Deep Brain Stimulation of Brainstem Arousal Circuits: Manipulating Sleep and Wakefulness



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For my family, the reason behind my entire body of work.

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Abstract

Arousal is modulated through the projections of brainstem areas, such as the Pedunculopontine Nucleus (PPN) and the Locus Coeruleus (LC), to a wider network of brain areas. These nuclei deliver activating afferents to cortical regions, while associated pathology and particularly neurodegenerative changes lead to disrupted sleep and diurnal patterns.

We sought to develop a method for surgical neuromodulation of arousal circuits in order to manipulate sleep states in the human, utilising overlaps between current therapeutic indications of deep brain stimulation (DBS) targeting and these crucial sleep-modulating networks for our pilot experiments. We additionally sought to explore the mechanisms that underpin transitions to quiet wakefulness both prior to and after brainstem stimulation, in order to further understand relevant circuit dynamics. Finally, we applied some of the insights from this combined process in an effort to ameliorate sleep and wake regulation pathologies in two patients who had been implanted with a novel device.

This work could help optimise stimulation protocols in disorders of sleep and consciousness, advancing device development and broadening the indications for DBS therapies.

(*175 words)*

Introduction

The present thesis is an account of the main initial experimental milestones along the way to developing an implantable device and associated algorithm(s) that selectively modulates sleep depth and arousal state *in vivo* in the human. Its organization follows the narrative from the start of this interdisciplinary effort, from the characterization of human oscillatory activity in the target brain region (2018) followed by the first sleep modulating stimulation protocol deployed in the same year, to the experiments using a novel device platform and configurable algorithm as a more recent collaboration with an engineering group (2022 and onwards). Therefore, it follows a narrative evolution from the description and understanding of network activity, to the exploration of how manipulation of network dynamics may lead to a desired effect in terms of arousal state.

Prior to discussions of dedicated experimental chapters, I will briefly review both key published scientific work, relevant to the experimental paradigms, as well as cultural content and historical events that might have influenced its original conception and could affect its subsequent reception by the reader. Any cultural and historical aspects are presented as a background of (conscious and unconscious) motivations and concerns, shared by many of those whose work is located at the junction of fundamental biological functions and brain-machine interfaces, since no work exists in sterile isolation from the cultural milieu of the people that created it.

Man and Tool, (Hu)man as Tool and the quest for Post-human transcendence

The current popular imagination has an established fascination with the prospect of neural cybernetics functionally merging with biological systems. Perhaps the most well-known and public instance of this was the cyberpunk revolution, heralded by fiction such as Samuel Delaney's 'Nova' (1968) and later works by William Gibson. These described implantable 'microchips' with the capacity for infinite memory storage and behavioural enhancement. Popular culture therefore started showing signs of engagement with considerably older questions of how technology is incorporated in human experience and if it can modify the beings that engage in its use. In a self-directed evolutionary process from Heidegger's concepts of 'tool-in-hand' (as opposed to the unintegrated, but readily available object at hand, first published in his seminal 'Sein und Zeit' in 1927 (Heidegger, 1927)), technology may be part of man even when the tool is not consciously used in goal-directed behaviour. Seamlessly interwoven in neural tissue, it may be used to add layers to cortical processing and participate in the formation of a novel conscious experience.

Thus far, real-life iterations of these imaginary 'enhancement' technologies involve extremely high costs and have not yet been widely commercialized. They are mainly being experimentally implemented in cases of debilitating conditions, such as quadriplegia secondary to trauma or neurodegeneration (the COMMAND trial, the first feasibility study of a commercially developed, permanent BCI implant was launched in July 2022 ("Synchron completes first-in-human implantation of Stentrode in US," n.d.). Such remarkable advances in the field of brain-computer

interfacing (BCI) involve the development of implants with the capacity of co-registering complex signals from multiple neuronal populations, creating activity maps that can then be decoded and integrated in closed-loop algorithms aimed at restoring function (for instance see (Silversmith et al., 2021)). For some, beyond restoration of function, exists a wish to favourably modify human capabilities. Despite the computational expenses and hardware challenges behind widely available applications, programming of the sensorium as a novel advance in biotechnology was recently placed in the same sentence as smartphone advances by Max Hodak, sparking a variety of public reactions (*see statement at the author's professional webpage* ("Max Hodak - A Science corporation," n.d.) *upon leaving Neuralink).* While a foray into the tenets and a critique of the goals of the transhumanist community is beyond the scope of this thesis, one should note that in addition to (or perhaps as a basis of) lofty ambitions of enhanced emotional and cognitive processing, transcendence of fatigue and its constraints is a proposed step on the way to post-human capabilities (Bostrom, 2003).

Besides the debate of whether its deliberate manipulation is ethical, it can be argued that complex, emotionally conceptualized experience and memory formation depends of the interplay of a multitude of cortical and subcortical brain regions and therefore may be significantly difficult to surgically modify with adequate precision (for an excellent overview see (Rolls, 2015). However, more primal functions may be significantly easier to manipulate through interfacing with key brain regions and besides –their modification may have already been experimentally tried in other species.

While we do not know how comparable our perceptual reality is that to the lizard and the fruitfly, we do know that we share at least one such common behaviour and function: sleep (Greenspan, Tononi, Cirelli, & Shaw, 2001; Mohanty, Wagener, Herrel, & Thaker, 2022). The ubiquitousness of a process driving an alteration between periods of wakefulness and sleep suggests the presence of fundamental circuits, capable of inducing state modulation across species with variable complexity. Regardless of the motivations behind the individual parts of a body of work spanning across decades and a multitude of research groups and individuals, experimental evidence of selective sleep modulation exists across species. A summary of this information and the potential surgically accessible circuits involved will be subsequently presented, including a cautionary note of how the sleep research community was inadvertently involved in historical violations of fundamental rights and moral codes. This is particularly important to remember in the general context (and even the early stages) of any investigational technologies which may be used to 'enhance' behaviour in lax regulatory frameworks.

War is the most notable case where prior ethical constraints can become irrelevant almost overnight. Therefore, it may come as little surprise that suppression of sleep need and maintenance of wakefulness, with simultaneously sustained enhanced performance, was the somewhat successful (albeit short-lived and eventually disastrous) result of one of the most morally questionable wartime state interventions, which dramatically changed European history. During the 3rd Reich, man was used as a military tool, hijacked for the propagation of expansive ideologies: state-mandated consumption of methamphetamine-based preparations

was the way of achieving fast troop and air force deployment, since the stimulants successfully decreased sleep time (see (Snelders & Pieters, 2011) and (Ohler, 2015)).

The documented addiction, increased aggression and other behavioural side-effects of this state-enforced stimulant abuse had a disproportionately destructive impact on both soldiers and the populations they came in contact with. Additionally, they contributed to the strong, long-term association of transhumanism with the atrocities of the 3rd Reich in the worldwide popular imagination. The prolonged misuse of Fritz Hauschild's patent and scientific research (Meyer, 2005) serves as a cautionary tale of how sleep-modulating technology can be egregiously exploited, outside of a framework guided by compassion and clinical need.

Clinical need for a sleep-modifying tool: the burden of Hypersomnia across conditions

There are a number of clinical pathologies and disorder spectra where excessive sleepiness can have severe implications, which I will briefly touch upon here. Central disorders of hypersomnolence are perhaps the most immediate category of conditions in need for safe and reliable sleep modulation. Among these, idiopathic hypersomnia is a common and debilitating disorder of bidirectional interactions with mental health complaints such as low mood (Dauvilliers, Lopez, Ohayon, & Bayard, 2013), that can further impact quality of life and daily functioning. There are currently no FDA-approved treatments for idiopathic hypersomnia, with patients relying on off-label stimulants with variable results and risks (Saini & Rye, 2017). Although considerably rarer, Kleine-Levin syndrome affects a predominantly adolescent

population, where severe hypersomnolence is correlated with behavioural disturbances such as compulsive eating and disinhibition. The mainstay of its management has been stimulant use and, despite the fact that recovery occurs often spontaneously (median disease duration reported as 4 years), in some cases excessive somnolence persists for decades into late adulthood (Arnulf, Zeitzer, File, Farber, & Mignot, 2005).

Further along the clinical spectrum of sleep disorders, patients with narcolepsy suffer from daytime sleep attacks, the consequence of a difficulty in maintaining vigilance states (Schoch et al., 2017) secondary to loss of orexin-secreting neurons in the hypothalamus. Unlike Kleine-Levin, narcolepsy subtypes in adults do not tend to resolve spontaneously as a general rule and require long-term pharmacological treatment (with a spectrum of agents from respectively 'milder' stimulants such as modafinil to the use of prescription amphetamines). Novel drugs such as pitolisant, an inverse histamine receptor agonist, are being developed in an effort to achieve therapeutic benefit with better management of the severe side effect profile of central simulants (Dauvilliers & Barateau, 2017). In 2023, a new single-dose preparation of sodium oxybate (ON-SXB) also gained FDA approval for treatment of excessive daytime sleepiness (EDS) as well as cataplexy attacks in narcolepsy, with a reportedly improved side-effect profile compared to its predecessors (Kushida et al., 2022).

Circadian rhythm disturbance in narcolepsy (stemming from its primary pathology and reinforced in untreated cases by disruption of the diurnal sleep-wake patterns)

also affects other physiological parameters, such as autonomic function (Plazzi et al., 2011), while its metabolic correlates include impaired glucose tolerance and altered inflammatory responses, leading to an additional comorbid burden (Depner, Stothard, & Wright, 2014). Therefore, agents reducing EDS may also increase overall quality of life in these patients.

Outside of primary sleep disorders, circadian rhythm disruptions such as decreased arousal during the day and increases in arousal at night are also seen in disorders of consciousness, such as vegetative and minimally conscious state. The level of impairment reportedly correlates to disease severity (with vegetative patients significantly more impaired than minimally conscious patients (Cruse et al., 2013). In addition, several studies have shown that brain activity patterns associated with drowsiness and sleep (such as slow wave activity) are increasingly present in patients with a disorder of consciousness outside of sleep or sleep-like behaviours (at resting-state, for example (Sitt et al., 2014)).

Dysregulated sleep and decreased arousal is also a common feature of neurodegenerative disorders (reviewed in (Bhat & Chokroverty, 2017)). This may be a secondary symptom due to neuronal loss and disease-related changes in key brain regions regulating sleep and wakefulness, however it may also stem from a common genetic predisposition: single nucleotide polymorphisms in ‘clock genes’ that regulate circadian function are also correlated with an increased risk for Alzheimer’s and Parkinson’s disease (Chen, Peng, Huang, Hu, & Zhang, 2015; Gu et al., 2015). Therefore, one may be able to appreciate that the presence of

sleep/wake disturbances is a complex and multifactorial issue in a variety of neurological pathologies, even in the absence of psychiatric comorbidities. While sleep complaints –both insomnia and hypersomnia- are a well-known feature of mood disorders (Geoffroy et al., 2018), an in-depth discussion about their epidemiology and psychopharmacological management is beyond the scope of this thesis.

As a brief note, in addition to disorders involving daytime drowsiness and prolonged/mistimed sleep, there are high-performance occupations where loss of vigilance and episodes of micro-sleep can have severe consequences, including loss of life. Measures monitoring vigilance in professionals such as non-warfighter pilots therefore remained of sustained interest (e.g. (Samn & Perelli, 1982)), but there is no safe, naturalistic, non-pharmacological way to maintain wakefulness while monitoring its decline in real time. It can be argued that, although invasive interventions in these cases would not be desirable or would require an extremely cautious regulatory approach in a very distant and technologically evolved future, any lessons learnt from clinical populations may be applied in the form of non-surgical neuromodulation (transcranial) in these high-risk populations, under very strict guidelines.

Surgical Sleep - Modifying Tools: Current Feasibility and Existing Need

Given the aforementioned burden of disordered sleep and reduced wakefulness in an array of patient cohorts, one could argue that there is potential for implantable bioelectronics to either supplement or provide alternative therapies to pharmacological interventions, since devices would promise specificity and potential ability to adjust settings on a microsecond scale. Such an argument should only however be considered ready for exploration in human populations if there is enough evidence that there is currently an adequate biotechnological platform to support it, while it also meets an unmet clinical need, or has an improved safety and availability profile compared to other current clinical interventions.

Central stimulant use in hypersomnia (be it a primary or secondary concern) is a complicated issue and its side-effect profile continues to drive the aforementioned, constantly evolving updates in novel agent development. One of the earliest concerns stemming from the clinical use of this drug category is addiction and abuse, the latter associated with their ability to induce euphoria as well as limit appetite (for instance see (Lepakhin, Babayan, Astahova, & Rudenko, 1986; Morton & Stockton, 2000)). Another concern arises from the fact that such agents may have side effects that could be detrimental in the case of associated comorbidity and frailty, especially in the case of hypersomnolence as a feature of advanced neurodegeneration and older age. Although in screened younger populations with primary hypersomnolence disorders the risk/benefit ratio may be attractive (Lavault et al., 2011), the autonomic side effect profile present even in some of the relatively

safer agents may not be tolerated in cases with cardiac risk factors (Binnenmars et al., 2012; Lakhan & Kirchgessner, 2012). Additionally, the risk of hallucinatory experiences and hyperarousal, as well as drug interactions with other psychoactive agents, may make this drug category prohibitively contraindicated in cases of comorbid dementia and altered cognition (Dequardo, 2002; Nieves & Lang, 2002). Therefore, in cases where use of these agents is absolutely not recommended, as well as in severe refractory cases of hypersomnolence despite trials of all available pharmacological measures, a surgical sleep modulatory intervention may be of added treatment benefit.

I herein argue that such a biotechnological advancement is currently feasible (as opposed to a product of the distant future) and –throughout this thesis- I provide evidence supporting this. On a starting theoretical premise (before the experimental evidence is presented), this argument about *in homine* feasibility stems from the fact that implantable neuromodulation devices are available for human use and that there also is a network of surgically accessible brain regions that has been well-proven to be crucial for sleep and wake modulation.

1. Deep Brain Stimulation (DBS): Existing Human Neuromodulation Platforms

The first formal description of a stereotactic frame used for human brain operations was published in 1947 (Spiegel, Wycis, Marks, & Lee, 1947) but was initially used for surgical lesioning in psychiatric inpatients (termed ‘stereoencephalotomy’). The first description of lead implantation with the purpose of electrical stimulation delivery came a year later in 1948 in a patient with severe depression (Pool, 1954).

However, the construct failed mechanically, with the wires breaking weeks after implantation. Ethical concerns surrounding patient consent (also voiced within the medical community during that period) and the advent of psychopharmacology led to only experimental uses of such practices, with ‘psychosurgery’-related investigations of subcortical electrical stimulation being carried out without regulatory approval as clinical treatments (for instance, Heath’s decades of work which were surrounded by a considerable amount of controversy, (O’Neal, Baker, Glenn, Conner, & Sughrue, 2017)).

Perhaps that was one reason behind the considerable delay from the first application of the method and the first Food and Drug Administration (FDA) approval for therapeutic use of deep brain stimulation (DBS) delivered through implantable devices in humans. This came decades later in 1997, following the publication of its efficacy in tremor control compared to thalamic lesions ten years earlier (Benabid, Pollak, Louveau, Henry, & De Rougemont, 1987).

Therefore, we are now in the third decade of non-experimental, clinically approved and widely used implantable human neuromodulation (with its experimental history extending further back in the past). Currently, clinical indications are expanding outside movement disorders in a return to neuropsychiatric diagnoses, including conditions such as obsessive compulsive disorder (Abelson et al., 2005) and anorexia (Wu et al., 2013) in a new attempt at complex behavioural modification. The current implantable devices utilized to achieve this are slightly primitive compared to the microelectrode arrays used in closed-loop BCI protocols, but still

capable of delivering electrical stimuli in subcortical networks with safety and relative precision. Specifically, electrode leads containing one or multiple contacts (made from platinum/iridium) are implanted in the brain parenchyma and connected with an implanted pulse generator (IPG) through subcutaneously tunnelled electrodes.

This is either performed over the course of two different operations (Stage 1 being lead implantation and Stage 2 being their subcutaneous tunnelling and connection) or in a longer single period of general anaesthesia. In the first case, there is a period of time when the leads can be accessible for recording and trials of different stimulation patterns through a detachable 'stimulation box', allowing for both research on human oscillatory activity and trial of settings in novel targets and/or challenging cases. 'Traditional' IPGs feature titanium enclosures of lithium batteries, which are connected to the extension wires and then placed subcutaneously below the clavicle. This battery-powered and voltage-controlled system functions therefore like a sort of 'brain pacemaker'. The IPG sends an electrical pulse to the parenchyma, with programmable parameters being its pulse width, amplitude and frequency.

The capabilities of DBS systems are continuously evolving, in terms of both hardware and software capabilities. There are different iterations of IPGs currently being marketed, such as rechargeable devices (to extend 'traditional' battery life from its starting point of 3-5 years) and novel cranially-mounted case systems (such as the Neuropace responsive neurostimulation (RNS) system (Neuropace, Inc., USA) and the platform described later in this thesis). In addition, material

compatibility with magnetic resonance imaging (MRI) has been another area of improvement, since an estimated 70% of patients will require an MRI within the first decade post-implantation (Falowski, Safriel, Ryan, & Hargens, 2016). A further field of hardware refinement is stimulation precision and timing, where a variety of developments have been made. In terms of the implanted leads, segmentation has allowed for shaping of the electric field in a directional manner relative to the target tract and/or nucleus, also allowing for a more energy-efficient approach since less current is required (Ramirez-Zamora et al., 2020). Further examination relevant to the efficacy of current-controlled stimulation modes has been made by device manufacturers, with one example being the Medtronic Activa PC system (Okun et al., 2012).

The temporal (as opposed to spatial) precision of stimulation delivery can be further controlled by embedded algorithms, with pulses delivered in relation to endogenous oscillatory activity features that serve as disease-related biomarkers. This might be done in a responsive mode, for instance subsequent to the detection of electrophysiological seizure precursors in cortical activity (Morrell, 2011), or following fluctuations of beta power-in-band (Little et al., 2016) or changes in its oscillatory phase (Cagnan et al., 2017) in patient with Parkinson's disease. Further published studies have incorporated cardiac activity monitoring (as a trigger for vagal nerve stimulation) or motion changes captured by accelerometery in their algorithms of stimulation delivery (summarised by (Bouthour et al., 2019) and further by (Krauss et al., 2021)).

In all these cases of closed-loop approaches (embedded algorithms as opposed to stimulation control by an external clinician or experimenter), the target is normalisation of aberrant/disease-related neurophysiology and behaviour, as opposed to changes aimed at altering arousal state. However, it can be argued that the platforms and algorithm types described offer a sufficient basis for the development of a system geared towards the modulation of sleep and/or wakefulness, in the presence of key neuromodulatory nodes that can be accessed surgically.

An additional feature that could further facilitate state-specific manipulations is the capacity of new generation devices to stream local field potentials wirelessly, therefore enabling the examination of long-term validity in the case of arousal-modulating closed-loop stimulation. Through longitudinal data sampling, one might in theory examine the evolution of neurophysiological activity over multiple diurnal and circadian cycles –although (as will be highlighted in the last portion of this thesis) current streaming capacities are short and data quality is still not of high enough quality. In this first introduction to sleep-modulating technological concepts however, a first overview of possible candidate target areas should first be presented.

2. Brief Overview: Surgically Accessible Sleep Regulatory Neuroanatomy

The pharmacological agents currently used as stimulants influence the balance between sleep and wakefulness through interfacing with a system of neurotransmitters, where acetylcholine, monoamines (such as dopamine, norepinephrine and histamine) glutamate and orexin are crucial players (Holst &

Landolt, 2018; Saper & Fuller, 2017). In addition to subcortical structures, neocortical neuronal layer 5 has also recently been implicated in state control, highlighting a level of distributed processing and a high level of complexity in terms of sleep/wake regulation (Krone et al., 2021). However complex this system may be, given the aforementioned technological specifications of implantable devices as well as based on a consideration of ergonomics and efficiency, a surgically implanted system might be effective by utilising key 'nodes' in this regulatory network.

Discrete subcortical nuclei therefore, whose projections to cortex modulate global states, might be a first candidate for sleep/wake modulation via implantable devices. Such a network indeed exists and was initially termed the Ascending Reticular Activation System (ARAS or RAS) by Moruzzi and Magun, who placed its central areas in the pons and midbrain (Moruzzi & Magoun, 1949). Rather than a clear bottom-up regulatory system, the ARAS network consists of a complex interplay between different systems of neurotransmission that exert both upstream and downstream effects, however key regions within it seem to have major arousal-related functions.

The hypothalamus is perhaps one of the most well-known centres of wake regulation, since orexin (hypocretin) peptides (orexin A and B subtypes) are produced in its lateral subdivision. Orexinergic projections regulate the activity of other subcortical regions of the pons and brainstem, as well as influence thalamocortical neuronal activity. This implies that the lateral hypothalamic

orexinergic system is central for arousal, as well as attention and emotion (Sakurai, Mieda, & Tsujino, 2010). Adjacent to the lateral hypothalamus is also another key player in sleep-wake regulation, the suprachiasmatic nucleus (SCN) or so-called 'pacemaker', which is responsible for circadian timing (J. S. Takahashi, Turek, & Moore, 2001), in a process not entirely independent but linked with sleep homeostasis (Achermann & Borbély, 1994). The SCN does not act as an isolated clock but is receiving feedback information through afferent connections with other cortical and subcortical brain regions and modulates its own activity on a diurnal basis (Deboer, Vansteensel, Détári, & Meijer, 2003).

Caudally in relation to these hypothalamic regions, the pedunculopontine nucleus (PPN) of the brainstem and laterodorsal tegmentum send cholinergic projections to cortex through an intra-thalamic and an extrathalamic pathway, the first through thalamic reticular neurons and relay cells and the second through the basal forebrain. This is another cholinergic region with direct activating projections to global cortical areas. Adjacent to the PPN is the locus coeruleus (LC), a major noradrenergic region with wide projections to thalamus, basal forebrain and cortex, as well as modulatory projections within the brainstem to the PPN. It has been claimed that the LC 'releases' PPN-mediated activation, through excitatory projections to the nucleus, while it inhibits GABA-ergic neurons in the ventrolateral preoptic area (Samuels & Szabadi, 2008). In keeping with the theory that this is the 'activating switch' between this pair of nuclei, the LC maintains quiescence during REM sleep (firing prior to its initiation), when the PPN becomes active (for instance see (K. Takahashi, Kayama, Lin, & Sakai, 2010)). Other important monoaminergic

nuclei located in the brainstem are the dorsal raphe nuclei, which are a serotoninergic region, while histamine is released primarily by the tuberomammillary nucleus of the hypothalamus.

Therefore, one can stipulate that by selective modulation of discrete wake-regulating neuronal populations in these subcortical centres it would be feasible to alter arousal state and induce wakefulness. Experiments in rodents have confirmed that ascending brainstem pathways, such as noradrenergic neurons originating from the locus coeruleus or cholinergic afferents stemming from the basal forebrain, can be used to achieve global cortical activation and wakefulness induction (Carter et al., 2010; Ozen Irmak & De Lecea, 2014). Optogenetic modulation however would not be easily feasible in humans for the purpose of planned experiments (viral transfection in humans might also be quite challenging to ethically approve).

Closer to the aim of sleep-modulating DBS in humans, some of the oldest published work in eliciting wake-like responses through electrical stimulation was performed in cats and therefore seemed very encouraging with regards to the feasibility of such a goal. Sleep state was modulated by electrical stimulation of the reticular activating system during slow wave sleep in cats by Benoit and Bloch in 1960 (Benoit & Bloch, 1960). Follow-ups of this work also provided classifications of the thresholding of stimulation parameters in order to elicit an ‘orienting response’ (pupillary dilatation, head turning and seeking behaviour) (Grahnstedt & Ursin, 1980).

Steriade’s classic work on thalamic patterns of activities in cats came a few years later (M. Steriade & Llinas, 1988; Mircea Steriade, McCormick, & Sejnowski, 1993),

showcasing the stage-dependent differences in cortical and subcortical oscillatory patterns (such as the burst-pause firing pattern noted in non-Rapid Eye Movement Sleep (NREM)). It could therefore be argued that the interfacing of electrical stimulation that was feasible in cats and caused state transitions, could be feasible in humans –if only the appropriate circuits were made available for such electrical stimulation. This could be done *de novo* (as in, during a specific trial where surgical planning would be based on arousal modulation) –however a review of the pre-existing literature at the beginning stages of this work revealed that some key hubs of the ARAS had already been accessed surgically, due to the multifaceted functions of these nuclei making them neurosurgical targets for non-arousal related symptom control. Additionally, due to shared circuit overlap between motor and non-motor regions, there was already an existing body of evidence (pre-2018) suggesting that DBS of various subcortical areas could have an effect on sleep.

