminergic function would be altered in subjects with EDS and we sought to investigate this by assessing [123 I]FP-CIT Single Photon Emission Computed Tomography (SPECT) data, which is a molecular imaging marker of dopamine transporter (DAT).

Patients/Methods

We performed a case-control study using people registered as healthy subjects in the Parkinson's Progression Markers Initiative database. We assessed and compared semi-quantified identified [123 I]FP-CIT-SPECT in two groups of 21 healthy subjects with and without EDS, who were matched for age, gender, age of diagnosis, years of education and disease duration.

Results

Our findings show increased thalamic DAT binding in people with EDS compared to matched healthy subjects without EDS. Higher thalamic DAT binding also correlated with worse EDS scores.

Conclusion:

Our findings provide evidence that increased dopaminergic function in the thalamus may mediate excessive daytime sleepiness in humans.

Keywords: Excessive daytime sleepiness; [123 I]FP-CIT; Thalamus; Dopamine

1.0 INTRODUCTION

Excessive daytime sleepiness (EDS) is defined as the inability to stay awake and alert during major waking episodes of the day, resulting into unintended lapses into drowsiness or sleep[1]. EDS is now acknowledged to substantially impair quality of life and work productivity, imposing a significant public health burden[2]. It has been reported to be implicated in up to 50% of work-related accidents, approximately 16% of motor vehicle accidents and 25% of home-based accidents [3]. Though EDS has strongly been associated with a range of neurological and psychiatric diseases including Parkinson's disease (PD), epilepsy and schizophrenia[4], its prevalence in the general population has been estimated to be as high as 18% [5]. Several risk factors have been found to contribute to EDS including obesity, depression and age[6]. Although EDS can commonly manifest secondary to nocturnal sleep abnormalities and poor sleep hygiene[7], it is not simply the consequence of sleep deprivation/disruption, hence potentially driven by distinct mechanisms[8].

Several brain regions, including the thalamus, hypothalamus, locus coeruleus and dorsal raphe are known to participate in the initiation and maintenance of alertness and sleep, though it remains to be ascertained the mechanisms underlying the development of EDS. The thalamus, specifically, has considerable nonphotic influence on sleep and circadian rhythmicity. The low- and high- frequency spindles generated by the GABAergic inhibitory reticular thalamic interneurons play an active role in inducing, maintaining and advancing non-rapid eye movement (NREM) sleep towards deeper stages[9].

Several neurotransmitters have also been implicated in playing significant roles in sleep and alertness, including norepinephrine, hypocretin, serotonin, GABA, glutamate and histamine. However, considerable evidence has emphasised the key role of the dopaminergic system in the sleep-wake regulation. Wisor, Nishino [10] demonstrated that mice with deletion of the

dopamine transporter (DAT) gene have 20% more wakefulness compared to controls and are unable to respond to psychostimulant drugs such as modafinil or amphetamine, suggesting that DAT may regulate wakefulness. Nearly all PD patients endure sleep disturbances, strongly suggesting the involvement of a dopaminergic component in the development of EDS. Studies have identified varying levels of extracellular dopamine in the terminal regions of ventral tegmental area (VTA) dopaminergic neurons across the sleep-wake cycle, with elevated levels of dopamine during waking and REM sleep[11].

The origin of the dopamine innervation in the thalamus is markedly diverse, including dopaminergic projections from the VTA, the hypothalamus, and lateral parabrachial nucleus (LPbN), highlighting the complex organisation of the thalamus in terms of DAT content, distribution, density and origin[12]. The thalamus is also innervated by wake-active dopaminergic neurons from the ventral periaqueductal gray (vPAG), which also project to integral components of the sleep-wake regulatory system such as the prefrontal cortex, the ventrolateral preoptic nucleus (VLPO), the hypothalamic orexin/hypocretin cells, the pontine laterodorsal tegmental nucleus cholinergic neurons and the basal forebrain cholinergic neurons[13].

A strong correlation has been identified between DAT binding and daytime sleepiness in PD[14], as well as a correlation between dopamine D₂/D₃ receptor binding and sleep deprivation[15]. Studies have also demonstrated a loss of vPAG gray matter dopaminergic neurons in patients with Multiple Systems Atrophy (MSA) and dementia with Lewy bodies (DLB), potentially contributing to excessive daytime sleepiness [16], as well as the dose of dopaminergic treatment predicting EDS in MSA [17]. Taken together, these results support the hypothesis that dopaminergic dysfunction may be associated with daytime sleepiness.

Here we hypothesised that thalamic presynaptic dopaminergic function would be altered in patients with EDS and we sought to investigate this by assessing [¹²³I]FP-CIT single photon emission computed tomography (SPECT) data, which is a molecular imaging marker of dopamine transporter (DAT).