Sleep-Regulatory Circuits: Overlaps with Existing Deep Brain Stimulation (DBS) Protocols.

The mainstay of DBS treatment is movement disorders, with main targets being regions associated with motor control such as the subthalamic nucleus (STN), the globus pallidus (GPi) and the motor thalamus (ViM). However, these are not the only surgical targets for symptom modulation and therefore some brain areas implanted for current clinical indications for deep brain stimulation (DBS) (including thalamo-cortical, basal ganglia, and brain stem regions) may show a degree of overlap with circuits involved in sleep maintenance and regulation (e.g. (Sharma, Sengupta, Chitnis, & Amara, 2018)). Symptom control may also be a factor influencing sleep

quality, especially in the case of Parkinson's disease where nocturnal freezing might limit the patients' capability to turn in bed and lead to relative disturbance. Additionally, DBS may modulate motor symptoms occurring at night (such as Restless Legs Syndrome in Parkinson's disease) and therefore enhance sleep quality (Chahine, Ahmed, & Sun, 2011).

However, reports of changes in sleep quality are based on studies with small populations, methodological differences and which in some cases have reported no substantial change despite an overall body of evidence pointing towards beneficial effects (review by (Eugster, Bargiotas, Bassetti, & Michael Schuepbach, 2016)).

With regards to primary, non-motor sleep pathology (such as Rapid Eye Movement (REM) sleep behaviour disorder), a more recent update on a review of current literature did not find evidence of any STN-DBS mediated improvements (Zuzuárregui & Ostrem, 2020).

This thesis however is not concerned with sleep modulation related to symptom control, but rather primary effects on sleep related to modulation of arousal-related pathways. Published cases suggestive of a primary effect and/or sleep modulation due to an overlap with arousal regulating pathways were therefore far more intriguing. One such study was the report of DBS-induced sleep disturbances (fragmentation, increased wake after sleep onset) in two patients who were receiving posterior hypothalamic (PH) stimulation for the treatment of cluster headaches, with a similar effect reported in both cases and reproduced over two nights of stimulation (Kovac et al., 2014). A prior study contradicted this and reported

an improvement in sleep quality in patients with PH stimulation, which the authors attributed to better symptomatic control overnight (Vetrugno et al., 2007). Another very exciting report described a series of nine patients where the anterior nucleus thalami was stimulated for intractable seizure control. The authors reported that their investigation was triggered by the patients' report of sleep disturbances post-DBS, while upon review of their polysomnographic evidence they noted an increased number of arousals in this cohort (Voges et al., 2015).

When cases of stimulation in brainstem regions were investigated, it was noted that there has only been one DBS trial of locus coeruleus to date, whose results were published over thirty years ago (1989) while the implantation of the DBS devices took part even earlier (1975-1977). The trial stated an aim of suppressing noradrenaline-mediated hyperactivation in epilepsy (results from two patients were reported) and cerebral palsy (one patient was recruited). The authors noted an encouraging improvement in symptom control with reduction of spasticity. Interestingly, it was reported that all cases experienced overnight sleep fragmentation (that one could argue may be attributed to noradrenaline-mediated hyperactivation instead of its suppression overnight) (Feinstein, Gleason, & Libet, 1989). In this interesting body of evidence however, enhancement of arousal is a by-product/side-effect of treatment in very small sample sizes –not the result of a specific tailored protocol with multiple repetitions over time.

Another brainstem target that has been more prominently featured in both literature and current practice is the aforementioned pedunculopontine nucleus (PPN). This

region is traditionally divided in two neuroanatomically distinct parts (the pars dissipata and the pars compacta), while its rostral region is rich in GABA-ergic neurons and its caudal end features more prominent glutamatergic and cholinergic populations (Mena-Segovia, Micklem, Nair-Roberts, Ungless, & Bolam, 2009), although the neuronal diversity of the region is more complex than this simplistic division (Pienaar, Vernon, & Winn, 2017). Subsequently, one could very broadly speculate that the effects of PPN stimulation could differ depending on which region is being electrically activated, with promotion of GABA-ergic rostral activity enhancing sleep and blockade of rostral activity or activation of the caudal PPN region leading to enhanced wakefulness and/or sleep fragmentation.

A line of relevant evidence in terms of potential PPN-DBS neuromodulation of sleep and wakefulness concerned the reported firing rates of this region. Although these were not measured in humans, there have been reports linking PPN burst activity to REM-sleep related ponto-geniculo-occipital (PGO) waves (M. Steriade, Paré, Datta, Oakson, & Curró Dossi, 1990). Additionally, while choline acetyltransferase (ChAT) negative neurons in the rat PPN were predominantly firing in bursts secondary to low-threshold calcium spikes, there was a population that was ChAT positive and exhibited more tonic properties (A-current), suggesting a complexity in firing rates and properties (Kang & Kitai, 1990; Takakusaki, Shiroyama, Yamamoto, & Kitai, 1996). However, it was subsequently reported that neuronal firing in the PPN plateaus at tonic rates between 20-80Hz for all neuronal populations, when exposed to depolarizing inputs (Simon et al., 2010).

These firing rates, centred close to the 40Hz frequency, are involved in cortical synchronization through influence exerted on cortical interneuronal activity by the intralaminar nucleus thalami, theorized by Llinas to be a basis for modulation of conscious experience (for instance see (Llinas, Grace, & Yarom, 1991; Llinás & Ribary, 2001)). Further highlighting this link were findings of electrical coupling of the PPN both downstream with the subcoeruleus region and upstream with the intralaminar nucleus thalami (both important sleep-regulatory regions), suggesting that this may be a central hub for potential sleep modulation through DBS (Garcia-Rill, Heister, Ye, Charlesworth, & Hayar, 2007).

The PPN is also linked to the regulation of posture and movement, being part of the mesencephalic locomotor region and densely interconnected with the basal ganglia (Mena-Segovia, Bolam, & Magill, 2004). Low frequency stimulation of the PPN (20-25Hz) has been reported to lead to postural and gait improvements in Parkinson's disease (PD) (Plaha & Gill, 2005). In a recent review of all 45 studies investigating the effectiveness of PPN-DBS in motor symptom regulation in PD, efficacy in fall and freezing reduction (but not bradykinesia, tremor or rigidity) was reported (Elias et al., 2021). Additionally, there were studies that reported an improvement in sleep quality (Lim et al., 2009; Stefani et al., 2010). However, it is unclear whether this was stemming from better symptom control (such as freezing reduction) or a primary modulation of PPN activity (such as enhancement of GABA-ergic neurotransmission).

Since it is also important in locomotion and autonomic function, the PPN is being surgically targeted in another Parkinsonian syndrome: Multiple Systems Atrophy (MSA), which is a lethal neurodegenerative condition characterized by debilitating autonomic, postural and gait impairments (Wenning, Tison, Ben Shlomo, Daniel, & Quinn, 1997). Surgical targeting of PPN in MSA is part of two Oxford-based clinical trials, since its stimulation has been reported to affect both motor and autonomic symptoms in synucleinopathies (Garcia-Rill et al., 2019; Hyam et al., 2019). From the perspective of accessing sleep-modulating afferents surgically in order to perform this body of work, this was a particularly important potential pilot population. Specifically, there was a keen interest in gait and autonomic modulation in MSA via PPN stimulation as a primary research goal of the main supervisor of this thesis (Prof Green, whose research interests and expertise lie in these two areas). Collaborative work with the Brain Network Dynamics Unit also explored the role of PPN oscillations in ambulatory-related function in this disease category (He et al., 2021).

Based on what has been summarised so far, despite the available technological platforms and potential candidate nuclei for surgical neuromodulation, there was still a high level of uncertainty with regards to providing justification for a dedicated trial of DBS in sleep disorders. Therefore, it seemed more logical to profit from an existing trial framework and the presence of patients with implants in regions important for sleep regulation, in order to investigate this thesis' aims.

Multiple Systems Atrophy (MSA) and Sleep Disturbance

Since patients with MSA were recruited for this work, the final part of this introduction should therefore briefly mention the links of this condition with specific sleep pathologies. An adult-onset neurodegenerative condition, MSA encompasses a multitude of previously considered independent syndromes (such as Shy-Drager and Sporadic Striatonigral Degeneration), since these can both co-exist and are mutually characterised by oligodendroglial cytoplasmic inclusions which are alpha synuclein immuno-reactive (Graham & Oppenheimer, 1969; Papp, Kahn, & Lantos, 1989). It is a relatively rare disorder, with reported incidence of 0.6 in 100.000, while currently there are no disease-modifying treatment options but therapies are aimed at symptom stabilization with low success rates (Krismer & Wenning, 2017). Symptoms in MSA originate from the degeneration of striatonigral and olivo-pontocerebellar structures, thus explaining its main reported symptoms of Parkinsonism (striatal degeneration, as first described by (Adams, van Bogaert, & van der Eecken, 1961), autonomic dysfunction (pontine degeneration) and ataxia (cerebellar changes, first noted by (Dejerine & Thomas, 1900)). Based on the main features of the disease phenotype, it is classified as MSA-P (Parkinsonian) or MSA-C (Cerebellar) (Jellinger, 2003).

A direct consequence of alpha synuclein aggregation in brainstem regions is the presence of neurodegenerative changes in parts of the RAS. Therefore, it may come as no surprise that MSA is also associated with discrete sleep pathologies as part of its sequelae. The most well-known and widespread of those is REM Sleep

Behaviour disorder (RBD), which consists of a loss of REM muscle atonia and subsequent motor disturbance secondary to dream re-enactment, posing significant risks to patients and bed partners. RBD may precede other disease-related symptoms and pathologies in rates as high as 76%, while seems to be a very prominent feature of MSA (64%-100% of patient cohorts having pathological REM sleep muscle tone) (Cochen De Cock et al., 2011).

RBD is not an exclusive correlate of MSA but is also present in Parkinson's disease, therefore making its study and neurophysiological correlates more attainable in terms of recruitable participants (Iranzo et al., 2005). The hypothesis that these symptoms must result from brainstem arousal circuit degeneration has been confirmed by various imaging studies, showing a higher degree of atrophy in the PPN region and loss of signal in the region of the LC in patients with PD and RBD, as opposed to those with PD alone (Boucetta et al., 2016; Gallea et al., 2017; García-Lorenzo et al., 2013). Insomnia as a feature in MSA is also reported, which could be attributed to both primary neuropathology as well as secondary factors (anxiety at bedtime, sleep fragmentation secondary to freezing in MSA-P, nocturia or presence of periodic limb movements) (Ghorayeb et al., 2002; Iranzo et al., 2004).

Other than the above, another important sleep pathology associated with MSA is the presence of sleep disordered breathing. Although in the case of our goals and hypotheses this may seem of secondary interest, it is worth mentioning in the sense that higher apnoea-hypopnoea indices may also correlate with sleep fragmentation (leading to respiratory-related arousals). Additionally, this pathology progresses to

stridor which is associated with sudden death and fast disease progression (Iranzo et al., 2004), while it can be of an insidious onset –in one study, it was an incidental finding in clinical polysomnography at 13% of presenting cases (Rekik et al., 2018).

Foundations for Human Surgical Sleep Modulation: Aims and Pilot Techniques

The aim of this thesis work was to examine the feasibility of inducing targeted sleep stage changes, enhancing wakefulness through an organised and dedicated protocol which could –if safe and successful- lead to further developments of implantable sleep modulating technologies for human use. In order to attempt this, in addition to personal prior expertise obtained in a specialised environment (as a member of Prof Kostopoulos' sleep research team during and shortly after my MD) and to obtaining new crucial guidance on neurosurgical and trial-related matters during my DPhil supervision, it was crucial to form links and seek advice by senior members of both the Oxford sleep community and the engineering field (as listed in the foreword of this thesis). Through mentorship offered at different stages during this degree, the general project aim was divided into discrete stages of experiments –as will be showcased in the subsequent chapters.

Utilising existing capabilities and patient populations, we therefore set out to apply the hypothesis that brainstem arousal circuit DBS could acutely modulate sleep, in a pilot cohort of MSA patients. This came with the intrinsic limitation that inherent pathology could influence our results to unknown degrees, while the fact that this was a rare patient population could also mean that matching patients with regards to multiple parameters (apart from age, sex and motor impairment) would be

challenging. An initial thought was to recruit a control group of PPN DBS cases with PD; however, this target was no longer routinely used in Oxford and therefore the two populations would not be stimulation naïve at onset and therefore potentially not comparable.

Given the complexity of the condition and the unknowns with regards to human oscillatory physiology in this target, we initially sought to characterize the activity of the PPN region (PPNR) in order to then further identify state-modulated oscillatory components (this will be explored in the first experimental chapter). However, our hypothesis was that, since prior evidence in various species reported a range of intrinsic activity patterns in the beta/low gamma range, PPNR DBS in associated frequencies would result in change of sleep stage (this will be presented in the second experimental chapter).

The fact that we had to combine these two approaches (passive recordings and stimulation trials) during data collection, which was incorporated as an amendment to an ongoing first clinical trial, designed prior to the author's arrival with the aforementioned motor and autonomic primary endpoints, meant that stimulation parameters had to be defined a priori. However, this perhaps added an additional layer of objectivity by setting a clear, hypothesis-driven set of stimulation parameters, as opposed to stimulation frequencies being modified on a patient-by-patient basis and therefore making pooling and further analyses challenging.

While the course of this thesis was being planned, my initial aim was to implement a closed-loop stimulation algorithm based on any significant observations (using an

interface between the externalized leads and an Arduino setup) and compare nights with such trials to continuous tonic stimulation and 'OFF' settings. This would apply to both periods of daytime sleep (undesirable sleep and/or drowsy periods) as well as discrete sleep stages on different days. However (as will be explained further in the third experimental chapter), although such an algorithm was coded and it is fully functional in its use on rodent local field potential (LFP) in vivo recordings, its implementation was rendered impossible by the specifications of a novel device that was utilized in the follow-up trial, at a time when externalised recordings also ceased to be performed in this patient population in Oxford. As a final step we therefore examine the implications of lessons learnt from both stimulation trials and passive recordings in the management of patients recruited in a follow-up clinical study, where device capabilities permit scheduling of stimulation parameters on a diurnal basis. By presenting what was feasible under these circumstances (which nevertheless showed some success and has also resulted in patented intellectual property), we are hoping that this pilot work might eventually become part of the surgical neuromodulation narrative and expand the boundaries of what is currently possible and clinically available.

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Chapter 1

Introduction

In order to develop a responsive deep brain stimulation system that modulates wakefulness, relevant intrinsic properties of cortico-subcortical networks need to first be studied. One of the earliest published attempts at such an understanding was the Baron von Economo's classic work on 'Sleep as a Problem of Localisation', based on the behavioural observations and post-mortem histology of patients with encephalitis lethargica (Von Economo, 1929). Therein lay the first seeds at defining a subcortical 'sleep center' which influences arousal-related behaviours. This led to later work over the course of almost a century and the definition of the 'ascending reticular activating system', which modulates wakefulness and sleep by bottom-up projections to cortex (Edlow et al., 2012). This system, connecting brainstem nuclei (such as the pedunculopontine nucleus and the locus coeruleus) to thalamus, hypothalamus and basal forebrain, projects to cortex through thalamic and extra-thalamic pathways to exert its control on arousal state (as reviewed more extensively in the introduction of this thesis) (M. Steriade, 1996; M. Steriade & Glenn, 1982).

However, even if a subcortical regulatory network has been anatomically defined in human patients, the circuit interactions of its components with cortical activity need to be further elucidated. This may facilitate a naturalistic approach in defining the

stimulation parameters of individual targets, which will utilise windows of excitability and therefore have a higher probability of transition to an awake state.

Sleep and Wakefulness: General principles of activity in cortical regions and brainstem nuclei

Broadly speaking (comparing wake and sleep states in general), cortical activity during higher levels of arousal is dominated by faster rhythms, contrasting with slower oscillatory activity during sleep. Cortical activity during wakefulness is rich in alpha and beta activity (8-30Hz), with gamma oscillations (>30Hz) characterising active wake (Cantero, Atienza, & Salas, 2002; Gross & Gotman, 1999). During deep sleep these are replaced by delta/slow oscillations (Achermann & Borbély, 1997). However, windows of cortical excitability exist even in deepest sleep, embedded in micro-architectural fluctuations of activity that could also arguably serve evolutionarily preserved, arousal or vigilance-promoting roles.

During slow-wave sleep (SWS), the membrane potential of neocortical neurons fluctuates between depolarized (UP) and hyperpolarized (DOWN) states, reflecting the balance between excitation and inhibition. In electroencephalography (EEG) these are seen as large-amplitude, low-frequency (~1 Hz) slow waves (M. Steriade, Nunez, & Amzica, 1993; Timofeev, Grenier, & Steriade, 2001). The emergence of cortical gamma oscillations, which are also associated with learning and information processing, occurs in phase with the UP-state during SWS –leading to this being described as the 'active' component of the slow oscillation (M. Steriade, 2006).

During the depolarised UP-state, both cortical and ascending brainstem circuit activity may cluster together in periods of heightened excitability (and therefore offer a potential for arousal-related manipulations). There is early evidence from rodent data that the activity of medial prefrontal cortex (mPFC), pedunculopontine nucleus (PPN) and locus coeruleus (LC) is grouped around the UP-state of cortical slow oscillatory cycles (Eschenko, Magri, Panzeri, & Sara, 2012; Mena-segovia, Sims, Magill, & Bolam, 2008). The maximum locking of LC spikes occurred relative to the transition from hyper- to depolarised state (slope leading to the UP-state). Thus, norepinephrine (NE)-related activity facilitates the recruitment of more cortical cells into an active (firing) mode. With regards to cholinergic networks, PPN neurons (ChATa) were shown to fire in temporal correlation with the cortical gamma oscillations, nested in the UP-state. These neurons had an overarching oscillatory period of their firing close to 1 Hz, which faded away during periods of absence of slow oscillations in the cortex. It therefore seems that based on evidence from nonhuman data, both norepinephrine and cholinergic activating outputs target the cortex in clusters of temporal precision, creating windows of higher arousal probability.

Whether these observations apply to human brainstem arousal circuit activity and its relationship with cortical excitability windows, based on human experimental data, has yet to be determined. Evidence may be scarce due to the difficulty in obtaining such recordings and determining implantation precision, while human recordings can also only be obtained in the presence of pathology, since implantation is carried out solely for therapeutic needs. Even in the presence of

these constraints, it is extremely crucial to establish the mechanisms of baseline circuit activity during excitable/wake-related and non-excitable/quiescent states, prior to the design and purposeful applications of dedicated stimulation algorithms aimed at state alterations.

Organisational Principles: Examination of Network Activity and Diurnal Electrophysiology

After baseline circuit dynamics are understood, frequency of stimulation and its timing may be tailored accordingly to achieve the desired outcomes, such as enhancing wakefulness (or preserving sleep). In order for such an understanding to be achieved (and to assist with further targeted surgical interventions), both the anatomy of the circuit –in terms of precise lead location- and its neurophysiology need to be examined.

Surgical targeting of brainstem nuclei: caveats of the pedunculopontine region

During lead implantation for this first cohort of four cases, which will be discussed in this chapter, a method based on anatomical landmarks apparent in clinical imaging was used and leads were placed in the PPN region (PPNR) (*further described in the 'Methods' section).* This surgical approach, although personalised –since planning takes place in native patient space, not MNI/normalised space coordinates- however comes with a multitude of caveats. First of all, it is impossible to visualise the boundaries of brainstem arousal nuclei in clinical imaging sequences. Histology remains the singular most reliable method of identification of these nuclei, each with

a few cubic mm in volume, while diffusion data can only be merged with ex vivo histological results post-mortem (Edlow et al., 2012).

Therefore, during this technique of surgical planning, where the only landmarks available are indirectly related to brainstem nuclei, any anatomical variations in grey matter structure (such as those related to patient's age and underlying neurodegenerative pathology) cannot be directly verified. Another source of variability comes from the fact that anterolateral trajectory planning is restricted, because CSF spaces need to be avoided, while regional vascular anatomy, whose perforators mainly originate from the basilar artery, must also be respected (Marinkovic & Gibo, 1993; Tatu, Moulin, Bogousslavsky, & Duvernoy, 1996). Thus, trajectory deviation due to variations in local vasculature might result in diverse electrode location in relation to target brainstem nuclei, if the only strict anatomical boundaries under consideration are those of the PPNR triangle.

Variation in trajectory within published, smaller cohorts has also been declared when other PPNR targeting approaches were used (Arnulf et al., 2010; Zrinzo et al., 2008). In addition to the fact that this will lead to a different composition of cholinergic versus other neuronal populations in the region sampled by each individual lead contact, one must also take into account that the area will contain a number of afferent and efferent fibres related to the nuclei (it will not be exclusively composed of grey matter). This fact is not only important in understanding the effects of electrical stimulation of these targets (which will be examined in the following

chapter), but also in order to plan and understand ‘passive’ electrophysiological signal sampling categorisation within the PPNR.

Acceptability of the implantation margins was assessed between surgical stages by the operating consultant Neurosurgeon (Prof A. G.), so that a revision could take place prior to lead internalisation (and connection with the battery), if necessary. No intraoperative electrophysiology took place (for instance, micro-electrode recordings to guide targeting). Given the aforementioned potential variability however, as well as the fact that contacts might be sampling afferent fibres from adjacent brainstem nuclei within a broader, surgically defined area, subsequent examination of local oscillatory activity was undertaken and also anatomically conceptualised, as will be further discussed in this chapter.

Electrophysiological grouping in the PPNR: Networks and Oscillatory Activity

Evidence of interplay between different neurotransmitter systems of the brainstem suggests that a network modulating slow-wave sleep might transcend regional anatomical boundaries. Activation of the cholinergic nuclei of the brainstem (the PPN and laterodorsal tegmentum (LdT) (Mesulam, Geula, Bothwell, & Hersh, 1989)) leads to activation of the locus coeruleus through muscarinic (m2) receptors (Egan & North, 1985). Furthermore, excitation of the PPN/LdT in frequencies shown to release acetylcholine in the cat (*further discussion of which will follow in the subsequent chapter)* led to changes in firing rates of the nucleus reticularis pontis, suggesting the presence of state-dependent functional connections (Garcia-Rill, Skinner, Miyazato, & Homma, 2001). Therefore, comparisons and grouping across

contact dipoles of leads implanted in the PPNR should transcend anatomical boundaries in order to offer information crucial in identifying regions of common, arousal-related network activity.

One way of furthering our understanding of network activity is examining regional local field potentials for the presence of similar patterns in oscillatory activity. Oscillatory synchrony between spatially distant neurons of similar properties was first identified in cells with a common orientation preference in cat visual cortex, occurring in response to global stimulus properties (Gray, König, Engel, & Singer, 1989). Subsequent studies across regions and species further supported the concept of spatially distributed, functionally organised neuronal networks, whose discrete roles in information processing are reflected in electrophysiological features – such as *when* populations fire and *at which frequency* (Csicsvari, Jamieson, Wise, & Buzsáki, 2003; Engel, Fries, & Singer, 2001).

Additionally, the role of oscillatory features within such networks exceeds information representation. State-dependent activity (described in the introduction of this chapter), which can generally be linked to intrinsic status of wake versus sleep, as opposed to the characteristics of discrete external stimuli, might act as a non-linear filter which controls the processing of salient inputs (Sejnowski & Paulsen, 2006). Emerging evidence also indicates the presence of a higher order loop, through which state-related activity fluctuations of brainstem circuits preferentially influence sleep stage progression, depending on previous global wake activity (Osorio-Forero et al., n.d.).

Lead dipoles may therefore be grouped together in terms of comparable diurnal activity patterns (frequency modulation between quiet wakefulness and sleep states). The determination of frequency parameters of interest (FOI) might depend on the presence of previously published evidence about regional activity patterns – although this is scarce where human brainstem ascending arousal networks (BAAN) are concerned. Based on non-human mammal literature, it appears that PPN/LdT, the subcoeruleus region of the cat (also termed as parabrachial complex in primates (Westlund & Dan Coulter, 1980)), as well as the intralaminar parafascicular thalamic nuclei (Pf, where PPN, LdT and the parabrachial complex send projections), are mainly activated in the beta/gamma frequency range during wakefulness and REM sleep (*summarised in* (Urbano et al., 2012)*).*

Activity range of the Pf was reported in the range of 15-45Hz, shifted closer to the higher end of this spectrum after the application of carbachol, which also increased firing rates in PPN from a baseline in the beta range (22-24 Hz) to 45-49Hz (in rats, (Urbano et al., 2012)). Furthermore, oscillations within the range of 30-80Hz in rat subcoeruleus were found after depolarisation in non-cholinergic and non-noradrenergic neuronal populations, suggesting that activity in the beta-gamma frequency range might be common across populations responding to different neurotransmitters and therefore the presence of a broader network (in rats, (Brown, Winston, Basheer, Thakkar, & McCarley, 2006)).

Therefore, the main focus of lead dipole grouping (*and subsequent stimulation, as will be explored in the following chapter),* was based on the diurnal variation of

contact dipoles in these (beta, gamma) frequency bands. We additionally attempted to conceptualise their anatomical location with a higher level of precision, in the absence of post-mortem histological results, through the localisation of maximally modulated contacts in comparison to established atlas co-ordinates of major brainstem arousal nuclei, which might be present in the PPNR surgical area (Edlow et al., 2012).

With these organisational principles in mind, we initially sought to establish an understanding of the bigger picture with regards to brainstem oscillations linked to circuit excitability and quiescence. Therefore, as a first step from an electrophysiological perspective, we examined activity during quiet wakefulness (at night-time with eyes closed) and slow-wave sleep (SWS) –two macro states corresponding to the extremes of this balance where cortex is concerned. We then explored how oscillatory activity co-varies with UP- and DOWN-states in SWS, since they are a reflection of cortical fluctuations between excitation and inhibition. Finally, we investigated underlying circuit dynamics and their correlation with sleep fragmentation.

Methods

Ethics

The present study was conducted in accordance with the Declaration of Helsinki, approved by the local ethics committees of the University of Oxford Hospitals NHS

foundation trust. All patients provided informed written consent before the experiment, which was re-visited by documenting verbal consent prior to each experimental night and recording procedure.

Participants and lead implantation

For this study, four male MSA patients with leads in brainstem arousal circuits were recruited (Supplementary Table 1, *Chapter Appendix*). They were implanted with 1.5 mm spaced St. Jude Medical Infinity directional DBS electrodes (Abbott) as part of a clinical trial examining autonomic and motor function in MSA (STAGG-MSA trial, Study Identification number: NCT03593512). Surgical approach involved defining the lead trajectory for implantation in the PPN Region (PPNR), with a dorsal boundary being positioned laterally to the decussation of the superior cerebellar peduncle, on an imaging plane of the dorsal inferior colliculus and dorsal pons (Hyam et al., 2019; Plaha & Gill, 2005).

In more detail, we identified the superior cerebellar peduncle, above the fourth ventricle in each patient's native space, on axial planes of fluid attenuated inversion recovery magnetic resonance imaging (MRI FLAIR). After finding the brachium conjunctivum, a triangular space was delineated between medial lemniscus (lateral side), decussating fibres (of brachium conjunctivum, medially) and peduncle (anteriorly). This triangular region has been purported to contain the PPN nucleus within its boundaries and therefore termed 'PPN region (PPNR)' in our unit's prior published work. Planned implantation trajectory was calculated so that an electrode entering the space through a frontal burr-hole would be placed on the anterolateral

side within this triangle. The lead co-ordinates were then advanced by 3-4mm, so that the second contact would sit within that axial slice. This would ensure that the lowest electrode contacts would be in the deepest part of what has been defined as the PPNR.

Electrode placement was clinically confirmed after implantation by the fusion of preoperative magnetic resonance imaging (MRI) and computed tomography (CT) scans, obtained post-implantation. For additional visualization of electrode positioning compared to the predicted location of important subcortical arousal-related nuclei, we reconstructed the electrode trajectories and location of different contacts using the MATLAB toolbox Lead-DBS (version 2.3.2) (Horn et al., 2019) based on preoperative MRI and post-operative CT. We used the Harvard Ascending Arousal Network (AAN) Atlas (Edlow et al., 2012) to co-register and normalise the contact locations in into the Montreal Neurologic Institute (MNI) 152-2009b space.

Data Acquisition

For the patient cohort in this chapter, trial questionnaires were collected by the designated recruiting STAGG trial Neurologist (*for the subsequent cohort in the next chapter, the author conducted a dedicated interview focused on sleep with use of clinical scales and questionnaires, for every recruited participant).* With regards to the cohort examined in this chapter, details of bedtime and wake-up time (as well as variation thereof) were obtained by the author for each STAGG patient upon enrolment during an additional structured interview, in addition to the trial

Neurologist's activities, in order to establish an order of habitual sleep patterns relevant to recording timing (Fig. 1).

Prior to implantation and after a night of habituation to the in-hospital environment, a polysomnographic recording was planned for every patient. Subsequently patients underwent electrode implantation with the electrodes remaining accessible (externalized) post-operatively, as opposed to connected to a subcutaneously implanted pulse generator (IPG). Baseline joint EEG/LFP passive overnight recordings were completed after at least 72hrs of recovery from general anaesthesia (GA), to ensure there were minimal carryover effects on slow wave activity. In addition to the consideration of the half-life of anaesthetic agents used at induction and GA maintenance, this window was based on a wealth of published protocols in studies performed over 40 years in small mammals, where day-by-day examination of slow wave feature and sleep architecture recovery is feasible.

A window of three to seven days is allowed as a general rule in rodent implantation studies prior to sleep recordings (Datta et al., 2000; Terrier & Gottesmann, 1978; Tsunematsu et al., 2011). However, the diameter of implants and the invasiveness of such procedures is orders of magnitude greater than their human equivalents. This relates to issues such as cranial volume versus implant diameter (rodent laminar probes and EEG/ECOG intracranial electrodes are large and secured with cement on extracranial sites). Additionally, the relative diameter of craniotomies which are performed in rodents is not comparable to the localised burr-holes

performed in humans, which also benefit from specialist input during peri-operative recovery.

Rodent and human physiology are not directly equivalent, while published human studies are of a far smaller number and do not always explicitly state the duration between implantation and recovery, despite focusing on slow-wave sleep (Geva-Sagiv et al., 2023). Therefore, time of recording setup and initiation matched patient bedtime and was only undertaken once sufficient recovery had occurred and sleep-wake behavioural patterns normalized to pre-operative baseline levels (*further detail offered in the 'Data Analysis' section that follows)*.

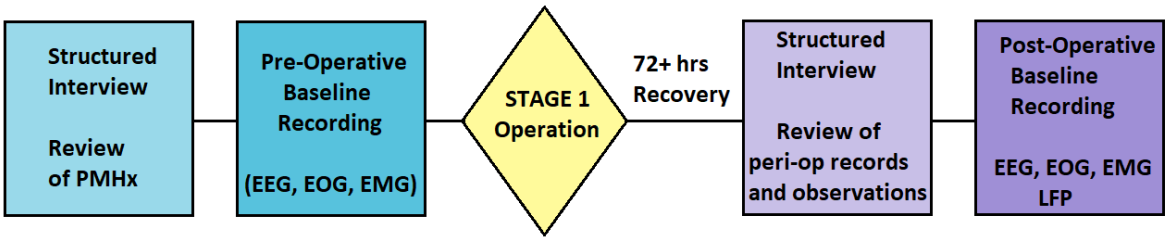


Figure 1. Planned patient recording trajectory for intracranial data. After habituation to the hospital environment, a pre-operative baseline recording night is arranged. During the day (in addition to any activities of the trial neurologist), a structured interview is undertaken to establish habitual bed time, any overnight arousals, as well as subjective sleep quality. Past medical histories are reviewed for evidence of sleep-related pathology, medication etc. Further review of peri-operative records, including operation notes, recovery entries and imaging, is undertaken at 72hrs post implantation. Vital signs and charted observations are also reviewed, prior to a structured interview about sleep onset and subjective complaints. If there are no concerns, a post-operative recording of EEG, intracranial signals and adjuncts (EMG, EOG) is arranged.

All data were recorded using a TMSi Refa system (TMS International, Oldenzaal, Netherlands) at a sampling rate of 2048 Hz. An electrode cap (Easycap, Herrsching, Germany) was fitted to each participant, designed for EEG data to be recorded from sixty-four silver/silver chloride electrodes placed according to the extended 10/20

system. Furthermore, a ground electrode was positioned on the midline centrally while the common channel average was used as acquisition reference.

Due to the presence of entry wounds at the implantation sites, we modified electrode placement to leave relevant sites undisturbed. Additionally, we decided to avoid mastoid electrodes altogether due to the fact that their placement interfered with comfort due to the externalised DBS electrodes in one patient. Electro-occulogram (EOG) was recorded from bipolar electrodes placed above and below the outer canthi of right and left eye respectively, while electrocardiogram (ECG, to assess/monitor for any cardiac events) and chin electromyogram (EMG) were also placed as per AASM guidelines (Berry, R. B. , Brooks, R. , Gamaldo, C. E. , Harding, S. M. , Lloyd, R. , Marcus, C. L. , Vaughn, 2017).

Data Analysis

1. Local Field Potentials: Implant-Specific Montage Considerations

We referenced our local field potentials (LFPs), which were recorded continuously across hours with a sampling frequency of 2048 Hz, in a combination of bipolar montage configurations that preserved lead directionality while controlling for volume conduction. This method has also been established practice in prior subcortical target literature (He, Deli, et al., 2021; Nazmuddin et al., 2018). In more detail, with specific consideration to the Abbott (St Jude) directional leads that were implanted, activity was recorded from eight contacts per hemisphere with locations that can convey spatial orientation on a 360 degree axis (Fig. 2).

Each lead consists of a localiser which is a non-recording part (helping orientation with regards to electrode contacts), a top and bottom ring contact which will sample from its surrounding tissue as well as two central segmented rings (Fig. 2A). These are particularly interesting in that, for the same coordinates along an axial plane, they will sample activity from different orientations (on different angles of the plane). Therefore, re-referencing them to either the top or bottom non-segmented contact, or their respectively orientated segment in their neighbouring ring ('cage-bar like' montage orientation) will create a localised dipole that can 'view' anatomical structures from a specific angle and capture activity from a specific portion of a nucleus (Fig. 2B).

Recent work in patients with Parkinson's disease has examined whether joint assessment of oscillatory activity from discrete directional electrodes and their anatomical position based on co-localisation with atlases in Lead DBS correlated with most efficient stimulation sites (Boëx et al., 2023). The conclusions were that oscillatory biomarker presence and distance from the target nucleus on Lead DBS were correlated, while also linked to optimal stimulation site selection. Although this work is related to a different clinical question and was published in July 2023 (significantly later than the onset of the research described in this thesis), it is aligned with –and may support the credibility of- the method of LFP evaluation that is also followed here.

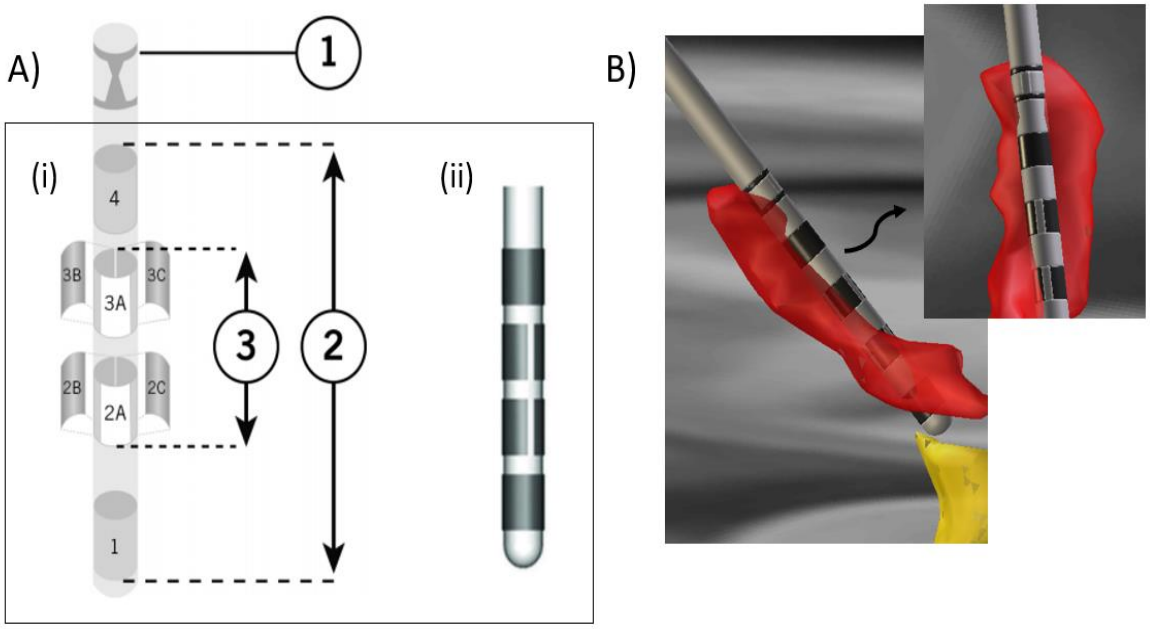


Figure 2. Directional lead (Abbott) and its relations to anatomy. A. Each lead consists of localiser/directional marker (1) and portion of the lead with a recording and stimulation capacity (2). The segmented nature of the two middle rings of electrodes (3) is shown along the axis of the lead (i). These are flush with the surface and seamlessly incorporated on the implantable structure (ii). B. Co-registration of the implant with anatomical atlases in Lead DBS. Note the localiser on top –while on the rotated view, it can be seen how different contacts in the segmented lead may sample from different angles (therefore also having potential access to different neuronal populations).

2. Data Pre-processing and Sleep Scoring

All analyses were carried out in MATLAB using bespoke in-house scripts, incorporating the FieldTrip (Oostenveld, Fries, Maris, & Schoffelen, 2011) toolbox. Individual channel data were de-trended to remove drift and filtered (high-pass Butterworth filter (3rd order) set at 0.2Hz and low-pass at 180Hz, with band-stop for 50Hz line noise and its harmonics) prior to any further analyses.

Both pre-operative baseline nights and post-operative recovery nights were visualised and scored as per American Academy of Sleep Medicine (AASM) criteria

(Berry, R. B. , Brooks, R. , Gamaldo, C. E. , Harding, S. M. , Lloyd, R. , Marcus, C. L. , Vaughn, 2017). A full description of these criteria and details of training necessary to obtain proficiency in the scoring methodology is available in the regularly updated, gold-standard handbook of the society. In the context of this thesis, two general concepts are of primary relevance: firstly, that the duration of AASM epochs for state scoring is 30 seconds (therefore when 'epochs' within a stage are mentioned in this thesis, this duration is implied unless otherwise specified).

Secondly, that sleep scoring (and therefore state evaluation) relies on the examination of features in neural (EEG) and non-neural (EMG, EOG etc) signals, exceeds simple power spectral plotting and requires relevant training and experience. As stated in the introduction, the author has multiple years of relevant experience in the field (having being taught sleep scoring methodology under the oversight of Prof Kostopoulos, head of the Neurophysiology Department at the University of Patras in the early 2010s). The author was also evaluated during joint scoring in the UK for relevant projects between 2019-2021, in collaboration with the University of Surrey (Surrey Sleep Research Centre, led by Prof Dijk) and has undertaken a number of courses and activities with the British and European Sleep Societies during the timeline of this thesis. However, patients with MSA present with a variety of atypical features (in terms of both NREM and REM sleep pathology, as highlighted in the introduction), therefore evaluation of architectural features outside SWS –particularly in REM- might be particularly challenging and their clinical conceptualisation in terms of links to sleep pathology deemed exploratory. The latter

is also the case given both the number of patients in this cohort, as well as the fact that these recordings do not constitute clinical polysomnography (with all respiratory, limb etc adjunct monitoring features).

3. Estimates of oscillatory modulation of LFP during different Windows of Excitability

Identifying opposite states: Quiet wakefulness versus Slow Wave Sleep

For our examination of LFP modulation, we sought to compare quiet wakefulness and a continuous slow wave sleep state. The latter was defined as having the highest levels of slow wave activity during Non-Rapid Eye Movement (NREM) sleep. Since slow waves have been classically described as 'traveling waves', originating in frontal cortex before spreading to other regions (Massimini, Huber, Ferrarelli, Hill, & Tononi, 2004; Murphy et al., 2009), we mainly focused on frontal and prefrontal cortical areas for our slow-wave sleep analyses.

First, we sought to identify the EEG channels with maximal slow-wave power during all slow wave or Non-REM Stage 2 or 3 (NREMS 2/3) sleep epochs, that had been visually scored and assessed for absence of artefact. To this end, power spectral density (PSD) of these selected signals were calculated using the short-time Fast Fourier transform (sFFT) with 4s of sliding-window and 3s of overlap and noise-floor normalisation. Then PSDs were averaged and collapsed across time, resulting in one mean value per NREMS cycle per channel, for selection of the main SWA frontal generator across all of NREMS.

From the EEG channels showing maximal SWA power and during these scored NREM2 and NREM3 epochs, slow waves were detected with the following criteria in addition to frequency (0.5-4Hz): peak-to-peak amplitude exceeding 50 μV as well as train (SWA trains or 'snippets') duration of 5sec. Such detected 'snippets' were visually inspected to ensure signal quality prior to further analysis. In addition, we selected control state 'snippets' from epochs scored as quiet wakefulness (eyes closed) of an equivalent number, where slow-wave activity was not detected. These control 'snippets' and their corresponding epochs, also equally distributed across patients, were also visually inspected for signal quality.

Identifying LFP Dipoles with Maximal State-Dependent Activity Modulation

First, we sought to identify the LFP channels with maximal power modulations between wake and slow-wave sleep. We therefore examined brainstem LFPs during scored epochs of quiet wakefulness and continuous slow wave activity during Non-Rapid Eye Movement (NREM) sleep stage 3. A total of 54 snippets were detected from different AASM epochs (EEG channels as identified above) during the part of sleep (NREM 3) with highest SWA power epochs within patients, with an equal number of control quiet wake segments. Power spectral densities (PSD) for this selection were calculated within each snippet, using the short-time Fast Fourier transform (sFFT) with a window of 0.5-60 Hz and a frequency step of 0.5, and normalised to the noise floor of the signal for each re-referenced dipole. The cut-off for higher frequency activity set since –as stated in the introduction and also further

elaborated on during the next chapter- previously published studies across species reported the majority of activity in the region to be within the beta/low gamma range.

Within each dipole, the mean PSDs across frequency range were then computed per state (wake vs SWS, with control for noise floor variation as stated) with a subsequent element-wise calculation of the difference in power across every frequency. These differences, capturing variability of activity across state, were subsequently compared across all dipoles within each patient with regards to activity across the beta and gamma frequency range (13-60 Hz), using a one-way ANOVA with Bonferroni correction for multiple comparisons. Clusters of dipoles that did not differ significantly between them were thus identified and categorised separately from re-referenced electrodes with higher absolute values of power modulation between states.

The latter were labelled on co-registered imaging of Lead DBS with the Harvard Ascending Arousal Networks atlas (Edlow et al. 2012), and interactive plots used to examine their relationship to key arousal-related nuclei at all reconstructed angles in 3D space. Additionally, their corresponding PSDs per state (not the differences) were plotted with addition of the ‘Boundedline’ package in Matlab, with the standard error of the mean calculated and visualised on the plots the for all dipoles within one modulation cluster. These plots, and associated statistical comparisons within them for the frequency band of maximal modulation (students t-test), were also calculated for the pooled data from all patient clusters across both hemispheres, as an estimate of total brainstem ascending arousal network activity (BAAN) in the PPNR.

Brainstem LFP power Modulation by UP- and DOWN-State

In examination of BAAN activity across excitability windows, we moved from a temporally broad base (slow wave sleep versus wake) and spatially focused aim (identification of specific identified dipole contact clusters, with maximal modulation within beta/gamma range) approach to a different perspective. We examined for common patterns of activity modulation across the entire lead during within-state transitions of excitability with a duration of milliseconds. As explained in the introduction, the UP- and DOWN-states of slow wave oscillations offer such a possible window.

However, the absence of direct measurements of membrane potential makes relating changes in EEG polarity during SWA difficult to directly relate to discrete UP- and DOWN-States, as these are defined in microelectrode animal literature. Nevertheless, if we consider EEG as a summary of temporally synchronous cortical events, the cycle between maximum negative and positive amplitudes of EEG SWA will reflect fluctuations between hyper- and de-polarisation (therefore excitability) of the underlying cortical region. Here we therefore term this as ‘putative’ UP- and DOWN-State, as also is the case in more recent publications where this distinction between direct (microelectrode) and global (EEG) measurements of the balance between excitation and inhibition is made (Bouchard et al., 2021).

We focused on slow waves detected during continuous 5 seconds trains from a frontal generator (as described) –with a total duration of an oscillation cycle between 25 milliseconds and 2 seconds. Therefore, each train could contain a maximum of

twenty oscillations (each with a putative UP- and DOWN- component). We considered one complete UP to DOWN-state cycle to be 2π for each time point, first finding all zero-crossing points from the bandpass-filtered EEG data to calculate its putative equivalent. The points where amplitude decreased maximally (corresponding to the negative peak of the DOWN-State) were assigned phase -π while positive maxima (peak UP-state) were assigned phase π. The phases of all other time points in the oscillatory cycle were determined through linear interpolation between –π and 0 (DOWN-State to zero-crossing) or between 0 and π (zero-crossing to UP-state). We further divided each cycle into 18 non-overlapping bins spanning from –π to π.

To calculate a 'phase/power modulogram' we then decomposed all filtered bipolar LFP channels from all patients in the time–frequency domain, by applying continuous complex Morlet wavelet transforms with a linear frequency scale, ranging from 1 to 95 Hz and a linearly spaced number (4–8) of cycles across all calculated frequencies. The LFP power spectral densities (PSDs) were further estimated by averaging the wavelet power across the time periods of interest and then normalized by the sum of the whole frequency band between 1 and 95 Hz. A broad range of frequencies was selected here, since there was a high degree of uncertainty with regards to what would be expected with respect to a cut-off in the gamma range due to a lack of relevant prior literature at the point of methodological planning (none in humans, scarce translatable equivalents in animals).

To quantify a modulation index (MI) per frequency, the mean power within each cycle phase bin per frequency was then normalized against the sum of all bins (to avoid negative values) to give a percentage of power for each phase bin. Then, the MI was defined based on the Kullback–Leibler (KL) distance between the phase-locked power at each frequency and a uniform distribution as follows (Kullback & Leibler, 1951; Tort, Komorowski, Eichenbaum, & Kopell, 2010):

$$\mathrm{MI} = \frac{D_{\mathrm{KL}}(P,U)}{\log(N)}$$

$$D_{\mathrm{KL}}(P,U) = \sum_{j=1}^{N} P(j)\log\left[\frac{P(j)}{U(j)}\right],$$

where P and U indicate the gait phase-locked power distribution at a specific frequency and a uniform distribution. N indicates the number of bins (i.e., 18). Here the KL distance is used to infer the amount of difference between two distributions, which has the property that DKL(P,U) ≥ 0 DKL(P,U) ≥ 0 and DKL(P,U) = 0 DKL(P,U) =0 if and only if when P = U (i.e., when the distributions are the same). Large deviations from the uniform distribution result in a large modulation index. Additionally, the mean power within each phase bin was calculated for each frequency and normalized against the mean of all bins, in order to give a percentage of power for each phase bin as an additional estimate of power changes across the SWA cycle.

The control wake state was not characterized by phasic changes in UP/DOWN State activity and had negligible SWA power content, therefore we calculated a ‘sham’ phase based on a random ‘transition’ point in the band-passed signal. The LFPs were then phase-locked to this random ‘sham’ phase for calculation of power modulation indices.

To assess the significance of power modulation indices for LFP contact pairs linked to UP- and DOWN-State, we used a non-parametric cluster-based permutation procedure while controlling for multiple comparisons (Maris & Oostenveld, 2007). The null hypothesis comparator distribution was by randomly shuffling the phase for each individual slow-wave cycle. The phase vector was re-calculated by selecting a random transition point for division of phase (at any point during UP- or DOWN-State transitions), then randomly shuffling phase for calculation of a new modulation index. This was repeated 1000 times to obtain a permutation distribution with 1000 samples. The permutation distribution mean and SD were then used to z-score the original un-permuted data, obtaining a p value for each data point.

Suprathreshold clusters were obtained for the original unpermuted data and each permutation sample by setting a pre-cluster threshold ($p < 0.05$). Statistical significance was considered if the absolute sum of the z-scores within the original suprathreshold clusters exceeded the 95th percentile of the 1000 largest absolute sums of z-scores from the permutation distribution (i.e., at $p < 0.05$). For comparisons of modulation indices in the control wake state, the additional null

hypothesis vector was calculated by a second random point for phase division in a similar way.