2.0 MATERIALS AND METHODS

2.1 Standard Protocol Approvals, Registrations, and Patient Consents

Clinical characteristics, imaging and CSF data was downloaded from the Parkinson's Progression Markers Initiative (PPMI) website on 15th of July 2016. PPMI is a five-year observational, international, multi-centre study designed to provide insight into disease aetiology by identifying PD progression biomarkers (<http://www.ppmi-info.org>). This study is registered with ClinicalTrials.gov, number NCT01141023. Institutional review boards approved the study and written informed consent was obtained from all participants. The present study was written according to the STROBE guidelines

We screened 189 people registered as healthy subjects in the PPMI database. We included healthy subjects older than 30 years, with no significant neurological dysfunction and no first-degree family member with PD. All participants had a Montreal Cognitive Assessment (MoCA) score greater than 26 and Geriatric Depression Scale (GDS) score less than 5.

EDS was defined according to the ESS. This widely used, validated, self-reported and self-completed instrument consists of 8 items, rating from 0: normal to 3: severe, with a maximum score of 24; a higher score corresponding to a higher degree of EDS[18]. Consistent with other studies, an ESS score of 10 or higher was set as a cut-off for EDS.

We identified 21 people with an ESS score of 10 or greater (≥ 10), satisfying the criteria for subjective EDS [18]. Using propensity scores [21], people with EDS were matched 1:1 for

age, gender, years of education and Rapid eye movement (REM) sleep behaviour disorder (RBD) Questionnaire scores with healthy subjects without EDS (Table 1). All 42 subjects studied here were non-demented, non-depressed and had no other neurological or psychiatric comorbidities. Depressive features and anxiety were assessed with the short version of the Geriatric Depression Scale (GDS) [15-item][19] and the State Trait Anxiety Total Score (STAI) [20]. Global cognitive status was measured using MoCA [21]. The following cognitive assessments were also performed: Benton Judgement of Line Orientation (BJLO) [22], Hopkin's Learning Verbal Test-revised (HVLRT)[23], Semantic Fluency Test (SFT) [24] and Symbol Digit Modalities Test (SDMT) [25]. Autonomic dysfunction was assessed with Scale for Outcomes for Autonomic Function (SCOPA-AUT) [26]. Olfactory dysfunction was measured by the University of Pennsylvania Smell Identification Test (UPSIT)). All 42 subjects enrolled in this study were non-demented, non-depressed and had no other neurological or psychiatric comorbidities.

Table 1. Demographic characteristics of healthy subjects with and without excessive daytime sleepiness.

| | HS without EDS (n=21) | HS with EDS (n=21) | <i>P</i> value* |
|--|--------------------------|-----------------------|-----------------|
| Age at screening ¹ (mean \pm SD) | 53.86 \pm 9.11 | 53.94 \pm 11.71 | 0.980 |
| Gender male ² , % (n) | 81.0% (17) | 74.4% (15) | 0.474 |
| Year of Education ² (mean \pm SD) | 15.05 \pm 2.54 | 14.48 \pm 2.77 | 0.279 |
| RBD Score ² (mean \pm SD) | 4.29 \pm 3.27 | 4.38 \pm 2.46 | 0.721 |

*All *P* values are uncorrected (¹*t* test and ²Mann-Whitney *U* tests).

RBD: Rapid eye movement sleep behaviour disorder

2.2 Dopaminergic imaging

SPECT images were obtained 4 ± 0.5 h after administrating an injection with approximately 185 MBq [^{123}I]FP-CIT, with a total scan duration of 30-45 minutes. Raw SPECT data was acquired into a 128×128 matrix stepping each 3° for a total of 120 (or 4° for a total of 90) projections in a window centred on $159 \pm 10\%$ KeV. Raw SPECT data for all subjects was transferred back to the Institute for Neurodegenerative Disorders Core Imaging Lab for standardised reconstruction, attenuation correction and quantification. Briefly, reconstruction was carried out using an iterative OSEM algorithm implemented on a Hermes workstation and subsequent processing was performed using PMOD (PMOD Technologies). A Chang 0 attenuation correction was applied using a site-specific mu empirically determined by the anthropomorphic brain phantom acquired at each site. A standard Gaussian 3D 6.0mm filter was applied to each image volume, followed by normalisation to standard MNI space. All SPECT images were visually interpreted by two experienced, independent readers who were blinded to the subject's clinical diagnosis.

2.3 Region of Interest analysis

For this study, normalised SPECT images were loaded together with a single subject MRI template in Montreal Neurological Institute (MNI) space using Analyze 11.0 software (Mayo Foundation). [^{123}I]FP-CIT SBRs in the thalamus, striatum, substantia nigra, globus pallidus, raphe nuclei, and locus coeruleus was calculated using a region of interest (ROI) analysis, with the occipital cortex serving as the reference region. ROIs were manually delineated on the MRI template using Analyze 11.0, before being applied on the SPECT images. ROIs were then manually adjusted on the SPECT images to compensate for individual variations, without altering the size or shape of the template.