4. Functional and Effective Connectivity Analyses

In addition to power spectral modulations in brainstem LFPs, we examined connectivity changes between PPNR and cortical regions, across sleep and wake. After sleep scoring, frontal channels during all NREM2 and NREM3 sleep epochs were further visually inspected for artefact presence and overall signal quality. We selected three main frequency bands of interest to compare state dependent signal changes: alpha, 8–12 Hz; beta, 13–29 Hz; gamma, 30–95 Hz (with a cut-off for cortical gamma activity set at 80Hz). For subsequent connectivity analyses across each frequency of interest (FOI), LFP montages per patient that exhibited the maximal modulation across NREM sleep and wakefulness for the respective FOI were selected.

For functional connectivity estimates, first we calculated the imaginary component of the coherence between selected bipolar LFPs and frontal EEG channels. Coherence estimates are usually presented as the magnitude-squared real part of coherence, however the complex coherency consists of both Cartesian and polar parts and is defined by its real and imaginary components. The real part, while larger than the imaginary, is strongly affected by volume conduction effects; the imaginary part is only sensitive to synchronization of time-lagged processes (Nolte et al., 2004).

For the two channels i and j let there be time series x̂ i(t) and x̂ j(t), with complex Fourier transforms xi(f) and xj(f). Therefore, the cross-spectrum is calculated as

$$S_{ij}(f) \equiv \langle x_i(f) x_j{}^*(f) \rangle$$

with $*$ denoting complex conjugation and $\langle\ \rangle$ expectation value. Complex coherency is defined based on the above cross-spectrum as

$$C_{ij}(f) \equiv S_{ij}(f) (S_{ii}(f) S_{jj}(f))^{1/2}$$

The imaginary part of coherence can be obtained by projecting the complex-valued coherency onto the imaginary axis (additionally see (Bastos & Schoffelen, 2016)). The imaginary coherence estimate values were therefore calculated individually for each combination between re-referenced lead contacts and/or EEG electrodes of two areas of interest, in a coherence matrix specific to condition (quiet wakefulness, NREM2/3 sleep). The magnitude of the imaginary component was used to indicate the degree of co-variability between the two signals over different frequency ranges in a manner least affected by volume conduction and artefactual changes. We used a multi-taper method based the FFT calculated, with 4s of sliding-window and 3s of overlap then subsequently normalised for the noise floor of the signals during each epoch, for the FOIs denoted before.

We also sought to establish the effective connectivity between subcortical and cortical areas of interest. To this end we used Granger causality (Granger, 1988),

which in its classical form is based on multivariate autoregressive modelling of data to infer the causal influence of one stochastic process on another.

Formally, Granger causality (GC) determines whether the predictions given by the autoregression of y:

$$y_t = a_0 + a_1 y_{t-1} + \ldots + a_n y_{t-n} + \varepsilon_t$$

are improved by inclusion of lagged values of x:

$$y_t = a_0 + a_1 y_{t-1} + \ldots + a_n y_{t-n} + b_p x_{t-p} + \ldots b_q x_{t-q} + \varepsilon_t$$

GC is widely considered an appropriate method of estimation of the directional information flow between two regions in Neuroscience, with studies of variable methodological quality using this method to bivariate GC in order to understand disruptions of directional cortical connections that are linked to changes in consciousness (for example see (Pullon, Yan, Sleigh, & Warnaby, 2020)) or apply GC on polysomnography-related variables in an effort to interpret data in a clinically-relevant fashion (for instance, (Orjuela-Cañón, Cerquera, Freund, Juliá-Serdá, & Ravelo-García, 2020)). There is however a legitimate concern in terms of the method's interpretation when used in this manner in multivariate, complex systems such as networks of the nervous system. Another issue is that autoregressive models are subject to overfitting, while different parameters may lead to different results (and therefore variable interpretations of significance). The caveats in assuming absolute directionality of information flow in GC estimates have also been highlighted, in relevance to its use in Neuroscience (Venkatesh & Grover, 2016). An

additional problem is that neuroscience researchers tend to sometimes interchangeably refer to all variants of this analysis as ‘Granger Causality’, further complicating interpretation of their findings (Stokes & Purdon, 2018).

Since a parametric estimate of Granger is often difficult to calculate accurately due to overfitting the autoregression model, we also calculated a non-parametric measure based on spectral matrix factorization (thus model-order independent), using the FieldTrip toolbox (Oostenveld et al., 2011). We also explored parametric approaches over different combinations of time window (twin) and model order (MO) over multiple iterations, to determine the best data fit, tailored to frequency of interest and both with and without down-sampling at 200Hz. The optimal parameters were selected based on which one provided the maximal values of the parametric and non-parametric model order, since they are inversely related to prediction error. In stable data, both parametric and non-parametric estimates should yield comparable results and since spectral matrix factorisation allows us to avoid overfitting and (sometimes more prone to bias) selection of MO parameters, we report non-parametric estimates in this chapter.

To accurately identify frequency bands with significant state-related coherence changes, we used the same non-parametric cluster-based permutation procedure while controlling for multiple comparisons as described shortly prior. To compare the group averages between two conditions (quiet wakefulness and NREM2/3), the condition labels of the original samples were randomly permutated 1000 times resulting in a null hypothesis distribution of 1000 samples, with a significance level

set as described in the prior section. We also compared for general inter-patient differences in GC values (regardless of presumed cause) with an ANOVA with sleep, wake and patient anonymised ID or group category as factors. A Bonferroni correction was used in all analyses to counter for multiple comparisons. The latter was an exploratory analysis, linked to concepts of sleep fragmentation (further explained in the following section).

5. Exploratory Analyses Related to Sleep Architecture

Sleep Fragmentation and Index Calculation

Sleep fragmentation was assessed per patient, in an effort to further understand the relationship between network activity changes and underlying pathology. Fragmentation was first examined based on number of spontaneous wake-scored epochs (as per AASM criteria) occurring after the first sleep episode, each defined as 'Wake after Sleep Onset' and counting towards a fragmentation score based on the Pittsburgh Sleep Quality Index Scoring Algorithm (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989).

After continuous sleep periods and frank epochs of wake after sleep onset (WASO) were assessed, relevant scores were calculated and used in later analyses and evaluations. A WASO (%) index was calculated as the percentage (%) of wake epochs occurring after the first sleep episode, divided by the total number of epochs from this episode until the first wake epoch with which sleep was ended for the night. Based on the Pittsburgh Sleep Quality Index algorithm, WASO ≤ 15% suggested minimally disrupted sleep while > 25% indicated highly disrupted sleep. WASO was

therefore categorized into three groups ≤ 15%, 15-25%, and > 25% to characterise sleep disturbance in this cohort.

In order to assess sleep continuity further, in addition to epochs scored as 'wake' based on AASM criteria, we also investigated the presence of Intra Sleep Awakening Reactions (ISARs) as sleep disrupting events and subsequently calculated continuous (>10mins) sleep episodes occurring after the first epoch of non-REM sleep (NREMS). These ISARs were defined as an abrupt, fast EEG frequency shift (including theta, alpha or frequency greater than 16 Hz with exclusion of spindles) following ten seconds of stable sleep, lasting more than ten seconds and followed by return to sleep ("EEG Arousals: Scoring Rules and Examples," 1992; Ruby, Eskinazi, Bouet, Rheims, & Peter-Derex, 2021). Thus, a continuity (%) index was calculated per patient, defined as the percentage (%) of epochs in continuous sleep episodes (as defined previously, exceeding 10 minutes in duration) divided by the total number of epochs from the first sleep episode.

To explore whether information flow estimates from PPN to frontal cortex during quiet wakefulness and sleep may be linked to sleep fragmentation, a factorial ANOVA was computed between directional Granger causality (GC) values, sleep continuity and WASO indexes for each macro-state (wake and sleep). A Bonferroni correction was used in all analyses to counter for multiple comparisons.

Post-operative Recovery of Prior Sleep Architecture Continuity

As stated previously in this chapter, sleep data collection after 72hrs post-implantation is considered acceptable in terms of recovery from potential peri-

operative insults. However in humans (and what is more, in this specific target region) there is little data to confirm the accuracy of this consensus. Time between recordings is constrained by the planned duration of peri-operative admission (which is usually somewhere between two days and one week, with longer times for patients undergoing staged procedures with externalisation). Additionally, in the case of rare targets, the low volume of cases may not facilitate the systematic characterisation of this phenomenon –especially in units where the neurosurgical team may not be collaborating with professionals with a specialist sleep-related interest (clinical or academic). Furthermore, co-morbid factors during admission, such as post-ictal drowsiness and associated sleep/wake disturbances in epilepsy populations, which are frequently accessed for intracranial sleep data, might further complicate the picture.

Given that the research in this chapter focuses on arousal-related concepts, not REM sleep characteristics, two main areas of consideration should be highlighted. First of all, that the most overt manifestations of fragmentation or insomnia would also be translated into behavioural observations, should they occur during an admission (with observed daytime somnolence, delays in sleep onset etc). Therefore, within a system of multiple healthcare observations per day (in addition to research-related visits) such parameters may be preliminarily captured. Prior to recording of post-implantation data, there was an extensive review of admission notes and observations, as well as a patient interview where the presence of features of new sleep disturbance was examined. Secondly, that the main analyses presented here have to do with within-night comparisons of network

activity, not stage changes from one night to another. Therefore, should there be an element of post-surgical influence on the network, this would affect the extent of translation of the findings outside the peri-implantation period and not the validity of state-dependent comparisons.

An additional concept of relevance is that of ‘stun’ effect. This relates to the presence of a temporary ‘lesion’, created during lead implantation and obvious clinically in the case of tremor-predominant movement disorders through the absence of tremor. Some electrophysiological studies have collected and presented data correlated with movement in the presence of stun effect (for instance (C. C. Chen et al., 2006; He, Baig, et al., 2021)). With regards to sleep dynamics, we did not feel that this would be desirable, since we wanted to capture dynamics as close as possible to normal. In the case of MSA, tremor is not often present and therefore stun might present with subtler signs –such as changes in autonomic symptoms with an immediate post-surgical onset, which resolve in a few days. In all cases and conditions in general, permanent lesions are excluded through further imaging, where there is a high index of suspicion with regards to an ischaemic or haemorrhagic complication. Prior to baseline recordings in externalised cases, all these factors were considered.

In terms of quantifiable evidence of sleep disruption, outside of AASM scoring and WASO indices, a decision to focus on occipital alpha (8-12 Hz) power spectral changes was made. This frequency is also termed ‘Posterior Dominant Rhythm (PDR)’ when most predominant in the occipital leads on polysomnography during

quiet wake and its presence is a crucial part of AASM scoring criteria for wake epochs (Kane et al., 2017). Not all alpha patterns are the same, since apart from wake, alpha can be present during other stages of the sleep cycle (Cantero et al., 2002).

Therefore, we normalised the power spectral values, obtained per frequency-in-band for occipital channels with a frequency step of 1 Hz, to the mean power for that frequency during quiet wake of equivalent duration, recorded prior to sleep onset for each night. Values close to 1 and exceeding it for periods consistent with AASM wake scoring would therefore indicate the presence of wakefulness in recordings. With regards to time compared, widely-cited papers have compared power spectra across periods shorter than the entire night's duration (such as for instance the first for NREM episodes, (Merica, Blois, & Gaillard, 1998) also between different patient cohorts with a direct intent of reaching clinically relevant conclusions). In this instance, we decided to focus on the first two hours from the first NREM-onset sleep epoch. Normalised occipital alpha values compared with SEM calculated using the 'Boundedline' Matlab package.

Results

Locally Distributed Network Modulates Oscillatory Activity based on State

While (based on operative landmarks as previously published) leads had been determined to be within acceptable locations of the PPNR, we noted that a detailed examination with regards to all adjacent arousal-related structures revealed a variability in implantation targeting (Fig. 3). In addition to the pedunculopontine

nucleus (PPN), grey matter regions with greatest proximity to lead contacts included the midbrain reticular formation (MRF), parabrachial complex (PBC), pontine oralis (PO) and locus coeruleus (LC). This observation persisted regardless of whether the atlas-based localisation was performed in native patient space or in MNI space.

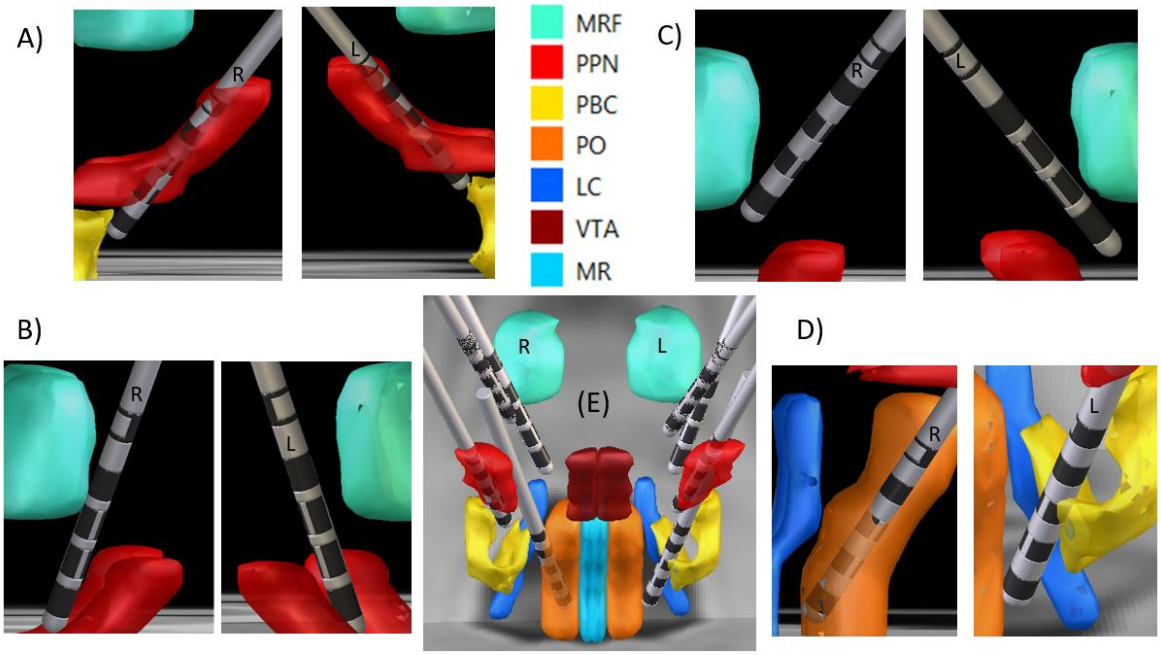


Figure 3. Relationship of lead placement with regards to arousal-related nuclei of the brainstem. Results are shown for each patient examined in this chapter, in addition to the group (centre, E). A. Lead placement of PPNR1 relative to pedunculopontine nucleus (PPN, red) and parabrachial complex (PBC, yellow). B. Lead placement of PPNR2 relative to top portion of PPN and ventral borders of midbrain reticular formation (MRF, turquoise). Based on LeadDBS estimates (more evident on rotational view), the lead is placed outside both nuclei. C. Lead placement of PPNR3 with regards to PPN (top) and ventral MRF. Some contacts are adjacent to MRF. D. Lead placement of PPNR4 relative to pontine oralis (PO, orange), locus coeruleus (LC, royal blue) and PBC. E. Group location, ventro-dorsal view. Ventral tegmentum (VTA, burgundy) and median raphé (MR, light blue) are visualised to assist with orientation with regards to the midline.

In addition to this anatomical localisation, we proceeded to examine contacts within every patient in terms of diurnal variability in the beta/gamma range and establish the location of maximally modulated contacts relative to brainstem arousal nuclei.

After identifying the clusters of contacts with maximal diurnal variation for each case, we plotted the comparative power spectra and statistically quantified the frequencies of maximal effect. In addition to within-patient analyses, the pooled contact clusters from all patients were examined to assess total diurnal network activity within the (surgically defined) PPNR region.

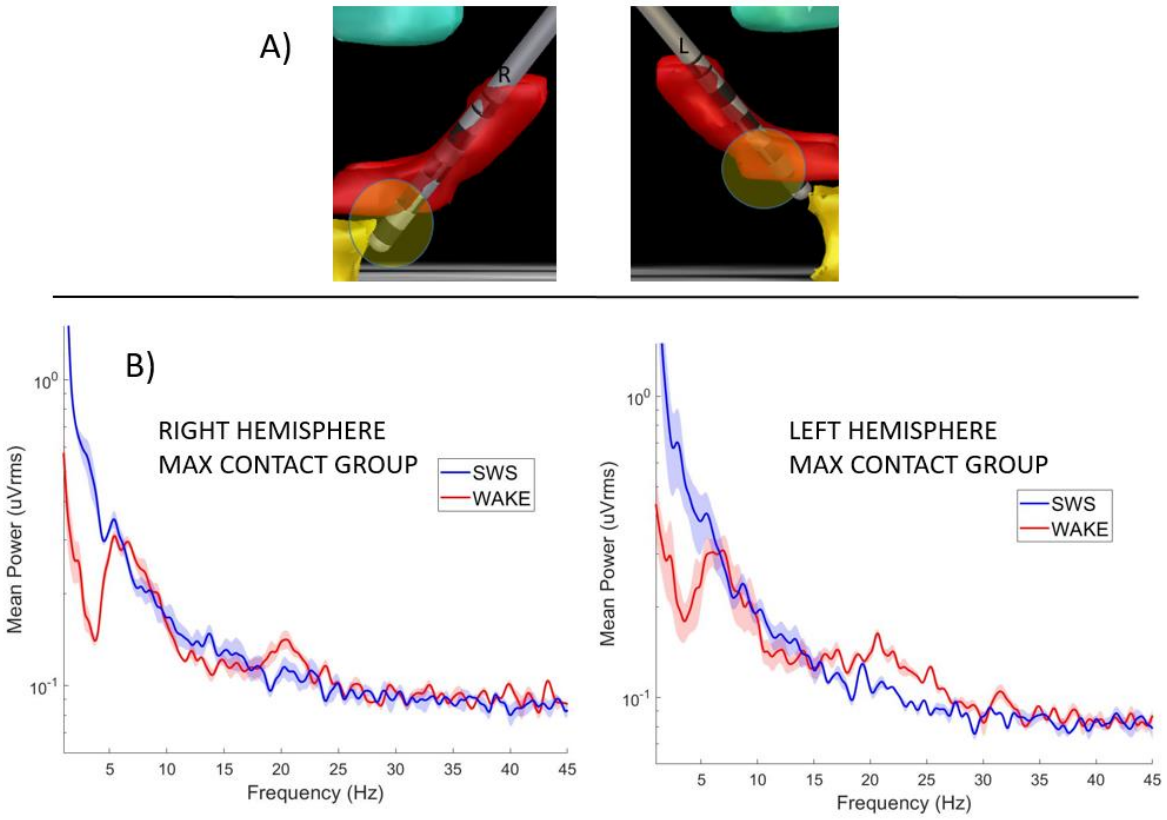


Figure 4. PPNR1: Maximally modulated diurnal contacts. A. Contact location in the caudal PPN, adjacent to PBC, in both hemispheres. B. Frequencies of maximal diurnal modulation per hemisphere cluster, with standard error of the mean (SEM) shown as shaded area around the cluster mean. Most noticeable is the modulation of beta centred around 20Hz. Modulation is generally noted in the beta and low gamma range (details in the text). SWS: slow wave sleep.

In PPNR1, examination of all directional dipoles across states (SWS and W) revealed statistically significant differences between contact clusters (Table 1a).

Maximally modulated/state-responsive contacts were located in the caudal portion of the PPN, adjacent to PBC (Fig. 4A). With regards to frequency modulation, there was a similarity in patterns of maximally modulated frequencies within the beta and low gamma band (Fig. 4B). Specifically, in the right hemisphere sub-bands within beta were at 15.8-29.6 Hz ($p<0.001$, CI 0.0196-0.0242) and low gamma at 30.8-32.6 Hz ($p<0.01$, CI 0.0116-0.0164), 35.9-38.3Hz ($p<0.001$, CI 0.0061-0.0097), 45-46.2 Hz ($p<0.001$, CI 0.0114-0.0186) and 53.3-55.2 Hz ($p<0.001$, CI 0.010-0.0145). Comparatively, in the left hemisphere corresponding bands were 18.6-22.2 Hz ($p<0.001$, CI 0.0205-0.0271) and 23.4-24.3 Hz ($p<0.001$, CI 0.0141-0.0227) in the beta band, with gamma band changes at 39.3-40.4 Hz ($p<0.001$, CI 0.0104-0.0165) and 42.9-43.7 Hz ($p<0.001$, CI 0.0104-0.0172).

Based on post-surgical LeadDBS co-registration of implants with the AAN atlas, lead placement in PPNR2 was at a greater distance from brainstem nuclei compared to other cases (Fig.3, Fig.5A). When diurnal oscillatory variation was examined, there was discrete clustering of activity –however, this involved smaller segments of the directional lead contents (only parts of the re-referenced directional rings) (Table 1b). In addition, these differences were less pronounced and centred around smaller frequency intervals compared to the broader modulation in other patients, while there was additional modulation within the alpha band (Fig.5B). Specifically, both hemispheres showed modulation in the alpha ($p<0.05$, left: 8.5-9.8Hz with CI 0.0359-0.0690, right: 8.9-10.4Hz, CI 0.0342-0.0763), as well as beta ($p<0.05$, left: 17-19.2 Hz with CI 0.0066-0.0107, right: 25.5-27.1Hz with CI 0.0038-0.008) and gamma range ($p<0.05$, left: 30.5-31.8Hz with CI 0.0045-0.0086, 41.5-42.2Hz with

CI 0.0016-0.0063 and right: 38.7-40.3Hz with CI 0.0031-0.0063, 45.3-47.7Hz with CI 0.0046-0.0078, 54.1-55.1Hz with CI 0.0038-0.0075).

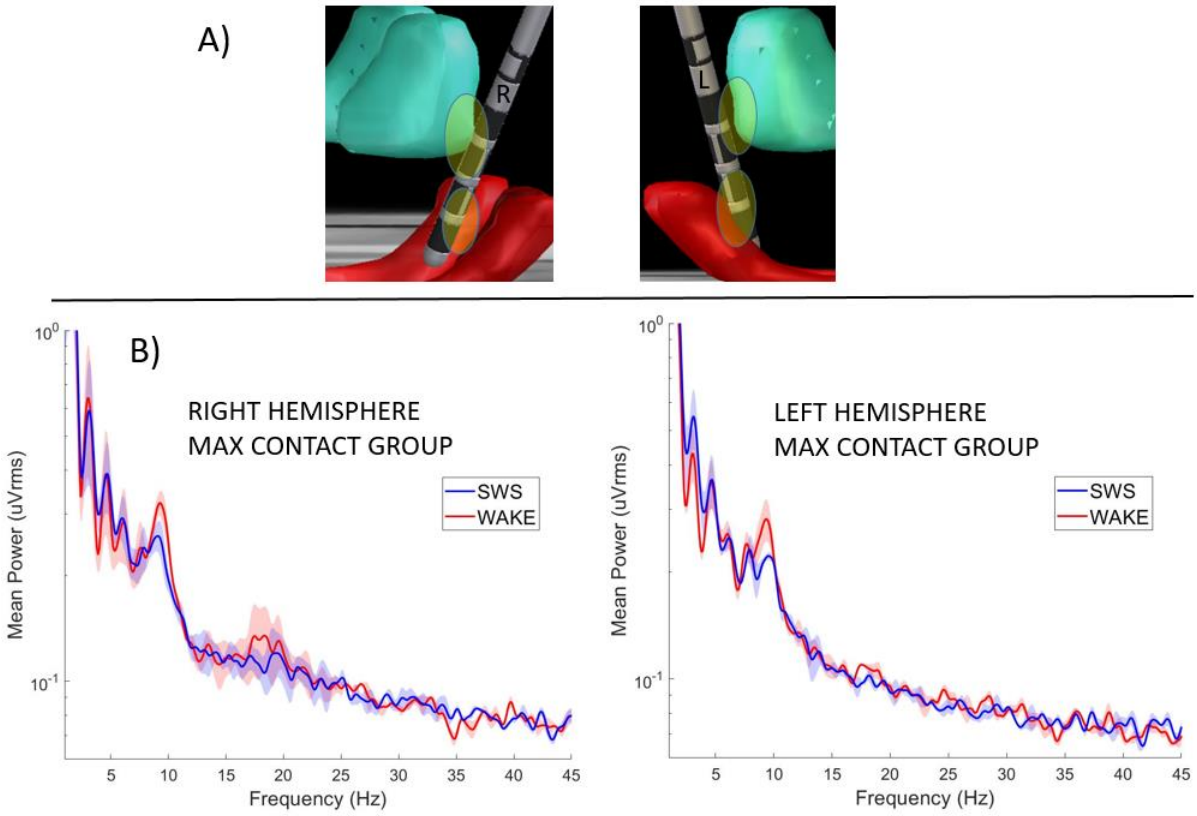


Figure 5. PPNR2: Maximally modulated diurnal contacts. A. Contact location did not appear to directly overlap with nuclei boundaries (this was more evident in rotational/interactive views). However, there were separate directional contacts that showed significant state modulation. B. Frequencies of maximal diurnal modulation per hemisphere cluster, with standard error of the mean (SEM) shown as shaded area around the cluster mean. Additional modulation can be seen in the form of an alpha peak, close to 10 Hz (details in the text). SWS: slow wave sleep.

In contrast to PPNR2, in the case of PPNR3 different implanted lead orientation meant that there was greater adjacency to the midbrain reticular formation (MRF, Fig.3 and Fig.6A). In this case, there was clear broadband beta and gamma

modulation of the caudal portion of implanted leads (Fig.6B) (Table 1c). This portion of the leads, closest to MRF, displayed most significant changes in the high beta/low gamma range. For the left hemisphere, these concerned the 19-20.3Hz and 23.2-27.3Hz range ($p<0.01$ with CI 0.0160-0.0397 and 0.0175-0.0272 respectively), while for the right hemisphere these frequencies were 19.8-24Hz, 26.7-30.4Hz and 38.5-39.4Hz ($p<0.01$ and CI 0.0424-0.0624, CI 0.0243-0.0398, CI 0.0053-0.0258 respectively).

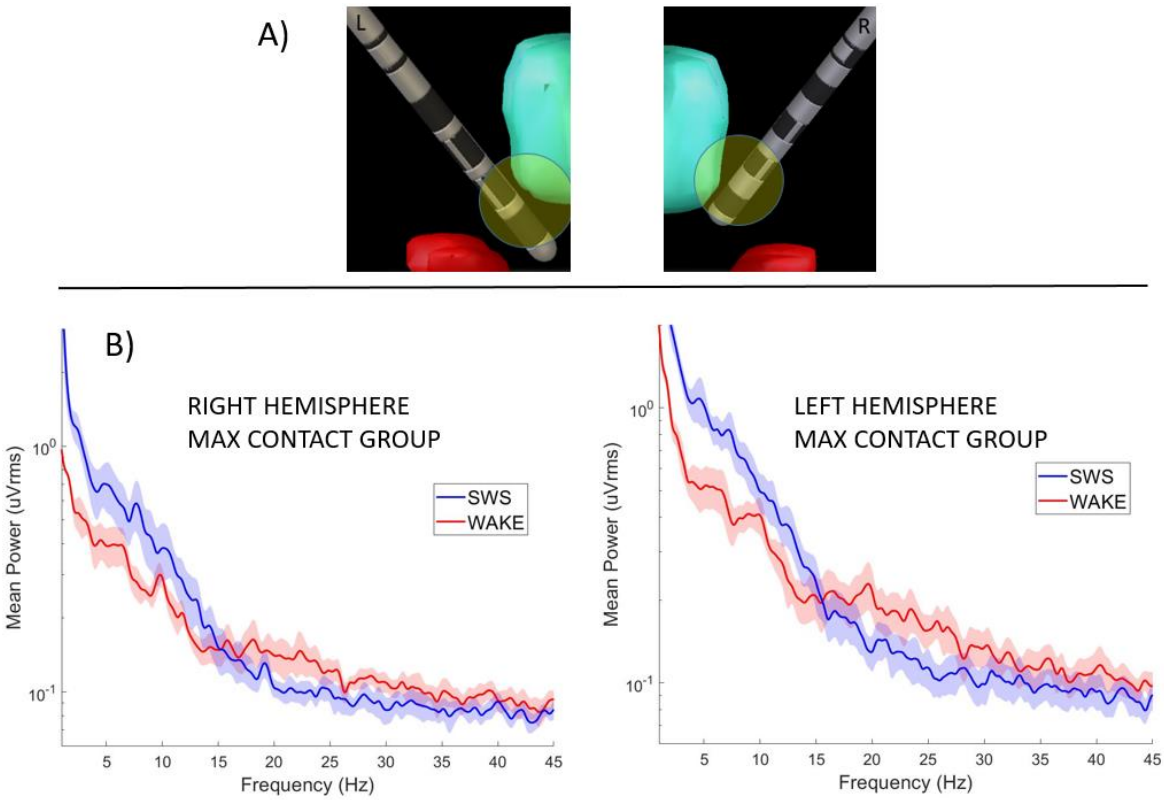


Figure 6. PPNR3: Maximally modulated diurnal contacts. A. Contact location was adjacent to the midbrain reticular formation (MRF), especially evident for contacts with highest diurnal modulation. B. Frequencies of maximal diurnal modulation per hemisphere cluster, with standard error of the mean (SEM) shown as shaded area around the cluster mean. There is broad beta/gamma increase during wake compared to slow wave sleep (details in the text). SWS: slow wave sleep.

The deepest implantation in this cohort was PPNR4, where active contact leads were in proximity –or directly within- pontine oralis (PO), parabrachial complex (PBC) and locus coeruleus (LC) (Fig.3, Fig. 7A). Here, there were large groups of contacts that clustered together in lead portions closest to these regions (Table 1d). A common PBC component might explain greater similarities with the electrophysiological profile of PPNR1, especially evident in the left hemisphere clusters (Fig. 7B, Fig. 4B). For both hemispheres, the most significant differences were within the beta range, 17-27.9Hz on the right ($p<0.001$, CI 0.0457-0.0591) and 18.8-22.9Hz ($p<0.001$, CI 0.0587-0.0855).

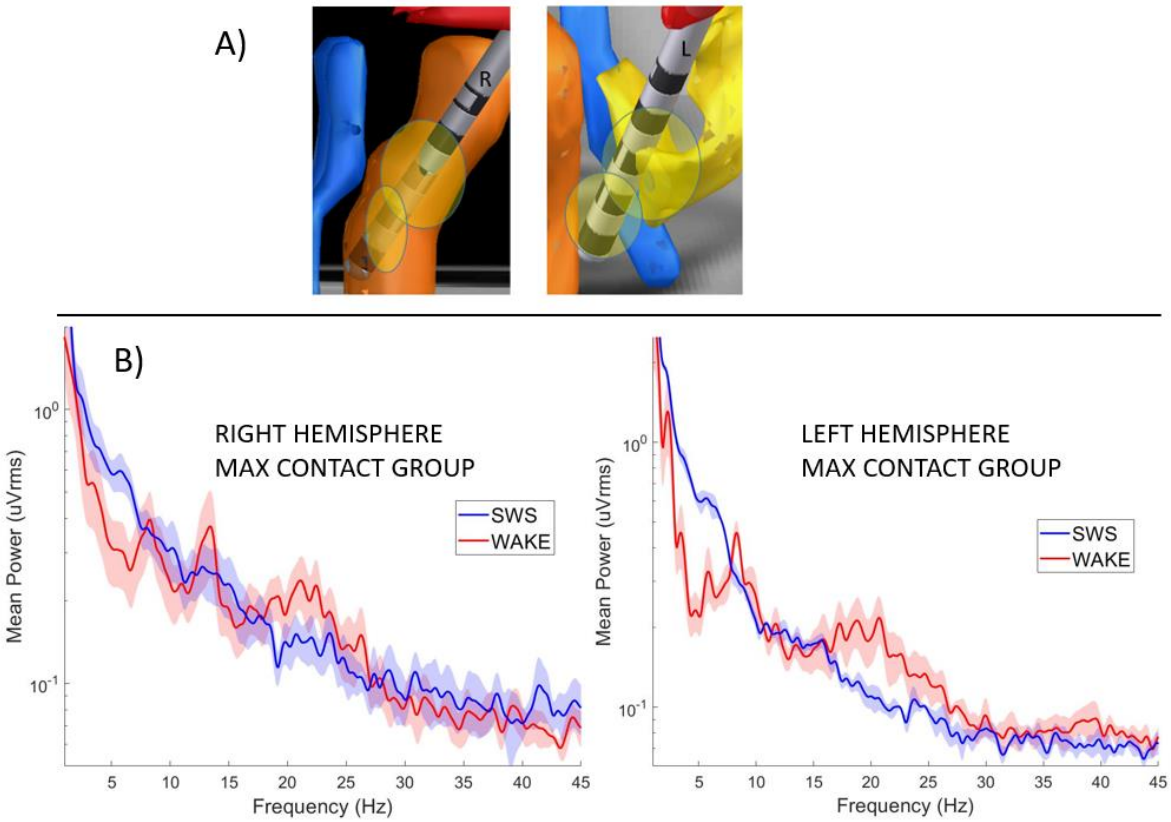


Figure 7. PPNR4: Maximally modulated diurnal contacts. A. Contact location was adjacent to the pontine oralis (PO, orange), especially with regards on the right, as well as parabrachial complex (PBC, yellow) and locus coeruleus (LC, blue). B. Frequencies of maximal diurnal modulation per hemisphere cluster, with standard error of the mean (SEM) shown as shaded area around the cluster mean. Most noted is the beta peak, which is centred broadly around 20Hz, in a manner similar to PPNR1 (details in the text). SWS: slow wave sleep.

Given both anatomical common factors –namely, the adjacency of maximally diurnally variable contacts to key arousal-related nuclei upon co-registration with the Harvard atlas (Edlow et al. 2012) and location within the surgically defined PPNR- as well as electrophysiological commonalities with common increases in the beta/low gamma band, we proceeded to network examinations of activity. Specifically, we co-analysed all contact clusters with maximal modulation between slow wave sleep and wake (Fig.8B), additionally pooling across both hemispheres to examine general patterns of activity in the PPNR region (Fig. 8A). Overall, two major frequency ranges showed increases during wake of maximal significance on the total network: one in the beta band, centred around 20Hz (17-27.3Hz, $p= 2.77 \times e^{-119}$ CI 0.0254-0.0299) and another in the gamma range between 38.5-40Hz ($p=7.96 \times e^{-12}$, CI 0.0058-0.0104).

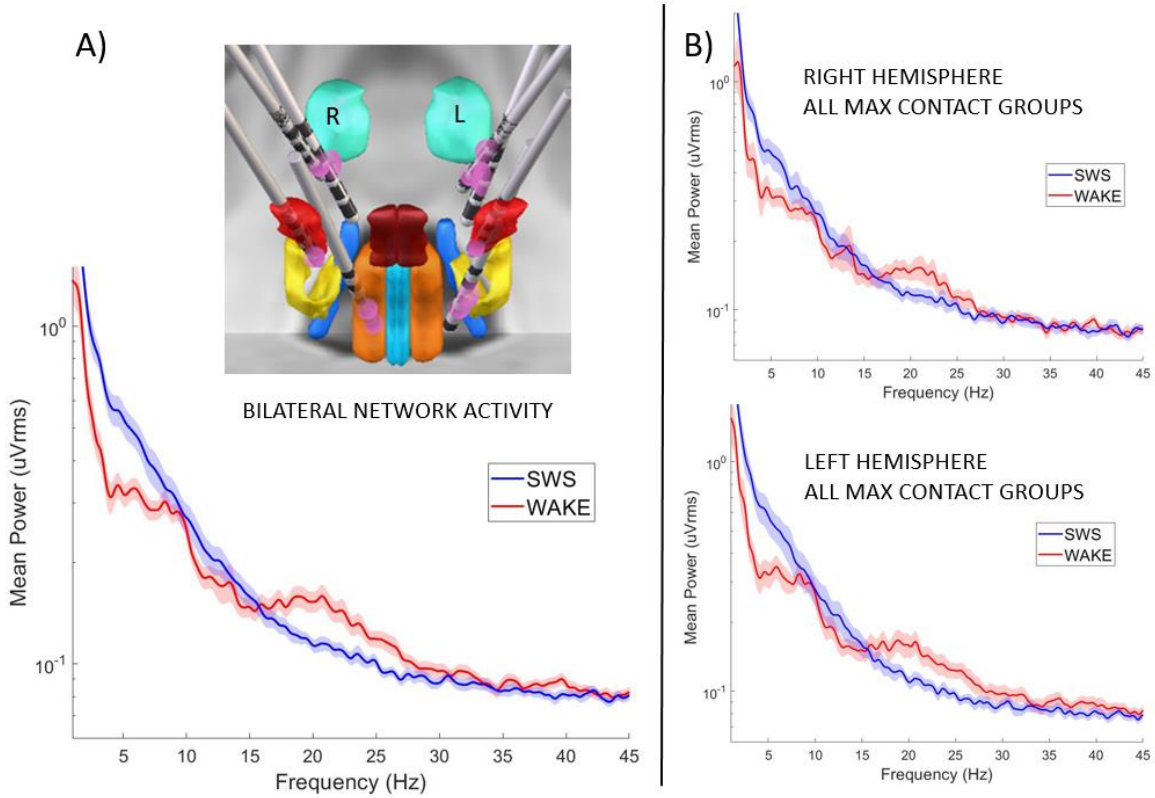


Figure 8. <u>Patterns of network modulation of neurophysiological activity between slow-wave sleep (SWS) and quiet wake</u>. A. Results for total network activity for both hemispheres are shown for pooled contacts from all patients (anatomical locations in A, top panel). Increases around 20Hz and 40Hz appear within the cohort during wake. B. Mean power across frequencies (up to 45Hz) are also plotted here for results pooled per hemisphere, with standard error of the mean (SEM) as shaded margins. It is again apparent that there is an increase in beta content (area centred around 20Hz) during wake.

Table 1. ANOVA results for dipole comparison per patient (with Bonferroni correction for multiple comparisons). Contact clusters show significant differences in the beta and gamma frequency range.

(a)	PPNR1					
	Left Hemisphere			Right Hemisphere		
	df	F	P	df	F	P
Beta	8	41.88	$1.26512e^{-63}$	8	22.52	$1.36694e^{-33}$
Low Gamma	8	49.02	$8.90222e^{-74}$	8	59.05	$1.47022e^{-102}$
High Gamma	8	103.23	$6.57029e^{-161}$	8	217.76	$1.15611e^{-321}$

(b)	PPNR2					
	Left Hemisphere			Right Hemisphere		
	df	F	P	df	F	P
Beta	8	93.44	$2.20652e^{-134}$	4	83.24	$9.86429e^{-63}$
Low Gamma	8	99.28	$1.9212e^{-139}$	4	126.17	$6.93983e^{-89}$
High Gamma	8	411.03	0	4	284.77	$3.80185e^{-202}$

(c)	PPNR3					
	Left Hemisphere			Right Hemisphere		
	df	F	P	df	F	P
Beta	8	10.33	$2.55665e^{-14}$	8	20.38	$4.2868e^{-30}$
Low Gamma	8	22.4	$4.16164e^{-33}$	8	25.77	$2.59388e^{-38}$
High Gamma	8	59.93	$9.05937e^{-94}$	8	97.62	$3.64186e^{-151}$

(d)	PPNR4					
	Left Hemisphere			Right Hemisphere		
	df	F	P	df	F	P
Beta	8	63.4	$1.43434e^{-87}$	6	18.59	$1.4391e^{-20}$
Low Gamma	8	100.34	$2.05313e^{-126}$	6	38.48	$1.1679e^{-41}$
High Gamma	8	247.51	$1.97104e^{-312}$	6	128.51	$7.68282e^{-138}$

Gamma and Beta Brainstem Oscillations are modulated by Cortical Putative UP- and DOWN-State transitions

In addition to broad observations across states, we investigated power spectral changes during within-state excitability windows, with a specific interest in (putative) DOWN- to UP-State transitions. We first quantified the power modulogram relative to pre-frontal EEG slow wave phase (calculated as described in *Methods* based on selected slow wave trains of 'snippets'), averaging across all bipolar LFPs and then across all of the lead/PPN region. We additionally made comparisons with power modulations during equivalent 2pi cycles in quiet wakefulness data, as an additional methodological control, testing the null hypothesis and suspending knowledge of prior biological data in an 'agnostic' blinded state. This was further facilitated through collaboration with a senior researcher experienced in oscillation co-variation with periodic biological phenomena (gait cycles) outside of the sleep field, who was also intentionally instructed to assist in the data analysis without prior insight in reports of FOIs from other published evidence with regards to UP- and DOWN-State cycles (Dr He, with additional thanks).

We saw clear PPNR power modulation by slow wave EEG phase during sleep (Fig. 9A, depicting two putative UP/DOWN State cycles and Fig. 9B, one 2pi cycle). In particular, alpha and beta modulation seemed to cluster during the first phase (DOWN) while low and high gamma power seemed to peak during the UP-State although (as mentioned in the prior section) only beta and high gamma modulation index differences survived statistical comparisons with the control ('sham' phase

during wake). Maximal differences in power modulation between sleep and wake were observed for beta/low gamma and high gamma (1934Hz, p=0.0311 and 77-95Hz, p=0.0195 respectively; *Fig. 9A*).

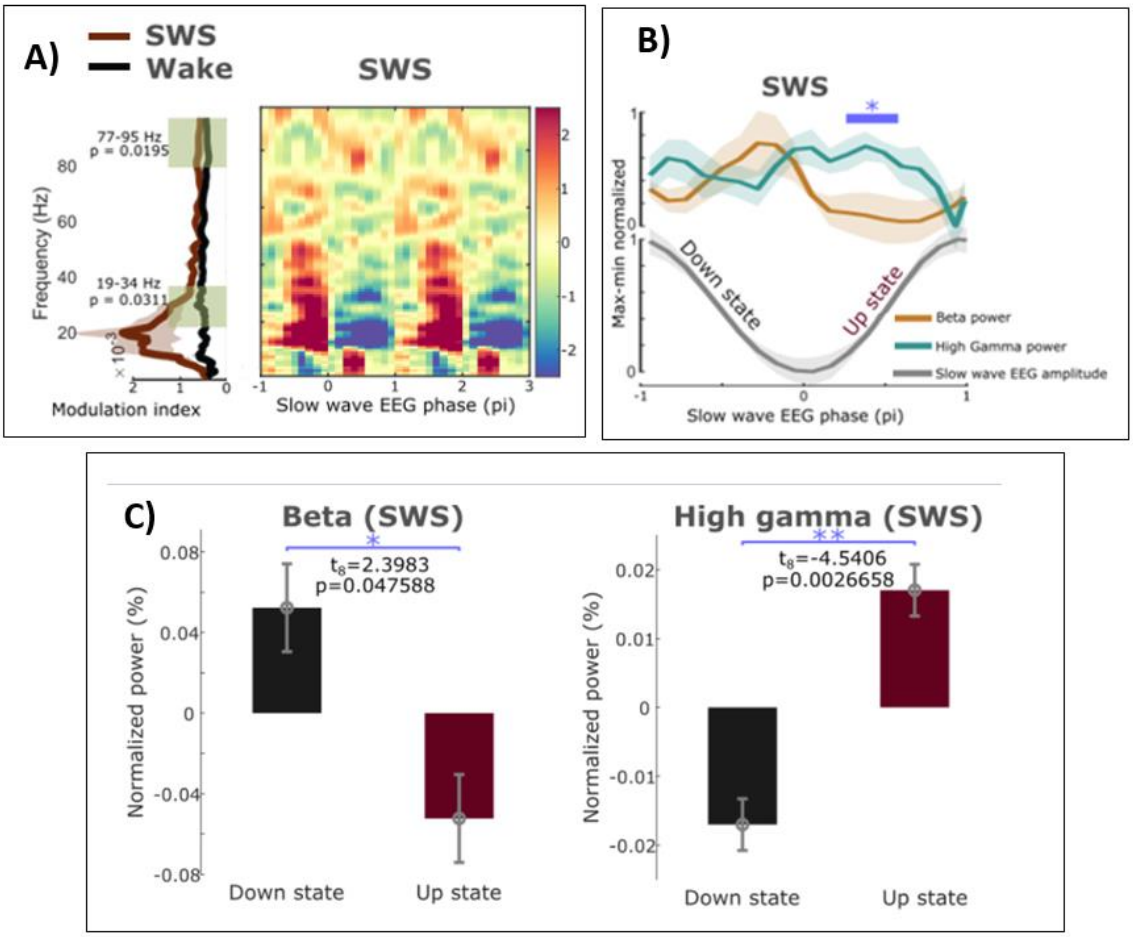


Figure 9. <u>Brainstem LFP power changes with regards to putative UP/DOWN State (Slow Wave phase)</u>. A. Power modulogram during slow wave sleep (slow wave snippets). PPNR activity clusters around the first phase of the slow oscillation cycle (DOWN-State) for beta and around the second phase of the slow oscillation cycle for gamma (UP-State). B. Statistical differences in modulation index differences (maximum minus minimum) for beta and high gamma, across slow-wave phase. Note the significant difference between the power in these two frequencies during the UP-State phase (p=0.0078). C. Further comparisons of beta and high gamma frequency (for signals with higher beta and lower high gamma frequency in DOWN-state), providing additional evidence of anti-phasing for these two oscillations.

When the modulogram values were compared across frequency bands between UP- and DOWN-State phases, we noted that beta and gamma power were also anti-phased with regards to slow wave period (Fig. 9B*).* Based on the total average of all re-referenced LFP signals, this resulted in a statistically significant increase in gamma compared to beta power (p=0.0078, 10000 times permutation test) during the 'excitable' part of the slow-wave cycle (transition to putative UP-State, Fig. 9B). In generated 2pi cycles of equivalent duration during quiet wakefulness data ('sham' phase), this effect was not observed (Fig. 10).

We further sought to quantify changes correlated to UP/DOWN cycles of alpha, beta and gamma frequency power-in-band, additionally separating gamma into low (below 45Hz) and high (above 55Hz) gamma bands. Specifically, for alpha and beta power, we selected the bipolar LFP channels with the maximum power during DOWN state while for low and high gamma power, we selected the bipolar LFP channels with the minimum power during DOWN state. We subsequently quantified the average power-in-band from the selected bipolar LFP channels and compared them between the two parts of the slow wave cycle. Beta power was significantly higher during the DOWN-State (p=0.04788, t=2.3983) while high gamma power significantly increased during transition to UP-State (p=0.002666, t= -4.5406). The differences in alpha and low gamma bands did not reach statistical significance.

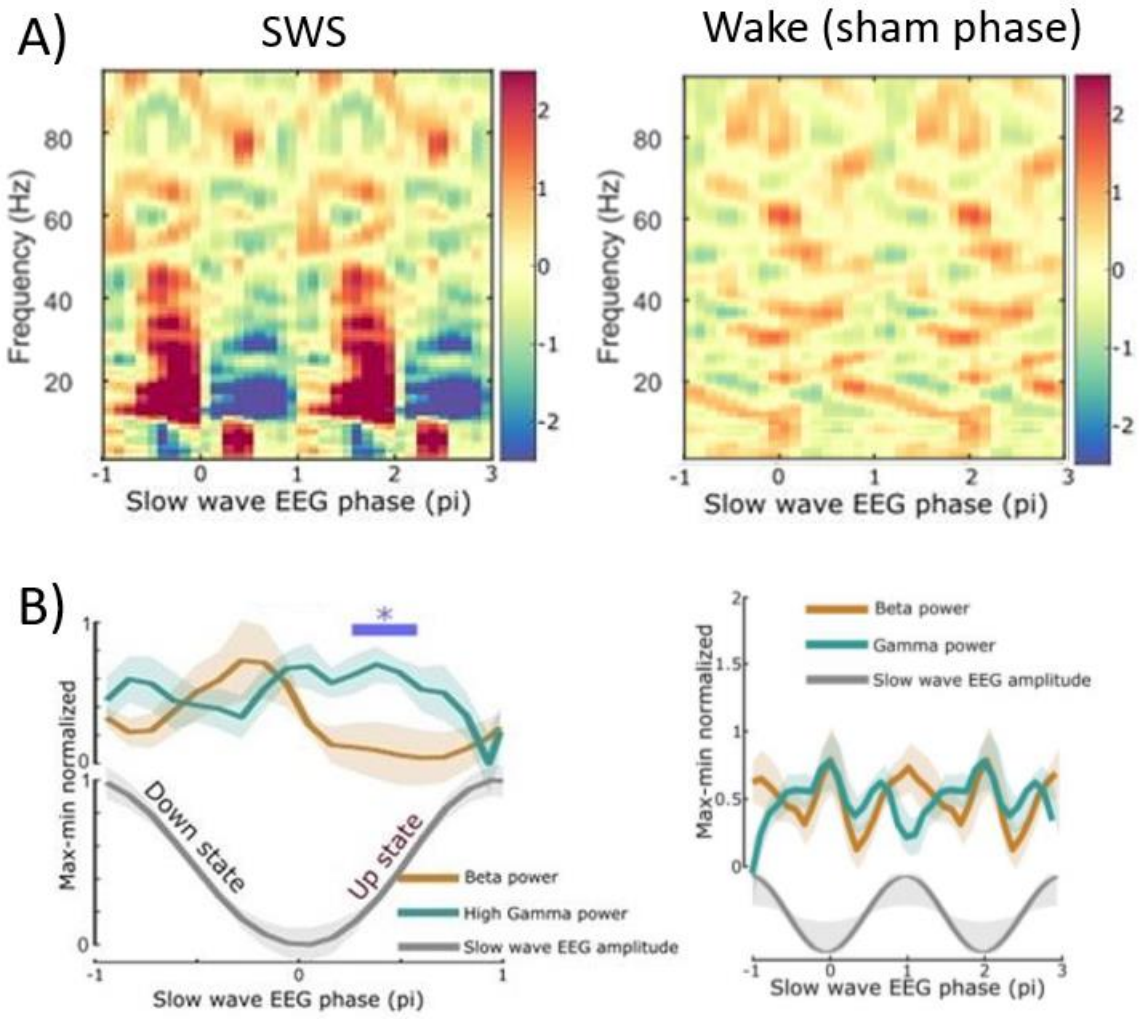


Figure 10. <u>Phasic Brainstem (PPNR) LFP power changes are state-specific</u>. Both in terms of plotted power spectral patterns (A) and modulation indices (B), the anti-phased patterns of beta and gamma activities are only present during SWS.