The thalamus, caudate, putamen, globus pallidus and substantia nigra, were manually delineated using a reliable, repeatable and robust technique[27]. The raphe complex was

drawn according to the anatomical location of the rostral and caudal raphe nuclei, as depicted by Hornung [28]. Regional estimates of Specific Brain Regions were calculated as the ratio of the mean ROI binding divided by the occipital cortex. This measure approximates the non-displaceable binding potential (BP_{ND}), when the radioligand is in equilibrium at the target site and has previously been reported with Ioflupane SPECT[29].

2.4 Statistical methods

Statistical analysis and graph illustration were performed with SPSS (version 20 Chicago, Illinois, USA) and GraphPad Prism (version 6.0c), respectively. For all variables, variance homogeneity and Gaussianity was tested with Kolmogorov-Smirnov test. Parametric (t -test) and non-parametric (Mann-Whitney U) test were used for between-group comparisons, as appropriate. Categorical variables were compared using a χ^2 test. Univariate and multivariate linear regression analyses were performed to assess the main effects of clinical and imaging variables between healthy subjects with and without EDS, controlling for age and gender. Sensitivity analysis was performed to evaluate the effect of gender on clinical and imaging variables. All data are presented as mean \pm SD, and the level α was set for all comparisons at $P < 0.05$.

3.0 RESULTS

In this study, we performed a case-control study in 21 people with EDS versus 21 healthy subjects without EDS, comparing semi-quantified [123 I]FP-CIT SPECT, neurobehavioural, neuropsychological, cognitive and autonomic features amongst the groups. There were no significant differences in neuropsychiatric, autonomic and cognitive assessments between the group of healthy subjects with and without EDS (all $P > 0.10$; Table 2).

Table 2. Clinical characteristics of healthy subjects with and without excessive daytime sleepiness.

| | HS without EDS (n=21) | HS with EDS (n=21) | <i>P</i> value |
|---|--------------------------|-----------------------|----------------|
| SCOPA-AUT ² (mean \pm SD) | 5.81 \pm 4.93 | 5.80 \pm 3.40 | 0.703 |
| GDS ² (mean \pm SD) | 1.81 \pm 2.14 | 0.81 \pm 0.87 | 0.205 |
| STAI Total Score ² (mean \pm SD) | 63.76 \pm 23.13 | 56.95 \pm 10.42 | 0.330 |
| MoCA ² (mean \pm SD) | 28.52 \pm 1.12 | 28.48 \pm 1.17 | 0.897 |
| BJLO ² (mean \pm SD) | 12.76 \pm 1.87 | 13.29 \pm 2.26 | 0.146 |
| HVLT Total ¹ (mean \pm SD) | 36.81 \pm 5.34 | 39.81 \pm 5.61 | 0.045 |
| SFT Score ¹ (mean \pm SD) | 51.14 \pm 12.16 | 55.19 \pm 12.59 | 0.871 |
| SDMT Score ¹ (mean \pm SD) | 50.14 \pm 12.31 | 50.00 \pm 11.61 | 0.950 |
| UPSIT ² (mean \pm SD) | 33.81 \pm 5.01 | 33.52 \pm 4.65 | 0.930 |

SCOPA-AUT: the scale for outcomes for PD-autonomic function; GDS: 15-item Geriatric Depression Scale, MoCA: Montreal Cognitive Assessment Scale; BJLO: Benton Judgement of Line Orientation; HVLT: Hopkins Verbal learning Test – revised; STAI: state and trait anxiety scale.

*All *P* values are uncorrected (¹*t* test and ²Mann-Whitney *U* test).

Thalamic [¹²³I]FP-CIT specific binding ratios (SBRs) was significantly increased in people with EDS compared to healthy subjects without EDS (1.78 \pm 0.21 vs 1.64 \pm 0.18, *P*=0.026; Table 3, Figure 1). Linear regression analyses revealed that increased thalamic [¹²³I]FP-CIT SBRs correlated with worse EDS scores (adjusted r^2 =0.128, *P*=0.012), which remained significant after adjusting for age (adjusted r^2 =0.106, *P*=0.013) and gender (adjusted r^2 =0.115, *P*=0.014) in people with EDS (Figure 1). No other associations were found between clinical and imaging data and EDS at the univariate level (all *P*>0.10).