Gamma Functional Connectivity significantly increases during Wake

After observing brainstem LFP power modulations between states, on both a gross level of arousal scoring (AASM) and during shorter durations of fluctuations in excitability, we explored how brainstem-cortical circuit connectivity may underlie such changes. We focused on the BAAN contacts (network of maximal diurnal

modulation) for states of SWS and quiet wake, given the overt behavioural correlations and distinct oscillatory profile. We selected wake and SWS (NREMS2/3) AASM epochs for comparisons (not just the concentrated activity snippets) and centred around EEG areas with maximal SWA to determine excitability-related differences. First, state-dependant modulation of imaginary coherence (ICoh) values between EEG channels (with maximal SWA power/ potential 'generators') and the selected BAAN contacts of brainstem was examined. For each channel combination, the ICoh values were calculated first. Then, the average of ICoh within all frontal combinations was calculated per state and each patient hemisphere. In the end, the cluster permutation method was used for all eight hemispheres. This revealed a significant increase in gamma ICoh during wake (compared to SWS), with the cluster of maximal importance centered around 68Hz (range 63-74Hz, p= 0.025 t= -3.1533). No statistical significant changes occurred for ICoh within the low gamma or beta frequency range for all gross epochs for these EEG channels.

Subsequently, ICoh values for interactions with all prefrontal and frontal channels (not just the ones with maximal SWA) were calculated during both SWS and wake and compared. This was performed in order to provide a more detailed picture of the functional connectivity between BAAN and frontal cortex. Gamma ICoh between cortex and brainstem was significantly higher during wake. This occurred again within the high gamma frequency range, especially for 61-64Hz (p= 0.0060 t= 4.6370). No statistical significant changes occurred for ICoh within the low gamma (30-55Hz) frequency range. There was an increase in ICoh between frontal cortex

and brainstem increased during sleep compared to wake for this collective channel group (unlike the frontal and prefrontal channels with maximal slow wave activity) for frequencies 15-16Hz (p= 0.001, t= 2.6439).

Exploratory Analyses: Beta and Gamma Information Flow from brainstem to cortex during Sleep correlates with Sleep Fragmentation

To further explore circuit connectivity during the two states, as well as identify any possible links between PPNR activity and fragmented sleep, we additionally looked at changes in information flow directed from brainstem to cortex. We therefore calculated Granger Causality (GC) during NREM and quiet wake epochs, analysing it as described previously (*Chapter's Methods*). We subsequently assessed sleep fragmentation and continuity in our cohort. We noted that in all cases there was poor sleep continuity; maximally only 1/3 of the sleep period (from first to last sleep epoch) consisted of continuous sleep epochs uninterrupted by ISARs, wake and significant artefact (Table 2).

Table 2: Sleep fragmentation indexes per patient. Calculated for the post-operative night (after recovery from Stage 1) when brainstem LFPs were sampled for analysis with simultaneous polysomnography. WASO: Wake After Sleep Onset.

Patient ID	WASO Index (%) ǂ	Continuity index (%) *
PPNR1	**24.48**	16.72
PPNR2	**13.75**	25.21
PPNR3	**12.44**	27.80
PPNR4	**34.65**	16.16

ǂ Percentage % of wake epochs/total Nº epochs from the first NREM episode
*Percentage % of epochs in continuous sleep episodes (exceeding 10 mins in duration)/ total Nº epochs from the first NREM episode.

We then calculated WASO indices per patient and subsequently pooled all wake and sleep epochs according to the degree of sleep disruption identified (*Chapter's Methods).* In two of our patients, WASO was < 15% (suggesting minimal presence of frank arousals after sleep onset), while one patient had moderate sleep disturbance (defined as WASO 15-25%) and one patient's WASO exceeded > 25% signifying severe fragmentation.

When epochs were pooled per category, we noted that there were no statistically significant differences between degree of sleep disturbance (WASO category) and values of wake Granger's Causality (GC) estimates of information flow from brainstem to frontal cortex channels ($p>0.1$ in all cases). However, in an exploratory analysis, we saw that increasingly pathological WASO was correlated to higher values of beta GC during SWS, with each tier/group having sequentially higher GC values than the prior (less severe) one ($p<0.0001$ with Bonferroni correction for all case comparisons, *Fig. 11 and Table 3)*. With regards to gamma GC, the

relationship between WASO index severity and increase in GC during SWS was less clear; although there was a significance in the interaction between WASO group and sleep status (Table 3), with the lowest WASO group having the lowest relative values of GC during sleep, the increase was not sequential for the next two groups.

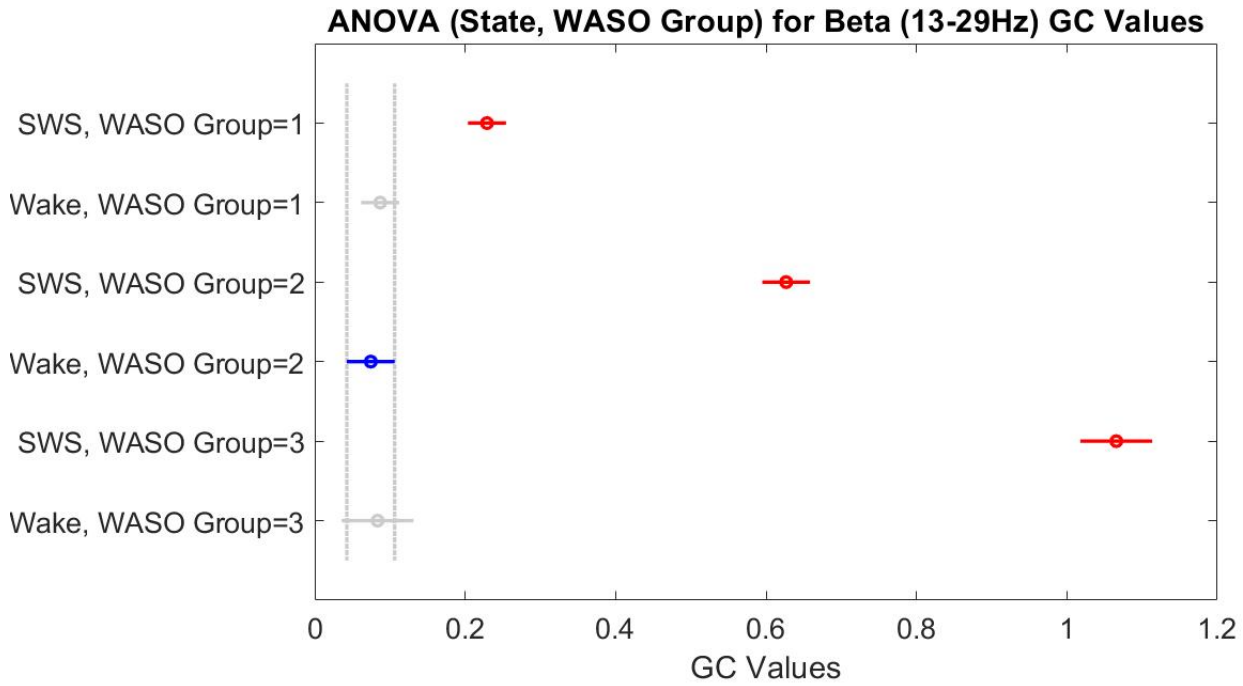


Figure 11. GC values increase during SWS in the beta range, correlating with degree of sleep disruption (WASO index). WASO Group 1 characterised by <15% of wake after sleep onset epochs, WASO Group 2 15%-15% indicating moderate sleep disruption and WASO Group 3 wake epochs >25% indicating severe sleep disruption. Groups between dashed lines: no statistically significant difference between sub-groups (here shown in blue and grey, with a vertical line standard error for the category surrounding the mean). Red: statistically significant difference between sub-groups.

Table 3: ANOVA results for comparisons between GC values for different WASO categories. WASO Groups as reported in the caption for figure 11. Sleep status: wake and NREM (2 and 3).

Beta (13-29 Hz)			
	df	**F**	**P**
Sleep status	1	$1.6040e^{3}$	$1.6227e^{-316}$
WASO status	2	301.6873	$1.7144e^{-126}$
Sleep status * WASO status	2	313.6100	$2.9371e^{-131}$

Gamma (30-95 Hz)			
	df	F	P
Sleep status	1	$4.0500e^{3}$	0
WASO status	2	108.8033	$9.5829e^{-48}$
Sleep status * WASO status	2	165.1184	$6.7216e^{-72}$

When differences in gamma and beta GC for both sleep and wake were examined between patients, we noted that there was no significant difference in wake GC values between any of our patients, across all examined frequency ranges ($p>0.1$ in all cases). However, we noted that in all cases information flow increased significantly across the beta and gamma ranges during SWS ($p<0.00001$ in all cases, after Bonferroni correction), suggesting the presence of a common process active in all patients. We also observed that overall, the highest values of beta and gamma GC during SWS were present in the case with the poorest capacity for sleep maintenance as signified by lowest sleep continuity score and highest percentage of wake after sleep onset (*Fig. 12 and Table 4*).

In order to be able to correlate the degree of sleep fragmentation to the baseline physiology of the cohort as much as possible, all recordings were performed when pre-admission sleep behaviours were re-established, based on both subjective

patient report and nursing observations. This meant that for one participant (PPNR4) there was a more prolonged interval between recordings (ten days from baseline pre-operative recording to baseline post-operative recording), due to a prolonged post-operative recovery secondary to respiratory sequelae. Although baseline behaviours were captured in all cases, one patient did not have a pre-operative baseline recording due to NHS bed pressures and limited pre-admission capabilities. However, in this case, the patient belonged to a low WASO category and did not have any post-operative complications or subjective persistent sleep disturbance.

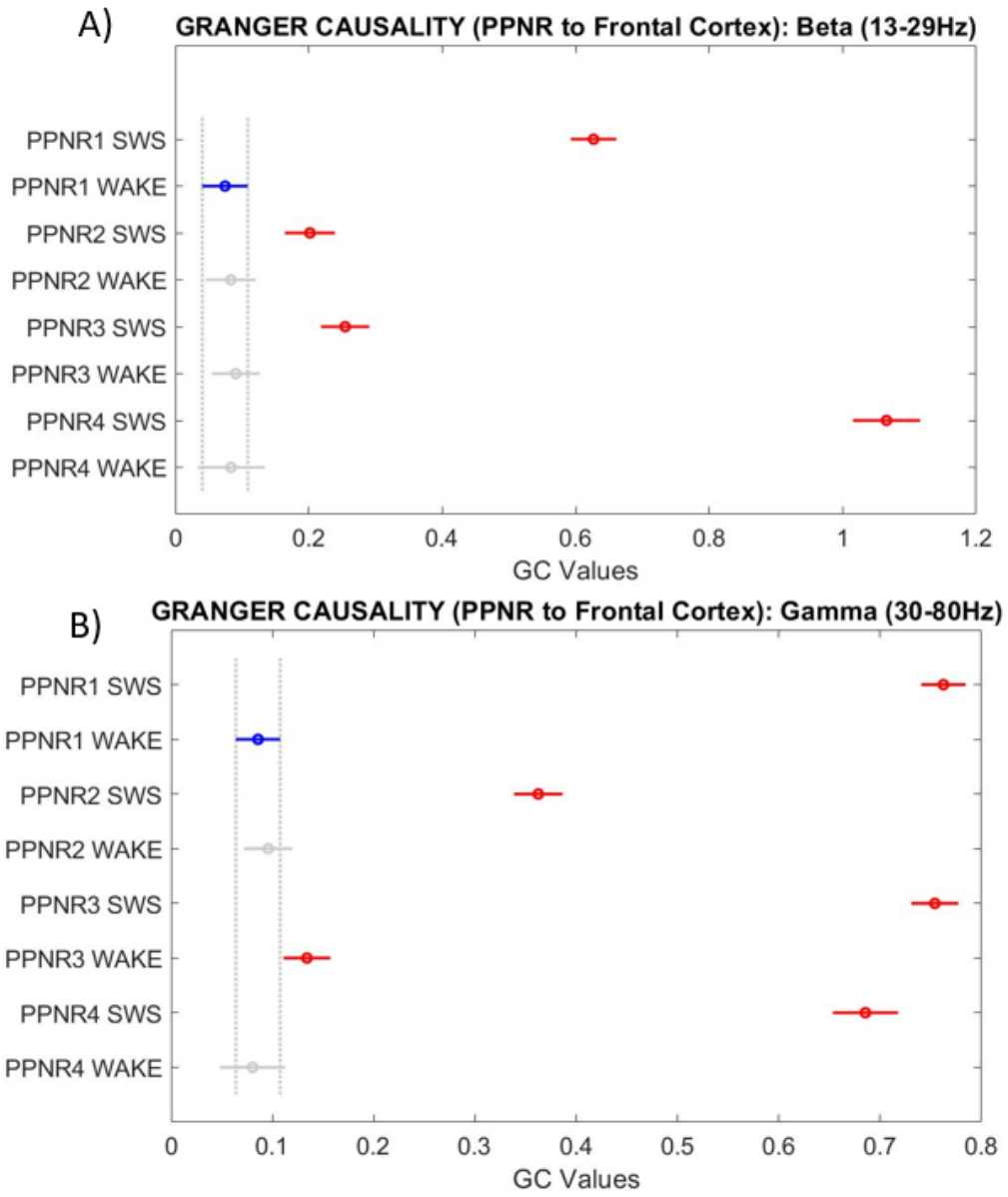


Figure 12. GC values (PPNR to Frontal Cortical EEG) for beta and entire gamma range. WASO values and sleep continuity indices per patient identity are recorded in Table 2.

Table 4: ANOVA results for comparisons between GC values for different patients (and Unified Multiple System Atrophy Rating Scale –UMSARS- status). Each patient is characterised by a different UMSARS total score of history and motor examination. PPNR1: 32 points, PPNR2: 35 points, PPNR3: 50 points, PPNR4: 67 points. Higher score indicates greater disability. Breakdown of this total score to score per history and examination can be found in the chapter's appendix table. Sleep status: wake and NREM (2 and 3).

Beta (13-29 Hz)			
	df	**F**	**P**
Sleep status	1	$1.2782e^{3}$	$1.2672e^{-257}$
UMSARS status	3	202.3065	$6.3467e^{-126}$
Sleep status * UMSARS status	3	209.7880	$2.1152e^{-130}$

Gamma (30-80Hz)			
	df	**F**	**P**
Sleep status	1	4435.79	0
UMSARS status	3	166.31	0
Sleep status * UMSARS status	3	151.09	0

In addition, although (as discussed in *Methods*) the concept of recordings after a 72 hr minimum period of recovery is based on considerable evidence across species and over decades, we sought to further verify whether –for our first case (PPNR1), which exhibited significant WASO and the recording was performed at 72hrs from operation- there were obvious post-operative electrophysiological sequelae linked to a higher posterior dominant rhythm (PDR) presence. We did not identify significant differences in PDR presence for the first two hours after sleep onset (NREM1 in both cases) (*Fig. 13)*, while total sleep time and number of arousals was also comparable. Therefore, we did not see a need of alteration in the planned schedule of recording events per patient, and proceeded to schedule post-operative baseline recordings in keeping with behavioural observations.

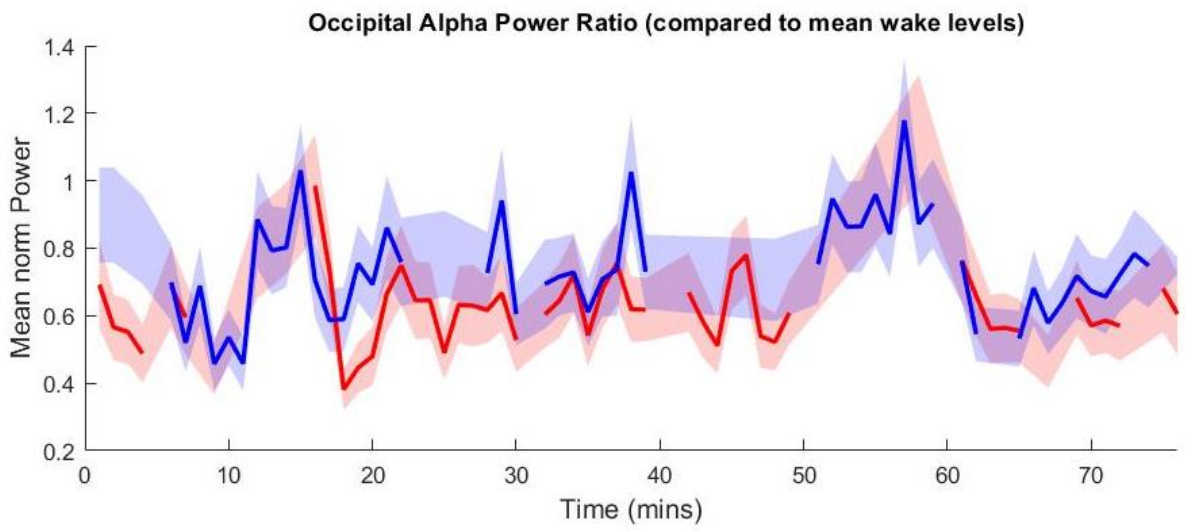


Figure 13. *Occipital Alpha Activity (PDR) comparisons between pre-operative and post-operative baseline (PPNR1). Time comparisons from NREM1 onset in both recordings, while alpha power (8-12 Hz) has been normalised to mean wake alpha power per AASM epoch during an equivalent time of quiet wakefulness. Where there was artefact with a duration greater than 1 minute (over 3 AASM epochs), the mean is not visualised with a bold line in the graph, to indicate interpolation –however the standard error (SEM) is shown. Blue: Pre-operative baseline. Red: Post-operative baseline. Values above/adjacent to 1 suggest the presence of arousals.*

There is a lack of data with regards to baseline daytime sleepiness and relevant sleep evaluation scales commonly used in Parkinsonian conditions, since they had not been collected during recruitment or clinical interviews by the trial neurologist (Supplementary Table 1 at the Chapter Appendix). However, since there was no planned longitudinal follow-up of these features for the cohort of this chapter, the lack of such data did not impede analyses (*in contrast to Chapter 3, where this data was collected by the author)*.

Discussion

We explored the link between MSA and sleep disorders in the introduction of this thesis, mentioning the evidence of neurodegenerative changes in brainstem/RAS nuclei in Parkinsonian conditions based on imaging studies. We also presented evidence of how connections between these nuclei, in addition to their complementary arousal-related functions, might lead us to consider network activities rather than each sub-region in isolation. Here we will try to conceptualise our findings with regards to specific subcortical oscillatory dynamics, based on comparisons with existing evidence on human subcortical LFP recordings during sleep (passive recordings without stimulation, since stimulation-related experiments will be presented in the next chapter). We will also discuss some of our findings (albeit originating from a compromised substrate and not healthy circuits) in the light of existing evidence of healthy activity patterns in brainstem nuclei, which has been obtained through various studies of animal electrophysiology and could be potentially preserved across species.

Brainstem circuit activity during SWS and Wake –evidence of universal physiological mechanisms

As previously touched upon during the introduction of this chapter, there is evidence of pedunculopontine region (PPNR) activity clustering around periods of higher cortical excitation. Indeed, very early evidence from animal studies showed that the cholinergic PPN neurons were firing preferentially during wakefulness and REM sleep (M. Steriade, Datta, Paré, Oakson, & Curró Dossi, 1990), which according to

the same group resulted in cortical excitation through enhancement of anterior thalamic synaptic responsiveness (Pare, Steriade, Deschenes, & Bouhassira, 1990). Diurnal firing patterns of the PPN with overall higher firing rates in activated states and relative quiescence during deep sleep were also reported in later studies (Simon et al., 2010). This seems to be a general pattern for multiple neuronal subtypes of the dorsal pons, some pertaining to PPN analogue areas while others distributed in the surrounding brainstem area and closer to the Locus Coeruleus (LC). GABA-ergic and Glutamatergic neurons both showed higher firing rates during wakefulness compared to sleep during in vivo recordings freely behaving mice, despite the fact that these showed different behaviours during transitions from REM sleep to wakefulness (Cox, Pinto, & Dan, 2016). In particular, glutamatergic neurons of the dorsal pontine region showed an increase in firing followed by quiescence, while GABA-ergic pontine neurons were firing at greater rates during wakefulness compared to REM sleep.

Furthermore, there is experimental evidence of orexinergic influence on excitatory activity in adjacent nuclei such as pontine oralis (PO), through which hypothalamic projections may exert a diurnal tone with higher firing rates during wakefulness and REM sleep (for instance in the cat, (Xi, Fung, Yamuy, Morales, & Chase, 2002)). In addition to state maintenance, there activity in brainstem arousal centers provides crucial modulation of arousal threshold in that it is crucial in the evaluation of potential threat across states, as for instance shown by lesion studies in the midbrain reticular formation (Hammond, 1973). In terms of translation to higher order mammals, diurnal activity patterns with increased firing of neurons located in the

PPN and adjacent areas, has been shown to be present in primates –therefore showing promise of such concepts being common across species (Goetz et al., 2016).

It has been argued that every PPN neuronal type fires maximally at a beta/gamma range (Urbano et al., 2012), which in patch-clamp recordings from rat pups were attributed to membrane calcium channel activity varying from only N-type, only P/Qtype or both N-type and P/Q-type Ca(2+) channels in various sub-populations

(Luster, D'Onofrio, Urbano, & Garcia-Rill, 2015). Overall, our findings from recordings in the brainstem ascending arousal network (BAAN) of the surgical PPNR area –despite in some cases shown to be located in adjacent, interconnected structures such as PO- support this diurnal preferential activity, with beta and gamma being the main oscillations which are modulated by wake versus SWS state. It would however be reductive to presume that the entirety of neuronal populations in this region follows exactly the same pattern. Indeed, some populations in the region may be preferentially active during NREM sleep (such as GABA-ergic neurons in the parabrachial nucleus, (Torterolo, Sampogna, & Chase, 2011)) and involved in controlling that group of states.

Furthermore, the fact that the PPN itself is a heterogeneous structure, comprised of both cholinergic and non-cholinergic populations, also translates to some differences in neuronal population dynamics in terms of excitatory modulation within slow-wave sleep. In an in vivo study of urethane-anaesthetised rats, where SWA and UP/DOWN states are present mimicking natural sleep, PPN neuronal firing was

primarily coupled to the peak of the UP-State of the cortical slow oscillation (44.2%), however in another main population firing was modulated by the DOWN-State (11.1%) (Petzold, Valencia, Pál, & Mena-Segovia, 2015). Given the fact that the main subcortical region in published literature, which is associated to study of UP/DOWN state dynamics, is the thalamic reticular nucleus and its function in spindle generation (which are coupled to slow oscillations, see (Destexhe & Contreras, 2011)), there is a comparative paucity of evidence with regards to how different brainstem structures organise their activity with regards to the phase of the slow wave. In our cohort of recordings in the PPNR, we found that beta oscillations clustered during the DOWN-state while gamma activity was more prominent during UP-states. This was a finding based on recordings not exclusively in one nucleus/neuronal population, but across nodes of modulation in the BAAN.