A

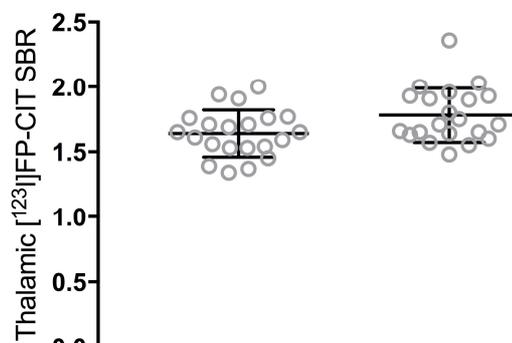


Figure 1 Comparison between people with EDS and those without EDS (A) Bar column graph showing that people with EDS have significantly higher thalamus [^{123}I]FP-CIT specific binding ratios (SBRs) than healthy subjects ($P=0.026$) **(B)** Thalamus [^{123}I]FP-CIT SBRs were positively correlated with increased EDS using linear, logarithmic and inverse models (adjusted $r^2=0.128$, $P=0.012$) in our cohort of healthy subjects.

We then assessed DAT uptake values in other brain areas with known rich DAT density, including the striatum, substantia nigra and globus pallidus, but found no significant differences in [^{123}I]FP-CIT uptake between people with and without EDS ($P>0.10$). [^{123}I]FP-CIT uptake in raphe nucleus and locus coeruleus has shown to provide indirect measures of serotonergic (serotonin transporter) and noradrenergic (noradrenergic transporter) system, respectively [29, 30]. We found no significant differences in raphe nucleus and locus coeruleus [^{123}I]FP-CIT SBRs between healthy subjects with and without EDS ($P>0.10$; Table 3).

Table 3. [¹²³I]FP-CIT imaging of healthy subjects with and without excessive daytime sleepiness.

| | HS without EDS (n=21) | HS with EDS (n=21) | <i>P</i> Value* | % change |
|--|-----------------------------|-----------------------|-----------------|----------|
| Striatum ¹ (mean ±SD) | 3.49 ±0.58 | 3.71 ±0.66 | 0.270 | +6.30 |
| Globus Pallidus ¹ (mean ±SD) | 3.34 ±0.52 | 3.58 ±0.58 | 0.178 | +7.19 |
| Substantia nigra ² (mean ±SD) | 1.41 ± 0.25 | 1.40 ± 0.26 | 0.855 | -0.71 |
| Thalamus ¹ (mean ±SD) | 1.64 ±0.18 | 1.78 ±0.21 | 0.026 | +8.54 |
| Rostral raphe nucleus ¹ | 1.40 ±0.17 | 1.35 ±0.18 | 0.321 | -3.57 |
| Caudal raphe nucleus ¹ | 1.15 ±0.19 | 1.12 ±0.28 | 0.604 | -2.61 |
| Locus coeruleus ¹ | 1.27 ±0.23 | 1.25 ±0.11 | 0.737 | -1.57 |

**P* values are uncorrected (¹*t* test and ²Mann-Whitney *U* tests.)

We also explored the effect of gender on clinical and imaging variables within the whole cohort and found no significant differences in DAT uptake in any of the regions of interest, as well as clinical variables, between males and females.

4.0 DISCUSSION

Our findings demonstrate increased thalamic DAT uptake in people with EDS, which further correlates with the severity of EDS, suggesting that increased dopaminergic function may be associated with EDS in humans. By using non-invasive SPECT molecular imaging we found no other changes related to DAT in DAT-rich areas such as the striatum, substantia nigra or globus pallidus. [¹²³I]FP-CIT SPECT of raphe nucleus and locus coeruleus provides indirect measures for the serotonin and noradrenergic transporters, respectively [31], however no uptake differences were found between people with EDS and healthy subjects without EDS. Moreover, no other associations were found between imaging and clinical data related to

neuropsychiatric, cognitive and autonomic function, suggesting that thalamic dopaminergic function may contribute to EDS development in humans.

There is growing evidence that dopamine modulates wakefulness, exerting a wake-promoting action. Studies have demonstrated that drugs that enhance dopaminergic signalling, through DAT blockade or inducing dopamine release, increase wakefulness in humans [32]. In fact, antipsychotic drugs that block dopamine D₂ receptors induce sedation in humans [33] and reduce wakefulness in rats [34]. Volkow, Wang [15] discovered a reduction in D₂/D₃ receptor binding within the thalamus and striatum after sleep deprivation, with the magnitude of this reduction correlating with sleepiness and fatigue. These findings indicate an association between increased dopamine release and sleep deprivation. Although reductions in DAT density are suggestive of loss of dopaminergic terminals, evidence has also suggested that an upregulation of DAT could be indicative of reduced synaptic dopamine levels. Radioligands including [¹²³I]FP-CIT have been reported to compete with endogenous dopamine, hence leading to an overestimation of DAT availability. Therefore, increased DAT could indicate a reduction of extracellular dopamine level in individuals with EDS [35]. Sossi, de la Fuente-Fernandez [36] reported that greater DAT levels were directly associated with lower dopamine turnover and lower changes in synaptic dopamine concentration. Thus, an increased uptake of [¹²³I]FP-CIT in the thalamus could be indicative of thalamic dopaminergic dysfunction in people with EDS.

Studies have also demonstrated, however, that DAT has direct implication in sleep and wakefulness. Qu, Xu [37] demonstrated the wake-promoting effect of GBR12909, a dopamine transporter inhibitor, in a dose-dependent manner. Therefore, if a reduction or inhibition of DAT causes wakefulness, we could assume that an increase in DAT may induce sleepiness.

The cellular basis underlying sleep regulation is complex, with much remaining poorly understood. Though dopamine has been implicated in regulating the sleep-wake cycle, the underlying dopamine pathway responsible for this remains unknown. Gonzalez, Moreno-Delgado [38] identified a potential mechanism for how dopamine can regulate the sleep/wake cycle via a circadian-controlled receptor heteromer. They described how the production of serotonin and melatonin by the pineal gland is modulated by a circadian-related heteromerization of adrenergic and dopaminergic D₄ receptors, through which dopamine inhibits adrenergic receptor signalling, blocking the synthesis of melatonin[38].

Furthermore, dopamine-containing neurons primarily arising in the VTA and substantia nigra *pars compacta* are recognised to innervate areas involved in sleep/wake regulation including the serotonergic cells of the dorsal raphe nucleus and medial raphe nucleus, the orexinergic arousal system of the lateral hypothalamus, the noradrenergic cells of the locus coeruleus, the histaminergic neurons of the posterior hypothalamus and the cholinergic cells of the basal forebrain[39].

DAT content of dopaminergic axons has been found to vary across the thalamus, suggesting that dopaminergic signalling is more temporally and spatially restricted in particular regions of the thalamus. Therefore, an overall increase in DAT binding may actually be an indication of alterations of dopamine in specific thalamic nuclei, potentially differing from those inducing arousal.

Behavioural arousal is impaired in dopamine D₁ receptor knockout mice, whereas systematic administration of selective dopamine D₁ agonists increases wakefulness and reduces slow wave sleep and REM sleep. Furthermore, administration of dopamine D₁ antagonist leads to sedation and reduces wakefulness, whereas slow wave sleep and REM sleep are augmented. Similar effects are observed when D₂ receptors are targeted [40]. Notably, Kimmel, Joyce

[41] reported that D₂ receptor agonists decreased the half-life of DAT, and D₂ receptor antagonist increased the half-life of DAT, showing that dopamine receptors influence DAT kinetics. These findings together not only demonstrate the unpredictable nature that varying dopamine concentration has on the sleep/wake cycle, but also highlight that dopamine transporters may play an imperative role in sleep regulation.

Further, EDS has been found to be exacerbated by dopamine agonists in Parkinson's disease patients and those suffering from restless legs syndrome. Specifically, Pramipexole, a dopamine agonist which is documented to increase sleepiness, exerts a potent agonistic effect on the D₂ receptor family with preferential affinity towards D₃ receptors [42]. The thalamus expresses a heterogeneous distribution of D₂ and D₃ receptors, as demonstrated by Rieck and colleagues *in vivo* by using positron emission tomography with radioligands [¹²³I]Epideopride and [¹⁸F]fallypride [43]. It could, therefore, be possible that D₂/D₃ agonists' mechanism of action is within the thalamus, causing sleepiness by reducing cAMP levels, thus, the global thalamic output to the cortex. However, the development of EDS in Parkinson's disease may be a more complex phenomenon that might involve a profound loss of D₃ receptors, independent of the treatment use [44].

It is important to consider that the subjective nature of the ESS means that it can often be misjudged as fatigue or lassitude, thus not only presenting a diagnostic challenge, but it also hinders the ability to determine the physiology of daytime sleepiness. Patients and clinicians often use other terms such as 'fatigue' or 'tiredness' interchangeably, thus leading to semantic confusion. However, despite some overlap, EDS and fatigue have different features [45]. It would, therefore, be both useful and informative if we had additional comparisons with the Multiple Sleep Latency Test (MSLT). This test is considered the gold-standard for objective assessment of sleepiness by the American Academy of Sleep Medicine [46], with advantages including measuring the functional consequences of sleepiness at two hour

intervals across the waking portion of the day and being scientifically validated to effectively detect varying degrees of sleepiness. Thus, this additional data could provide additional insight into the pathophysiology of sleepiness. It is notable, however, that in light of recent observation, the robust status of MSLT diagnosing EDS and narcolepsy has been questioned. Johns [47] reported that the MSLT was the least accurate measure of daytime sleepiness compared to ESS and Maintenance of Wakefulness Test (MWT), with ESS posing as the most accurate assessment of EDS. Further, Goldbart and colleagues concluded that the MSLT was had a large number of false positive results [48].