Since our recordings were performed in a population with brainstem circuit pathology, where beta oscillatory activity could be linked to dysfunction (see later section in the discussion), it is difficult to say with a great degree of certainty whether these two different oscillatory patterns are a result of two different neuronal populations or influenced by a pathological process. In the case where the first is true, one could theorise that two processes/population samples have joint role in promoting excitation: one first 'driving' transition to excitation (with beta accumulation during DOWN-state). Subsequently, after a threshold is crossed, a second population is activated and permitted to fire during the excitatory phase, whose increased activity maintains it and coincides with the peak of the cortical UP-state (gamma burst during UP-state).

Indeed, the presence of discrete populations with the capability of tuning arousal has been investigated in BAAN nuclei. In locus coeruleus, where a GABA-ergic subpopulation directly inhibits a local activating noradrenergic sub-population based on environmental cues, tuning the nucleus' response and effects on arousal (Breton-Provencher & Sur, 2019). Currently the interactions of different populations of the LC with PPN populations in wake- and REM-promoting interactions are currently been studied, with preliminary data showing that LC neurons can act as a 'switch' to PPN and other activating pontine populations in the adjacent region, releasing their activity during the UP-state (Prof. A. Luethi, unpublished observations and preliminary data presented during the EBRS conference 2022). Additionally, similar interacting gating mechanisms exist between cholinergic populations and GABA-ergic neurons in pontine oralis (PO), where a 'switch' may result in progression to either REM or wake (Xi, Morales, & Chase, 2004). It must be highlighted however that without definitive post-mortem histological analyses, the degree of erosion of such interacting populations due to an ongoing neurodegenerative process (and therefore the level of maintenance of such physiological interactions) is unclear.

A further finding from animal studies that could shed some light on our causality results comes from a non-pathological perspective, from the aforementioned urethane study. There, during pedal pinch eliciting a state without slow wave activity and a greater degree of desynchronised, high frequency cortical oscillations (closer to higher arousal state), it was noted that PPN activity became less synchronous with cortical activity –despite the fact that PPN is more active during wake states. This phenomenon (in PPN and/or adjacent nuclei) could explain a decoupling

between higher levels of a particular frequency in the power spectra during one particular state, and a lack of elevation in levels of coherence with specific cortical regions in that frequency compared to sleep.

'Phasic' PPN neurons were all classified as cholinergic; they tended to respond with increased firing during transitions to wakefulness but thereafter their firing rates relatively dropped and their activity became desynchronised compared to that of the cortex. This same neuronal population was the one whose activity was closely coupled to the phases of the slow oscillation. It could therefore be that our overall higher causality values during sleep, for both beta and gamma oscillations, reflect this phenomenon. More specifically, that the predominant neuronal population that we are sampling shows gamma activity that is most closely correlated to cortical activity during its contribution in grouping of cortical excitation around the UP-state, with higher degrees of desynchronization during wakefulness meaning that this causal interaction is less clear in terms of changes in coherence.

Additionally, the fact that higher degrees of GC between cortex and PPNR are present in cases of greater sleep fragmentation (higher WASO indices) could reflect that there is a greater propensity for such activation to occur and is not contradicting the above hypothesis. Although our findings do seem to fit with physiological RAS and brainstem circuit activity patterns as evidenced from healthy animal data, it is however crucial to conceptualise them in the context of human LFP studies available as well as specific evidence of pathology present in human recordings. This could help us understand how this data can be interpreted in the context of Parkinsonian

syndromes, without the surrogate of animal models, which are often limited in how they can be translated to humans. It can also help us comprehend how this particular dataset can contribute to the existing body of evidence from human subcortical LFP recordings in a novel and innovative way.

Human Subcortical LFPs during Sleep –Evidence in Parkinsonian syndromes and from ARAS targets

One of the earlier motivators of this body of work was the first large-scale multimicroelectrode study of human cortical activity during sleep (in patients undergoing pre-operative evaluation for epilepsy surgery), establishing that animal study findings reporting gamma clustering during UP-states was translated to human data (Van Quyen et al., 2010). Despite the fact that in this study authors speculated that such transient activity was restoring 'micro-wake' windows, this data was not complemented by subcortical recordings of areas modulating wakefulness and sleep.

With regards to comparable human subcortical LFP data, recordings have been obtained during sleep in patients with Parkinson's disease (PD). However, evidence still remains scarce and focused on common DBS targets such as the subthalamic nucleus (STN), despite the first published evidence being issued more than a decade ago (Urrestarazu et al., 2009). In that early study, higher STN beta power during sleep was exhibited in patients with a REM-Sleep Behaviour Disorder (RBD) diagnosis, compared to patients with PD but no RBD. Lower STN beta power during NREM sleep compared to wakefulness was also reported, bearing in mind that the

nature of this study meant that there is no ‘healthy control’ comparator. Beta power modulation by state in the STN, reported in this first cohort, were additionally corroborated by later studies [(Y. Chen et al., 2019; Thompson et al., 2018) also reviewed in (Baumgartner et al., 2021)].

Despite the fact that this body of evidence refers to a different target than the group of nuclei that we are examining, these reports seem to converge with our results on a broader theme. Specifically, our findings of higher beta power in the PPNR during wakefulness also support a broad similar trend of higher beta subcortical power during wakefulness in Parkinson’s and related syndromes (since MSA traditionally falls under this wider umbrella). However, directional interactions between sleep and wake with regards to possible information flow from subcortical regions to cortex were not examined in these publications.

In addition, evidence with regards to oscillatory disruptions in Parkinsonian syndromes with regards to other subcortical targets (directly linked to the ARAS such as the brainstem nuclei or thalamus) remains unknown –the latter a fact noted in more recent reviews of the topic (Zahed et al., 2021). One joint MEG-LFP study examining brainstem-cortical coherence has been performed during wakefulness, reporting that beta coherence within the PPN region (PPNR) was increased with posterior mid-cingulate, supplementary motor area and primary motor cortex during wakefulness (resting data) on the OFF state (without any dopaminergic medications), compared to other cortical areas (Jha et al., 2017). However, no such

PPNR LFP data were obtained during sleep for comparative purposes between states.

Given the scarcity of published evidence on human subcortical LFP activity during sleep, we examined publications of RAS-related target activity regardless of condition. In epilepsy, patients implanted with either macro/microelectrode arrays or deep brain leads are monitored in a dedicated in-hospital unit, with video-EEG recordings also covering extensive (over 24hrs) periods. However, brainstem nuclei have not been traditionally targeted for seizure control; a group of regions that are conversely commonly targeted are thalamic nuclei. The most prevalent practice here is selecting anterior nuclei thalami group for stimulation, while the centromedian group (part of the RAS together with centralis lateralis nucleus thalami) are regarded as off-label targets (Fasano et al., 2021).

When this body of existing reports is viewed, one main criticism is that recordings from different regions/nuclei are jointly reported, not due to post-implantation anatomical differentiation (as in, post-mortem/imaging evidence that, despite aiming for a single target, different outcomes were reached) or common electrophysiological patterns sampled from these regions, but rather due to the overall low number of patients accessible for such recordings. As per the general theme observed in studies obtaining human LFPs, patients with implants in different thalamic nuclei are often collected in an effort to increase sample size, even though recordings are performed in discretely different anatomical areas. Therefore, it is more difficult to distinguish any neurophysiological differences due to direct ARAS

projections and/or explore effects dedicated to one group only (that would probably require years of pooling data or multi-center collaborations to reach sample size comparable to existing publications).

This is true from the first-recorded study of thalamic invasive recordings (shortly after WW2, where also a variety of different surgical approaches is described, in a variety of patients and conditions) (Williams & Parsons-smith, 1949) to the latest report occurring over seventy years later, which is a description of activity recorded from both patients with recording electrodes in medial (mediodorsal) and patients with anterior thalamic nuclei implants (Ujma, Szalárdy, Fabó, Erőss, & Bódizs, 2022). An additional issue presents where contacts of one lead/array may span multiple target areas is the possibility of some effects being attributed to volume conduction from one predominant source/activity generator, further confusing the picture (Wennberg, Pohlmann-Eden, Chen, & Lozano, 2002). Despite these limitations however, it is perhaps worth presenting some of the published evidence here, so that activity reported in the thalamic region can also be compared and contrasted to our findings in another RAS-related subcortical target.

Earlier studies examined the presence of differences in thalamic activity patterns between sleep and wakefulness. Interestingly, the first report of such differences came from a single patient with chronic pain, implanted across 14 different thalamic regions where single neuron activity was recorded (including somatosensory and motor regions however, which are not part of the RAS). Overall, tonic firing patterns were reported during wakefulness and replaced with burst firing, suggesting state

modulation of activity (Tsoukatos, Kiss, Davis, Tasker, & Dostrovsky, 1997). Over the decades though, most studies focused exclusively on sleep, performing detailed correlations with cortical sleep architectural events –especially focused on thalamic activity during cortical sleep spindles, since early seminal work by Steriade suggested a thalamic generator for these oscillations (Hu, Steriade, & Deschênes, 1989; M Steriade & Amzica, 1998; Mircea Steriade, 2003). In the context of comparisons with the present study of PPNR, we will however only focus on those studies that examined human thalamic activity (potentially related to ARAS projections) in relations with the phases of the slow oscillation.

Two studies (one including pulvinar recordings and one from centromedian thalamus) examined synchrony of delta (vertex) waves and DOWN-states between cortex and thalamic LFPs. They both reported lags in the emergence of such activity patterns, suggesting a cortical provenance, however the presence of nested oscillations was initially not explored (Mak-Mccully et al., 2017; Velasco et al., 2002).

However, a later study, using the same pulvinar dataset, analysed oscillations nested in and around the DOWN-State in this cohort, reporting that thalamic theta bursts (5-8Hz) preceded and also occurred on the peak of DOWN-States (Gonzalez et al., 2018). A lack of overlap with cortical theta events suggested a thalamic generator as per the authors, while the distinct temporal relationship with regards to slow wave phase was interpreted as theta bursts having a role in DOWN-State generation and grouping of spindle activity (that temporally followed). When anterior and mediodorsal nuclei activity was examined though in the context of the

DOWN/UP-State cycle, it was noted that, although theta activity clustered in the DOWN-State, it was preceded by cortical events. Additionally, it was observed that beta oscillations in the thalamus occurred during UP-States while –importantly- thalamic LFPs did not exhibit significant gamma clustering during the UP-State period (Ujma et al., 2022). The latter lack of effect could be due to the intrinsic properties of human thalamocortical circuits, but also due to the fact that any ARAS effect is diluted by adjacent area activity and/or pathology present in this patient cohort.

In the light of the above, our present study not only adds to the further body of evidence of human cortico-subcortical circuit interactions during SWS (and respectively slow wave phases), but it can also be contrasted to the findings reported in thalamus. In the case of our brainstem ascending arousal network (BAAN)/PPN region recordings, beta and gamma oscillations were significantly anti-correlated and linked to two discrete phases of the slow wave cycle, with UP-States correlated with higher gamma activity while beta activity clustering around the DOWN-State. Despite the fact that beta oscillations cluster around period of low cortical excitability, we theorise that they do not promote/sustain DOWN-States (unlike the authors' hypothesis about thalamic theta). This is based on a variety of cues: the fact that PPN beta oscillatory activity power is maximal in BAAN during wake –suggesting it is a rhythm linked to higher arousal- and the correlation of increases in GC values during SWS with the degree of sleep fragmentation being the ones originating from our data. Additional evidence can be based on animal models of circuit dysfunction, while the fact that beta (wake-related) clusters with

the excitable UP-State in the thalamus in a human cohort where there is no pathological sleep also seems to be pointing towards this direction.

Beta and Gamma Hyperactivation during SWS -Indicators of Pathology

Evidence of increases in burst firing of PPN and adjacent regions of caudal mesencephalic ARAS during 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication has been reported in primates, where this compound was used to damage the nigrostriatal pathway and induce Parkinsonian-type symptoms (Goetz et al., 2019). This fact further supports that the pattern of hyperactivity in these regions, correlated to sleep pathology (as evidenced by increased beta information flow with increased WASO indices in our study) is due to a pathological neurodegenerative process, present in MSA. There is additional experimental evidence linking subcortical increases in beta connectivity during sleep and pathology. In a relatively recent study of Parkinsonian primates (MPTP-induced model), beta coherence between basal ganglia and cortex increased prior to spontaneous awakenings compared to healthy (non-MPTP) controls, while comparatively higher subcortical beta power during sleep in the affected animals was correlated with decreased NREM sleep and more frequent episodes of fragmentation (Mizrahi-Kliger, Kaplan, Israel, Deffains, & Bergman, 2020).

Despite the fact that we recorded in a network of ARAS targets in humans (a fact that also comes with the conundrum of there being no control ‘healthy volunteer’ group) our findings are following the same vein. In addition, we noted that higher beta coherence during sleep (even compared to wake) was present between frontal

EEG channels and PPN area –however this finding was not significant where EEG channels/regions with maximal slow wave power were concerned. This could perhaps signify that where SWS integrity is highest, pathological beta hyper-connectivity with subcortical areas is less present.

Furthermore, an increase in excitatory information flow from brainstem nodes to cortical areas in a circuit translates directly to higher propensity for cortical oscillatory activity in the respective (beta and gamma) frequency range. Examining the consequences of this from a surface EEG perspective is relatively easier, given the abundance of human data obtained in exploration of beta and gamma cortical power increases and their link to a variety of sleep pathologies. In a high-density EEG study involving two age, comorbidity and medication-matched control groups, RBD patients exhibited higher beta power during transitions from tonic to phasic REM sleep, with higher beta content during phasic REM sleep being proposed by the authors as a factor contributing to higher possibility of pathological arousals in this cohort (Valomon et al., 2021).

Evidence of the fact that higher beta and also gamma oscillatory content is not limited to fragmentation secondary to movement disorders. For instance, patients with primary insomnia exhibited more average NREM activity for Beta-1 (14-20Hz), Beta-2 (20-35Hz) and Gamma activity (35-45Hz) when polysomnography findings were compared with good sleepers/secondary insomnia patients (Perlis, Smith, Andrews, Orff, & Giles, 2001) while intra-hemispheric symbolic transfer entropy in cortical gamma increased during arousal events in patients with OSA (obstructive

sleep apnoea) (Zhou et al., 2020). We may therefore argue that these oscillatory disturbances are a more global marker of fragmentation/circuit dysfunction as opposed to a marker of a specific condition. In our cohort, despite that evidence of directional flow from brainstem network nodes to cortex correlated directly with increases in WASO, findings with regards to gamma GC were not that clear-cut (although the lowest GC did belong to the group with lower WASO scores). This could be directly linked to the small sample size of this study, as well as that the GC was calculated during one externalized night when both LFP and EEG could be captured. Furthermore, although there are common oscillatory patterns and behaviours between the implanted regions, while there is a presence of common neuronal populations in the region, target-related variability and variations of population activity in the gamma range might also contribute to this result.

Limitations of the Study

As also stated elsewhere in this chapter, one main concern with regards to the generalization of such findings is the fact that they were obtained in a compromised substrate, where neurodegenerative circuit changes are a given. Unfortunately, this cannot be overcome, since it is ethically impossible to perform invasive recordings in healthy human participants. Comparison with another control group (non-neurodegenerative) would be feasible should PPNR be targeted in conditions other than PD or MSA. We hope that data gathered during our upcoming BRAINSTATE trial, which will focus on minimally conscious state in younger populations, may answer some comparative questions with regards to what portion of these findings can be translated across a range of pathologies (“Funding to investigate role of

brainstem nucleus in human consciousness," n.d.). Furthermore, direct comparisons in small sample sizes might be further complicated due to the variable structures that can be accessed within the PPNR surgical triangle, depending on surgical technique and regional anatomy within each patient. With definitive confirmation of lead location being possible only post-mortem, there is a degree of uncertainty with regards to which exact neuronal populations are being sampled.

Another limitation has to do with the joint interpretation of functional and effective connectivity results in the cohort. As discussed previously in the chapter, Granger causality as a general methodology presents specific challenges in its directional interpretations, even in model order-free approaches. In addition, it must be highlighted that in this chapter we present interactions from brainstem targets within the PPNR to frontal and prefrontal areas only (since the overall focus is cortical slow wave activity). Furthermore, comparisons are made on data scored based on AASM epoch guidelines (with a 30 sec scoring window). Although wake was juxtaposed with NREMS 2 and 3, the level of fragmentation in this cohort implies that there might be an element of micro-arousals present in those epochs. The author is currently following up these observations with a more in-depth analysis, involving both a broader spatial exploration in terms of EEG regions, as well as a more fine-grained temporal approach. Additionally, a more detailed study in terms of post-implantation recovery across various targets is under way.

Although recordings through externalized leads have the great advantage that prolonged, high-quality data capture (over multiple hours) can be obtained in perfect

synchrony with EEG for connectivity analyses, a significant limitation of externalization using the Abbott/St Jude device is that after leads are connected to the battery, LFP recordings are no longer possible. Therefore, we had no way of longitudinally measuring brainstem activity and characterizing fragmentation-related oscillopathy over prolonged periods of time and in relation to therapy and disease progression. Such a possibility may exist with novel devices that allow streaming of LFPs (such as the Activa PC&S which is more widely used in the USA or the DyNeuMo device (described in more detail in later chapters) in the UK).

However, LFP streaming at present cannot be performed with either of these devices for prolonged periods of time since the battery life is severely impacted with repeated drainage –a fact that is prohibiting full overnight recordings. In addition, synchronization and quality of streamed LFPs is greatly problematic, with synchronization requiring co-acquisition in a mutual registering computer which is currently not facilitated by device manufacturers, whose proprietary software is installed in dedicated tablets provided by those companies with programming as their main function. Additionally, sampling rates during streaming are considerably lower that what is possible in externalised cases, while recording quality is much lower and contains significant artefacts (*further elaboration in Chapter 3).*

The fact that externalization allows for short-term prolonged recordings of optimal signal quality could be capitalized by multiple days and nights devoted to EEG/LFP recordings. However, there are two main roadblocks to this, even in the case where patients are happy with and formally consent to protocols with prolonged periods

between the two stages of their operations. The first being incurring additional cost for prolongation of hospital stay, as well as the fact that –in a climate of severe NHS austerity and crises with regards to bed availability- appropriately purposed bed space may be unavailable for such prolonged periods of time. Since these patients require nursing care and monitoring between their operations, this issue cannot be easily solved without utilizing dedicated clinical space, required for epilepsy-related polysomnography. This was prohibitingly inaccessible during the immediate post-COVID climate and subsequent wait list crisis in the UK. An additional concern is that prolonged externalization may increase the risk of infection –although there has been published evidence that the risk of inoculation in externalized lead use does not result in higher infection rate compared to both Stage 1 and 2 procedures being completed on the same day (Mostofi et al., 2021). However, such hypothetical risks together with the higher costs and complexity of co-ordination (one must calculate theatre and staff use over an additional day etc) meant that we could not study patients in our cohort for a prolonged period of time.

The small sample size (and lack of a control group) is a definitive limitation with regards to generalization of the findings –despite levels of significance. Unfortunately, another consequence of the concerns around externalization of patients means that this is now no longer practiced regularly in our Trust (over one and a half years since the pandemic there had only been one case of an externalized tremor patient, which was permitted based on complexity of aetiology and need for prolonged programming sessions). In addition, our new MSA trial (with arousal-related investigations described in later chapter) was not designed around

externalization due to the use of a novel investigational device (DyNeuMo device) for which externalization kits are still not available (*further on this during the third experimental chapter*). We do hope however to resolve device-related limitations to an extent and expand this cohort accordingly, including -at the very least- brief synchronized recordings in MSA patients with DyNeuMos. Another option would be outsourcing to centers that still practice externalization in this cohort, a feat that would involve seeking further funding for international collaborations.

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Chapter 1 Appendix

Supplementary Table 1: Patient Enrolment Variables and Demographics for this chapter

Score/Variable	PPNR1	PPNR2	PPNR3	PPNR4
Age	72	74	54	65
Sex and Gender	Male	Male	Male	Male
Disease Duration	2 years	3 years	7 years	4 years
Disease Subtype	MSA-P	MSA-P	MSA-P	MSA-P
UMSARS Hx	20	18	23	27
UMSARS Ex	12	17	27	34
COMPASS-31	57	31	30	33
RBD Dx	No formal Dx	No formal Dx	No formal Dx	No formal Dx
RBDSQ	N/A	N/A	N/A	N/A
PDSS	N/A	N/A	N/A	N/A
ESS	N/A	N/A	N/A	N/A
Stimulants (current or prior use of amantadine or modafinil)	No	No	Not current (prior trial of amantadine, discontinued)	No
GFQ	21	12	27	28
FOGQ	12	10	17	17
PDQ-8	N/A	N/A	N/A	N/A
ADL	80%	70%	50%	50%
EQ-5D VAS	50	70	30	37
Zarit Burden				
ACEI-III	-	100	93	93
MOCA	25	-	-	-
HAD Scores	HADS-A: 10 HADS-D: 7	HADS-A: 8 HADS-D: 4	HADS-A: 5 HADS-D: 13	HADS-A: 2 HADS-D: 9
L-Dopa post-surgically	No	Yes	Yes	Yes
L Dopa cumulative dose (if yes)	-	300mg DA	800mg DA	100mg DA

Abbreviations: MSA-P: Multiple system atrophy- parkinsonian type, UMSARS: Unified Multiple System Atrophy Rating Scale, COMPASS-31: Composite Autonomic Symptom Score-31, RBD: REM-Sleep Behaviour disorder, RBDSQ: REM Sleep Behavior Disorder Screening Questionnaire, PDSS: Parkinson's Disease Sleep Scale, ESS: Epworth Sleepiness Scale, GFQ: Gait and Falls Questionnaire, FOGQ: Freezing of Gait Questionnaire, PDQ-8: Parkinson's Disease Questionnaire, ADL: Activities of Daily Life, EQ-5D VAS: EuroQuol-5D Visual Analogue Scale, ACE I-III: Addenbrooke's Cognitive Examination I-III, MOCA: Montreal Cognitive Assessment, HADS, DA: Dopamine, Dx: Diagnosis, N/A: Not Available.

Chapter 2

Introduction

Our explorations of endogenous brainstem arousal circuit activity, as delineated in the previous chapter of this thesis, cannot lead to a well-rounded understanding of such circuit function in the context of neuromodulation of wakefulness and sleep if examined in isolation. It could be argued that, should one wish to optimise and/or build on an existing system of invasive neurostimulation, objective neurophysiological evidence of perturbations' effects on oscillatory activity should be obtained, in addition to an evaluation of endogenous baseline dynamics. Subsequently, a more direct way of tuning a system of state modulation may be attempted based on the combination of such evidence, as opposed to current practices of trial and error of a variety of possible settings. Therefore, an endpoint in our externalised patient population cohort was testing the effects of stimulation parameters on slow wave sleep, following a controlled protocol within clinically permissible boundaries, which would allow for efficacy evaluation.

The experimental period, when both local field potential (LFP) sampling and delivery of stimulation during sleep would be feasible in this population, was limited to each admission period between Stage 1 (implantation of leads) and Stage 2 (internalisation and connection to the implanted pulse generator –IPG). After Stage 2, sampling of LFP from the pedunculopontine nucleus region (PPNR, as defined in the prior chapter) would no longer be feasible. Furthermore, upon discharge patients

would be ‘switched on’ at a pre-determined clinical deep brain stimulation (DBS) protocol, aimed towards the improvement of autonomic and gait-related outcomes. Therefore they would no longer be stimulation-naïve during subsequent follow-up and it could be argued that plasticity effects on the network (secondary to that clinical DBS protocol, (McIntyre & Anderson, 2016)) could interfere with the generalisation of the results of sleep-related PPNR stimulation trials to wider patient populations. Additionally, it would not be possible to wait for baseline LFP data collection for the cohort and subsequent group analyses, prior to deciding on common stimulation parameters (namely, stimulation frequency and pulse width). Subsequently, we first examined both available published evidence of human stimulation studies in the pedunculopontine nucleus region (PPNR) and adjacent nuclei, as well as some relevant animal studies.

The effects of subcortical stimulation of cholinergic regions on cortical dynamics and behaviour have been examined during a period spanning over the last two decades, using techniques with a variety of neuronal target specificity. Initial early evidence comes from trials of electrical stimulation applied to the PPNR in freely-moving cats (as also mentioned in the introduction of this thesis), with increased REM sleep time as a result of PPNR stimulation (Thakkar, Portas, & McCarley, 1996). Since electrical stimulation may affect a number of neuronal populations, this was later followed by microinjections of glutamate in areas with cholinergic PPN neurons, where it was observed that at lower concentrations REM sleep would be induced while wakefulness ensued upon higher levels of imposed excitation (Datta & Siwek, 1997).