It is also imperative to recognise that EDS has a multifactorial aetiology. Although our cohort did not report any sleep-specific comorbidities and were matched for RBDQ scores, it does not exclude the possibility of these subjects experiencing an unreported underlying sleep disorder such as sleep apnoea or restless legs syndrome, as well as mood disorders or side effects of medication. Therefore, although increased DAT uptake in the thalamus appears to be associated with EDS, it may be causally associated with other potential sleep disorders, which are inevitably reported to cause daytime sleepiness. Furthermore, Koch and colleagues demonstrated gender-specific differences in DAT uptake within the thalamus. Specifically, females were reported to have 31% higher DAT uptake within the thalamus compared to males [49]. Although the EDS cohort only encompassed an additional two females compared to the cohort without EDS, we took this notion into consideration and explored the potential effect of gender on DAT uptake. We did not find an association between gender and DAT uptake in any of the regions of interest, as well as gender having no effect on the association between thalamic DAT uptake and daytime sleepiness.

Furthermore, the complexity of EDS means that neither the sleep-state nor wakefulness can solely be explained by the action of a single site, but is presumed to arise as a result of the intimate interplay between many regions and distributed systems, including the orexinergic

neurons in the hypothalamus [50], cholinergic neurons in the basal forebrain and serotonergic neurons in the raphe [51]. It would, therefore, be interesting to determine how the dopaminergic system interacts with non-dopaminergic systems to induce EDS. [¹²³I]FP-CIT is not specific for serotonin or noradrenaline, hence may need future Positron Emission Tomography (PET) scan to elucidate our results further. It is also possible that people with EDS share a common increase in thalamic [¹²³I]FP-CIT binding, but their underlying EDS aetiology may vary substantially. Sleep is a structured process, with the sleep-wake cycle being governed by a complex, multilevel neuronal system in the brain stem, thalamus, hypothalamus and basal forebrain [52]. A disruption in any of these regions could potentially cause EDS, which could vary from individual to individual. Additionally, we included subjects with a pathological increase of ESS score. Though the ESS is widely used and recommended for screening for EDS, the subjective nature of the instrument inevitably introduces bias according to the subject's motivation, fatigue or recall.

Understanding the pathophysiology of EDS, including determining which systems are involved in this specific phenomenon, will not only heighten our understanding of sleep, but will encourage the development of therapies, with reduced side-effects, to tackle this symptom in the general public, as well as multicentric diseases such as PD. A variety of sleep-waking agents, including modafinil and methylphenidate are recognised to induce wakefulness via the dopaminergic system. Therefore, increasing our knowledge surrounding the mechanisms underlying sleepiness will allow selectivity for these effects, as well as tailoring these treatments based on the patient's neurophysiological alterations. Dopaminergic function, specifically DAT, should be further investigated to confirm its role in regulating the sleep-wake cycle.

ACCEPTED MANUSCRIPT

REFERENCES

- [1] Medicine AAoS. International Classification of Sleep Disorders, Diagnostic and Coding Manual. 2nd ed. Westchester, I11, USA2005.

- [2] Haavisto ML, Porkka-Heiskanen T, Hublin C, Harma M, Mutanen P, Muller K, et al. Sleep restriction for the duration of a work week impairs multitasking performance. *Journal of sleep research*. 2010;19:444-54.
- [3] Pagel JF. Excessive daytime sleepiness. *American family physician*. 2009;79:391-6.
- [4] Slater G, Steier J. Excessive daytime sleepiness in sleep disorders. *Journal of thoracic disease*. 2012;4:608-16.
- [5] Swanson LM, Arnedt JT, Rosekind MR, Belenky G, Balkin TJ, Drake C. Sleep disorders and work performance: findings from the 2008 National Sleep Foundation Sleep in America poll. *Journal of sleep research*. 2011;20:487-94.
- [6] Bixler EO, Vgontzas AN, Lin HM, Calhoun SL, Vela-Bueno A, Kales A. Excessive daytime sleepiness in a general population sample: the role of sleep apnea, age, obesity, diabetes, and depression. *The Journal of clinical endocrinology and metabolism*. 2005;90:4510-5.
- [7] Van Dongen HP, Maislin G, Mullington JM, Dinges DF. The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep*. 2003;26:117-26.
- [8] Simuni T, Caspell-Garcia C, Coffey C, Chahine LM, Lasch S, Oertel WH, et al. Correlates of excessive daytime sleepiness in de novo Parkinson's disease: A case control study. *Movement disorders : official journal of the Movement Disorder Society*. 2015;30:1371-81.
- [9] Steriade M, McCormick DA, Sejnowski TJ. Thalamocortical oscillations in the sleeping and aroused brain. *Science*. 1993;262:679-85.
- [10] Wisor JP, Nishino S, Sora I, Uhl GH, Mignot E, Edgar DM. Dopaminergic role in stimulant-induced wakefulness. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2001;21:1787-94.
- [11] Lena I, Parrot S, Deschaux O, Muffat-Joly S, Sauvinet V, Renaud B, et al. Variations in extracellular levels of dopamine, noradrenaline, glutamate, and aspartate across the sleep--wake

cycle in the medial prefrontal cortex and nucleus accumbens of freely moving rats. *Journal of neuroscience research*. 2005;81:891-9.

[12] Sanchez-Gonzalez MA, Garcia-Cabezas MA, Rico B, Cavada C. The primate thalamus is a key target for brain dopamine. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2005;25:6076-83.

[13] Lu J, Zhou TC, Saper CB. Identification of wake-active dopaminergic neurons in the ventral periaqueductal gray matter. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2006;26:193-202.

[14] Happe S, Baier PC, Helmschmied K, Meller J, Tatsch K, Paulus W. Association of daytime sleepiness with nigrostriatal dopaminergic degeneration in early Parkinson's disease. *J Neurol*. 2007;254:1037-43.

[15] Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Wong C, et al. Sleep deprivation decreases binding of [¹¹C]raclopride to dopamine D2/D3 receptors in the human brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2008;28:8454-61.

[16] Benarroch EE, Schmeichel AM, Dugger BN, Sandroni P, Parisi JE, Low PA. Dopamine cell loss in the periaqueductal gray in multiple system atrophy and Lewy body dementia. *Neurology*. 2009;73:106-12.

[17] Moreno-Lopez C, Santamaria J, Salamero M, Del Sorbo F, Albanese A, Pellecchia MT, et al. Excessive daytime sleepiness in multiple system atrophy (SLEEMSA study). *Archives of neurology*. 2011;68:223-30.

[18] Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep*. 1991;14:540-5.

[19] Weintraub D, Oehlberg KA, Katz IR, Stern MB. Test characteristics of the 15-item geriatric depression scale and Hamilton depression rating scale in Parkinson disease. *The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry*. 2006;14:169-75.

- [20] Kendall PC, Finch AJ, Jr., Auerbach SM, Hooke JF, Mikulka PJ. The State-Trait Anxiety Inventory: a systematic evaluation. *Journal of consulting and clinical psychology*. 1976;44:406-12.
- [21] Nasreddine ZS, Phillips NA, Bedirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *Journal of the American Geriatrics Society*. 2005;53:695-9.
- [22] Benton AL, Varney NR, Hamsher KD. Visuospatial judgment. A clinical test. *Archives of neurology*. 1978;35:364-7.
- [23] Brandt J. The Hopkins Verbal Learning Test: Development of a new memory test with six equivalent forms. *The Clinical neuropsychologist*. 1991;5:125-42.
- [24] Troyer AK. Normative data for clustering and switching on verbal fluency tasks. *Journal of clinical and experimental neuropsychology*. 2000;22:370-8.
- [25] Smith A. Symbol Digits Modalities Test. Western Psychological Services, Los Angeles 1982.
- [26] Rodriguez-Blazquez C, Forjaz MJ, Frades-Payo B, de Pedro-Cuesta J, Martinez-Martin P, Longitudinal Parkinson's Disease Patient Study ELdPcEdPG. Independent validation of the scales for outcomes in Parkinson's disease-autonomic (SCOPA-AUT). *European journal of neurology*. 2010;17:194-201.
- [27] Tziortzi AC, Searle GE, Tzimopoulou S, Salinas C, Beaver JD, Jenkinson M, et al. Imaging dopamine receptors in humans with [11C]-(+)-PHNO: dissection of D3 signal and anatomy. *NeuroImage*. 2011;54:264-77.
- [28] Hornung JP. The human raphe nuclei and the serotonergic system. *Journal of chemical neuroanatomy*. 2003;26:331-43.
- [29] Qamhawi Z, Towey D, Shah B, Pagano G, Seibyl J, Marek K, et al. Clinical correlates of raphe serotonergic dysfunction in early Parkinson's disease. *Brain : a journal of neurology*. 2015;138:2964-73.

- [30] Isaias IU, Marotta G, Pezzoli G, Sabri O, Schwarz J, Crenna P, et al. Enhanced catecholamine transporter binding in the locus coeruleus of patients with early Parkinson disease. *BMC neurology*. 2011;11:88.
- [31] Politis M, Loane C. Serotonergic dysfunction in Parkinson's disease and its relevance to disability. *TheScientificWorldJournal*. 2011;11:1726-34.
- [32] Killgore WD, Rupp TL, Grugle NL, Reichardt RM, Lipizzi EL, Balkin TJ. Effects of dextroamphetamine, caffeine and modafinil on psychomotor vigilance test performance after 44 h of continuous wakefulness. *Journal of sleep research*. 2008;17:309-21.
- [33] RJ B. *Drugs and the treatment of psychiatric disorders*. New York: Pergamon Press; 1990.
- [34] Ongini E, Bonizzoni E, Ferri N, Milani S, Trampus M. Differential effects of dopamine D-1 and D-2 receptor antagonist antipsychotics on sleep-wake patterns in the rat. *The Journal of pharmacology and experimental therapeutics*. 1993;266:726-31.
- [35] Nikolaus S, Beu M, Hautzel H, Silva AM, Antke C, Wirrwar A, et al. Effects of L-DOPA on striatal iodine-123-FP-CIT binding and behavioral parameters in the rat. *Nucl Med Commun*. 2013;34:1223-32.
- [36] Sossi V, de la Fuente-Fernandez R, Schulzer M, Troiano AR, Ruth TJ, Stoessl AJ. Dopamine transporter relation to dopamine turnover in Parkinson's disease: a positron emission tomography study. *Ann Neurol*. 2007;62:468-74.
- [37] Qu WM, Xu XH, Yan MM, Wang YQ, Urade Y, Huang ZL. Essential role of dopamine D2 receptor in the maintenance of wakefulness, but not in homeostatic regulation of sleep, in mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2010;30:4382-9.
- [38] Gonzalez S, Moreno-Delgado D, Moreno E, Perez-Capote K, Franco R, Mallol J, et al. Circadian-related heteromerization of adrenergic and dopamine D(4) receptors modulates melatonin synthesis and release in the pineal gland. *PLoS Biol*. 2012;10:e1001347.
- [39] Steriade M. Synchronized activities of coupled oscillators in the cerebral cortex and thalamus at different levels of vigilance. *Cerebral cortex*. 1997;7:583-604.

- [40] Monti JM, Monti D. The involvement of dopamine in the modulation of sleep and waking. *Sleep medicine reviews*. 2007;11:113-33.
- [41] Kimmel HL, Joyce AR, Carroll FI, Kuhar MJ. Dopamine D1 and D2 receptors influence dopamine transporter synthesis and degradation in the rat. *The Journal of pharmacology and experimental therapeutics*. 2001;298:129-40.
- [42] Newman-Tancredi A, Cussac D, Quentric Y, Touzard M, Verrielle L, Carpentier N, et al. Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. III. Agonist and antagonist properties at serotonin, 5-HT(1) and 5-HT(2), receptor subtypes. *The Journal of pharmacology and experimental therapeutics*. 2002;303:815-22.
- [43] Rieck RW, Ansari MS, Whetsell WO, Jr., Deutch AY, Kessler RM. Distribution of dopamine D2-like receptors in the human thalamus: autoradiographic and PET studies. *Neuropsychopharmacology*. 2004;29:362-72.
- [44] Pagano G, Molloy S, Bain PG, Rabiner EA, Chaudhuri KR, Brooks DJ, et al. Sleep problems and hypothalamic dopamine D3 receptor availability in Parkinson disease. *Neurology*. 2016;87:2451-6.
- [45] Lebowitz SM, Brooks SN, Black JE. Excessive daytime sleepiness: considerations for the psychiatrist. *Psychiatr Clin North Am*. 2006;29:921-45; abstract viii.
- [46] Littner MR, Kushida C, Wise M, Davila DG, Morgenthaler T, Lee-Chiong T, et al. Practice parameters for clinical use of the multiple sleep latency test and the maintenance of wakefulness test. *Sleep*. 2005;28:113-21.
- [47] Johns MW. Sensitivity and specificity of the multiple sleep latency test (MSLT), the maintenance of wakefulness test and the epworth sleepiness scale: failure of the MSLT as a gold standard. *Journal of sleep research*. 2000;9:5-11.
- [48] Goldbart A, Peppard P, Finn L, Ruoff CM, Barnet J, Young T, et al. Narcolepsy and predictors of positive MSLTs in the Wisconsin Sleep Cohort. *Sleep*. 2014;37:1043-51.

- [49] Koch W, Unterrainer M, Xiong G, Bartenstein P, Diemling M, Varrone A, et al. Extrastriatal binding of [(1)(2)(3)]FP-CIT in the thalamus and pons: gender and age dependencies assessed in a European multicentre database of healthy controls. *Eur J Nucl Med Mol Imaging*. 2014;41:1938-46.
- [50] Baumann CR, Bassetti CL, Valko PO, Haybaeck J, Keller M, Clark E, et al. Loss of hypocretin (orexin) neurons with traumatic brain injury. *Ann Neurol*. 2009;66:555-9.
- [51] Zeman A, Britton T, Douglas N, Hansen A, Hicks J, Howard R, et al. Narcolepsy and excessive daytime sleepiness. *BMJ*. 2004;329:724-8.
- [52] Pace-Schott EF, Hobson JA. The neurobiology of sleep: genetics, cellular physiology and subcortical networks. *Nature reviews Neuroscience*. 2002;3:591-605.

- People with excessive daytime sleepiness exhibit higher thalamic [^{123}I]FP-CIT uptake
- The severity of sleepiness correlates with increased thalamic [^{123}I]FP-CIT uptake
- Age or gender does not affect this association

ACCEPTED MANUSCRIPT