Since viral transfection (and subsequent selective stimulation) of cholinergic populations using in vivo optogenetic stimulation protocols affords an even greater degree of specificity, such approaches were later used in rodents, investigating the basal forebrain (Ozen Irmak & De Lecea, 2014) and pedunculopontine tegmentum (PPT) (Cissé et al., 2018; Van Dort et al., 2015). With regards to the latter, cholinergic-specific stimulation of PPT increased number of REM sleep episodes and elicited cortical shifts to theta and gamma activity (albeit in one study without being followed by behavioural arousal). In the case of basal forebrain stimulation, when the light pulse frequency mimicked endogenous firing patterns associated with wake state, behavioural arousal was observed.

While evidence from animal studies converges on outcomes (both across experimental modalities and across species), published results from human trials are sparse, and in some cases even contradictory. In one single-participant report, where longitudinal polysomnographic changes were monitored, continuous PPN stimulation with a frequency of 25Hz was reported to increase total REM sleep time (a finding in keeping with animal data) while over time low frequency PPN DBS reportedly promoted greater nocturnal sleep efficiency (Romigi et al., 2008). On the other hand, another publication where stimulation occurred during the day (two patients, who had been receiving long-term DBS with some variance in their clinical motor outcomes) reported that at 25Hz stimulation, wakefulness was enhanced while when stimulated at higher frequencies (75-80 Hz), the patients experienced bouts of ‘irresistible sleep’ that resembled narcolepsy’ (Arnulf et al., 2010).

One could putatively argue that such a dramatic result could be due to inhibition of the PPNR (since such a result being due to activation of cholinergic neurons would disprove decades of intra-species evidence in addition to well-established cholinergic pharmacology). However there is also evidence that a lesional effect may actually have the opposite result; in a study of 35 Wistar rats where the PPNR was lesioned, sleep became disorganised with higher probabilities of Wake/REM transitions, without any acute 'irresistible sleep' effects (Petrovic, Ciric, Lazic, Kalauzi, & Saponjic, 2013). However, the region implanted in this human study could potentially significantly vary from its rodent homologue and/or what other centres currently use as a target for PPNR implants.

Generally speaking, stimulation parameters utilised clinically in movement-disorders vary from 15-40Hz, with the latter being the preferred frequency in our centre based on a combination of long-term practices and patient reports (Ferraye et al., 2010; Stefani et al., 2007; Thevathasan et al., 2012). At the beginning of our study, an additional compelling argument for the use of a 40Hz stimulation frequency was the published evidence that cholinergic PPN neurons had membrane oscillatory activity that ranged between 40-50Hz based on patch-clamp recordings (Simon et al., 2010). A 40 Hz stimulation frequency in the PPNR (and its potential ability to influence cortical activity in that frequency) is additionally appealing when taken into context with Llinas' theory of cortical binding and in particular published work tying this cortical frequency to wake and dream states (Llinas & Ribary, 1993; Llinás, Ribary, Contreras, & Pedroarena, 1998).

Even in the case where the sum of published evidence with regards to intrinsic PPNR oscillatory patterns could be translated across species, the degree of expected variability that could be present in the cohort (due to different implantation locations, disease state etc) made the presence of control settings crucial in evaluating the effectiveness of a proposed stimulation paradigm. Therefore, we planned for two control settings. First, we selected another stimulation frequency in the gamma range (therefore having some common features with the 'homologue/physiological' oscillatory pattern we wished to evaluate) but significantly higher in frequency, so that it would be far removed from intrinsic network activity. For that purpose, we planned for trials at 100 Hz to be thresholded and delivered, alongside 40 Hz trials, during 'stimulation nights' (*further details in '*Methods'). Additionally, we planned for the 'delivery' of trials where no electrical stimulation would be applied (*further described in 'Methods').* Although this was not a clinical trial, but rather an exploratory study, we took measures to ensure patient blinding. This was both in terms of necessary thresholding of the settings (which also ensured the preservation of a state of sleep) but also in terms of avoiding anticipatory effects with regards to the sequence of experimental events (*further details in '*Methods').

Another variable that needs to be taken into account, before any trials between patients are compared, is the underlying cortical state at the time of stimulation delivery. It has been argued that the effects of subcortical ascending neuromodulatory pathways may vary due to concurrent cortical states present upon pathway activation (Tantirigama, Zolnik, Judkewitz, Larkum, & Sachdev, 2020). Subcortical input needs to be processed by pyramidal neurons, whose dendritic tree

spans multiple cortical layers, while it's coupling with the soma changes during states of decreased arousal, such as loss of consciousness in general anaesthesia (Suzuki & Larkum, 2020). In a sleep-related context with regards to further evidence of variable brainstem ascending arousal network (BAAN) manipulation outcomes through interventions within the PPNR, it has been shown that alterations of orexinergic activity within pontine oralis can have different effects on subsequent REM and wake induction, depending on the administration of micro-injections during NREM or quiet wake (Xi & Chase, 2010).

Based on these indications one could hypothesise that, in addition to controlled experimental state conditions across patients (that is, delivering stimulation trials during a specific sleep stage as per American Academy of Sleep Medicine (AASM) scoring guidelines), cortical state during the epoch preceding trial delivery needs to be robustly controlled for within each patient (where hypnotics and/or other medications affecting sleep depth remain a constant). This was a factor that we decided to strictly control for, selecting not only for trial delivery within NREM during the experimental phase of our protocol, but also controlling for slow wave power within these epochs during subsequent analyses.

Overall, based on the body of published data and should existing animal evidence be translational, we would expect to see a shift away from NREM, towards cortical states with an oscillatory profile linked to lower sleep depth and/or frank arousal, subsequently to a PPNR stimulation protocol within a physiological range. In order to add another layer of understanding of circuit dynamics, we also sought to examine

the effects of PPNR stimulation on brainstem-cortical interplay; therefore, part of our experimental design incorporated the collection of brainstem LFPs at the time of stimulation to calculate connectivity measures. Finally, we also sought to gain some understanding of the mechanism with which the phase of a stimulation frequency interacts with endogenous cortical rhythms present during the stimulation period. This would help us further inform frequency selection for clinical parameters, for the cortical state during which our trials are applied.

Methods

We sought to deliver stimulation trials (homologue pattern/physiological frequency, supra-physiological control frequency, control trials where no electrical stimulation was delivered) during a homogeneous cortical state (same depth of slow wave sleep). To ensure this, both live scoring of cortical EEG during experimental trial delivery as well as post-hoc trial examination took place, during a strict pre-defined protocol.

A. Experimental protocol

Patients' bed space was separated from the experimenter, so that monitoring could be performed in the ante-room space without immediate proximity to the patient, while all stimulation trials could be delivered through remote programming. The latter was also feasible since the stimulation box could be connected to a wire extension of sufficient length to allow connection to the monitoring setup, while –for

bilateral trials- the St Jude/Abbott device also allows for sufficiently prolonged capabilities of remote connection through the internal pulse generator (IPG) implanted in the chest. This –in contrast to other devices with shorter, less energy-efficient capabilities of remote connection- meant that there was no need to physically connect the patient programmer to the IPG (which is done through a dedicated device extension, placed on the patient's body over the IPG site), throughout the night, for any investigations after Stage 2 had taken place.

With regards to stimulation frequency, during stimulation nights the experimental trials were randomized to two gamma stimulation protocols. One was selected on the basis that frequency would mimic endogenous PPN activity, as also previously reported in the literature (Edgar Garcia-Rill et al., 2019; Simon et al., 2010) and mentioned in the introduction; this protocol was set at 40Hz with a fixed pulse width of 60 microseconds. As also stated, for a 'supra-physiological' gamma stimulation of the circuit, a frequency of 100Hz was selected with the same pulse width. Prior to each stimulation night, thresholding of the amplitude of stimulation parameters took place, to ensure that sleep depth would not be changed by sensory/other side effects of stimulation. This was carried out while patients were awake, by incrementally increasing the stimulation amplitude for a defined pulse width and stimulation frequency, until the first amplitude was reached where sensory side effects (such as facial or arm tingling, visual disturbance etc) were experienced by the patient. Subsequently, amplitudes were decreased with a smaller increment until side effects were no longer experienced and the patient could not identify whether the stimulation was switched on or not.

A first thresholding session was performed during a post-recovery admission morning, after baseline post-implantation recordings had been completed. Furthermore, the resulting settings per frequency were briefly tested prior to each experimental night. In addition to preservation of behavioural state, so that any changes thereof could be attributed to network effects of the stimulation, this served as an additional level of patient blinding, since patients would not be able to distinguish between what type of trials (stimulation versus non-stimulation trials) would be administered overnight, based on differences in the testing routine during the periods prior to their bedtime.

In addition to this element of patient blinding, the behavioural state of the patients during the experimental night (asleep) also meant that there was no need to create additional programmes with zero amplitudes in the device or stimulation box, and 'trigger' them during non-stimulation trials. This is a common practice in clinical DBS studies while patients are awake, with such non-stimulation trials termed 'SHAM'. Instead, we proceeded to indicate the start of such a non-stimulation trial by pressing a marker button on the experimental laptop, indicating its presence in the recording. It is worth noting that in animal studies during sleep (where stimulation is controlled remotely through a headstage connected to a computer), this planning factor for 'SHAM' trials through an external programming device also does not apply. Therefore, we will therefore refer to non-stimulation trials as 'SHAM' in subsequent sections of this chapter.

To fully ensure that ‘SHAM’ trials were examining circuits which were stimulation naïve, stimulation and ‘SHAM’ trials were delivered on separate nights. This was due to the fact that in published literature there was evidence of subsequent changes in sleep architecture following stimulation –while washout periods for post-stimulation neurotransmitter release would not be known a priori. Therefore, to ensure that ‘SHAM’ trials would be exactly comparable as a pool, we did not co-randomise with real stimulation trials. The night of ‘SHAM’ trials always preceded the first stimulation night, during which stimulation was delivered unilaterally through a dedicated stimulation box, while the contralateral PPN activity was captured from the other DBS lead. This was also decided in order to avoid potential carry-over effects secondary to stimulation-induced sleep deprivation, since it has been demonstrated that slow wave activity is very sensitive to prior sleep deprivation with rebound effects (D. P. Brunner, Dijk, Tobler, & Borbély, 1990; Rechtschaffen, Bergmann, Gilliland, & Bauer, 1999).

Additionally, by convention we decided to stimulate the hemisphere corresponding to handedness, while recording from the contralateral hemisphere. Therefore, left PPN was stimulated in right-handed cases (which were 3/4 in this cohort). Although this did not guarantee we were stimulating the patients’ dominant hemisphere, it was performed to account for lateralisation effects as much as possible. Furthermore (as also discussed in more detail in the previous chapter, where it was explained that a minimum of 72 hrs intervened between Stage 1 implantation and baseline passive recordings), a period of over five days followed Stage 1 procedures and all unilateral stimulation trials. This not only ensured that recovery from post-surgical events

would be achieved, but also allowed for baseline recordings and identification of diurnally modulated contacts within patient. Thus, although cohort analyses could not be performed and anatomical localisation was not investigated prior to the completion of the experimental schedule, contact characterisation allowed for the selection of dipoles for programming the pre-selected stimulation parameters and their thresholding, as described earlier in this section.

Besides these unilateral stimulation experiments, further stimulation trials were delivered on a second stimulation night after recovery from Stage 2, when both leads had been connected to the implantable pulse generator (IPG) (Fig. 1, *top panel*). Since Stage 2 procedures involve the connection of previously externalised leads to the chest IPG, not further intracranial manipulations, bilateral stimulation trials followed one night of post-surgical recovery. Sleep pattern recovery was also confirmed through a review of recorded events and after discussion with each patient, in a manner similar to what was described in Chapter 1.

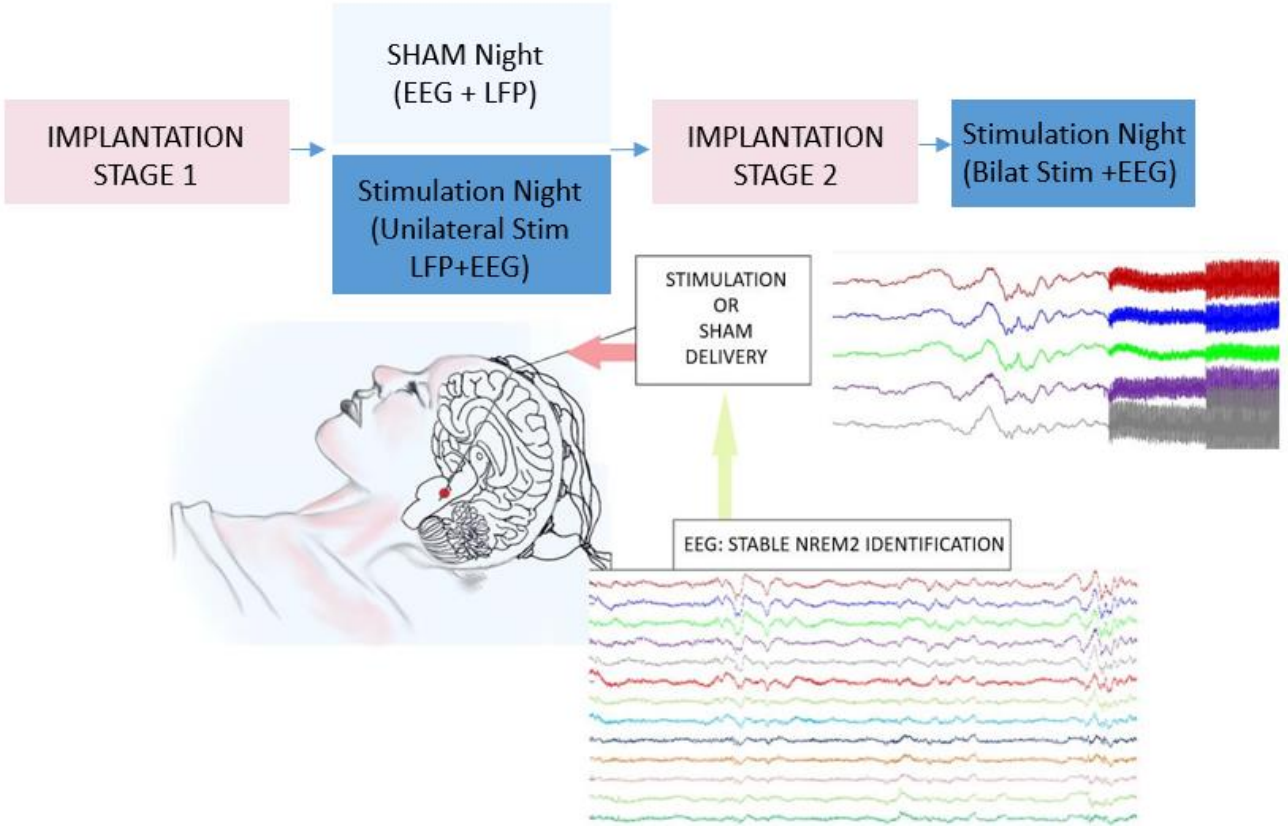


Figure 1. Schedule of experiments and visualisation schematic of an experimental trial. After Stage 1 procedures (and a window of recovery, as outlined in the main text and in Chapter 1 where we described baseline post-operative recordings), 'SHAM' stimulation nights and unilateral stimulation nights were scheduled. Subsequently, an additional night of stimulation followed recovery from Stage 2 implantation. During stimulation nights, upon stable NREM2 identification (note presence of slow wave activity on a real epoch of data, bottom center) as per described criteria, stimulation or a 'SHAM' trial is triggered. Stimulation artefact can be visualized in the data, with the amplitude ramp-up period set at a pre-thresholded value to avoid sensory disruptions (top right, where preceding slow wave activity can also be seen).

For stimulation delivery after sleep induction in both cases ('SHAM' and stimulation nights), hallmarks of NREM2 sleep (K complexes/spindles and slow wave activity) were identified in a minimum of two AASM epochs preceding stimulation delivery during real-time sleep scoring. Upon the appearance of a slow wave in the

subsequent epoch, stimulation was triggered through the patient programmer from the connected ante-room/separated space, where experimental equipment was located (Fig. 1, *bottom*).

Pre-set rules guiding the duration of stimulation for both protocols were set in advance to randomization. Should overt wakefulness occur, the stimulation trial would be terminated, while upon the observation of either stage shifts or Intra Sleep Awakening Reactions (ISARs, defined in the previous chapter), the total duration of stimulation would be limited to a period of up to 3 minutes. Even in the event where no effect would be observed, stimulation trials to be included should not exceed 5 mins of total stimulation duration. Additionally, there should be a washout of at least 5 mins between trials, even if sleep stage remained unaffected.

B. Trial data analysis

Trial data (EEG and LFP) were visually inspected after the experiments to identify artefacts (motion, stimulation-related etc) and optimal frontal channels for analyses, as well as to formally score sleep depth of the epochs preceding stimulation. Exclusion criteria of trial data from further analyses included delivery outside of slow-wave sleep, excessive artefact presence during the post-stimulation period (for instance due to movements, cap disconnection etc) and/or any trials where stimulation duration exceeded standardised protocol parameters. Trial data were assessed with regards to presence of wake, ISARs and stage shifts.

In more detail, for each individual stimulation trial, channel data were visualised after de-trending and filtering (high-pass Butterworth filter (3^{rd} order) set at 0.2Hz and low-pass at 180Hz, with band-stop for 50Hz line noise and its harmonics). Frontal and prefrontal channels were assessed for signal quality and presence of artefact within the three AASM sleep epochs preceding and following the stimulation period, identified both by experimental records (recorded markers) and the presence of stimulation artefact at the DBS frequency.

EEG channels with minimal artefact were then selected and re-referenced (central reference of Cz, FC1 or FC2 where that was not available due to artefacts). In addition, during unilateral stimulation trials, PPN LFP channels of the contralateral hemisphere where maximal diurnal modulation had been identified during passive recordings were assessed for signal quality and re-referenced in the same manner as in prior baseline analyses (described in Chapter 1, where bands of interest, their relevance to wake status and general methods for spectral analyses are also discussed). As a brief reminder, cortical power spectra were calculated for bands of interest, with the Fourier transform parameters being a time window of 4 sec and overlap of 3 sec (since the longest frequency cycle was that of slow waves).

As described, experimental stimulation trials had a pre-stimulation duration of 90 seconds (one formal AASM epoch being thirty seconds, as defined previously), with an equivalent (90-second) comparable time duration after stimulation had ceased. Therefore, as a further clarification, each individual trial would result in 87 discrete values per re-referenced EEG channel for each individual frequency within a band

of interest. For instance, for each frequency step within an 8-12 Hz alpha band, 87 values would be generated for the pre-stimulation period and 87 values for the post-stimulation period. These values could be averaged within band per analysis bin per channel (87 'alpha power' values pre-stimulation, 87 values post-stimulation) for one single experimental trial. Therefore here it is explicitly clarified that one experimental trial does not result in one 'value of interest' (as is, for instance, the case with studies with questionnaire data or laboratory values associated with every trial) –it rather a results in a pool of observations across a cortical region of interest, as per an extended '10-20' EEG channel setup montage (*'EasyCap' setup and associated references in the prior thesis chapter*).

Finally, we only included in our analyses those trials (SHAM or stimulation) that (in addition to the above exclusion criteria) could belong within the same distribution and therefore would not differ to a statistically significant degree in terms of pre-stimulation cortical slow wave power in between the groups. Therefore we would be additionally safeguarding the fact that pre-stimulation cortical states could be considered comparable. This was performed through the exclusion of outliers, defined as those trials whose average slow wave power in selected frontal channels, over three AASM epochs preceding stimulation onset, exceeded the bounds of three standard deviations surrounding the larger group mean.

Functional Connectivity Analyses

We calculated coherence (imaginary component) between cortical (frontal EEG) and brainstem (PPNR) activity for each pre- and post-stimulation period, using the PPNR lead channels where oscillatory power showed the greatest degree of modulation by state (wake/slow wave sleep), as identified in the previous chapter of this thesis. In addition, we assessed Granger causality between PPNR and EEG to determine if changes in information flow from brainstem (contralateral PPNR to the stimulation site) to cortex occurred after gamma stimulation. These analyses were executed using the methods described in the previous chapter (*Chapter 1, where we have also discussed caveats and limitations)*, while computed values were compared between SHAM and stimulation trials.

Phase-Locking Value Calculation

The phase locking value (PLV) was also used to estimate the synchronization of cortical rhythms of interest to the DBS frequency and to subharmonics of that frequency. We denoted as primary 'bands of interest' the alpha (8-12Hz) and beta (13-30Hz) frequency ranges. There was a priori knowledge of the importance of these intrinsic cortical frequencies for excitability and sleep depth modulation, as discussed in the previous chapter. In addition, following all cohort baseline analyses, important findings of beta modulation in the brainstem ascending arousal networks (BAAN) of the implanted PPNR region arose in this patient group. Therefore, the impact of DBS gamma stimulation paradigms on these frequency bands was evaluated. With regards to gamma frequency range, we focused on the lower end

of that frequency band, where the main stimulation frequency was included, to examine the possibility for 1:1 entrainment. Very high gamma (over 90 Hz) is not assessable in scalp EEG due to the higher potential presence of noise, even with very low impedances. Therefore, 1:1 entrainment at a 100Hz frequency range was not examined.

To this end, we first considered re-referenced EEG signals to minimize stimulation artefacts, for the channels where we would examine the effects of DBS on intrinsic activity (Fig. 2). For each individual trial, data were visualized across individual channels to select appropriate re-referencing pairs. Additionally, we examined non re-referenced channels, to identify that with the maximal presence of stimulation artefact. Based on that channel, the number of stimulation pulses was computed by obtaining individual peaks after crossing the threshold of the local maxima, using the raw acquired signal of the channel ('trigger signal' with the maximal stimulation artefact) as the input signal vector for the detection (Fig. 2). The result was verified both by visual examination of each trial as well as by ensuring that the number of peaks detected after threshold specification corresponded to the stimulation frequency that had been experimentally applied (Fig. 3).

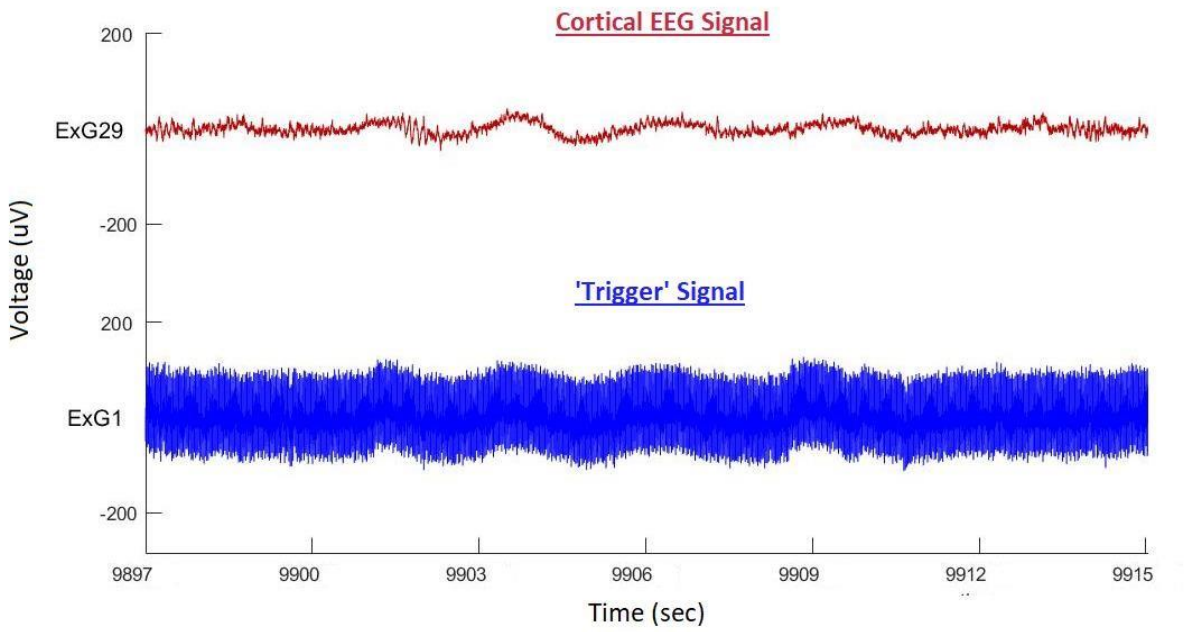


Figure 2. <u>Artefact rejection and 'trigger' signal.</u> Re-referenced pair (channel 29 –channel of interest- in a dipole with channel 12), compared with 'trigger' signal, later used for peak detection (channel 1, not re-referenced). Note how stimulation artefact is not present in the dipole containing the channel of interest, where oscillatory activity can also be visually discerned.

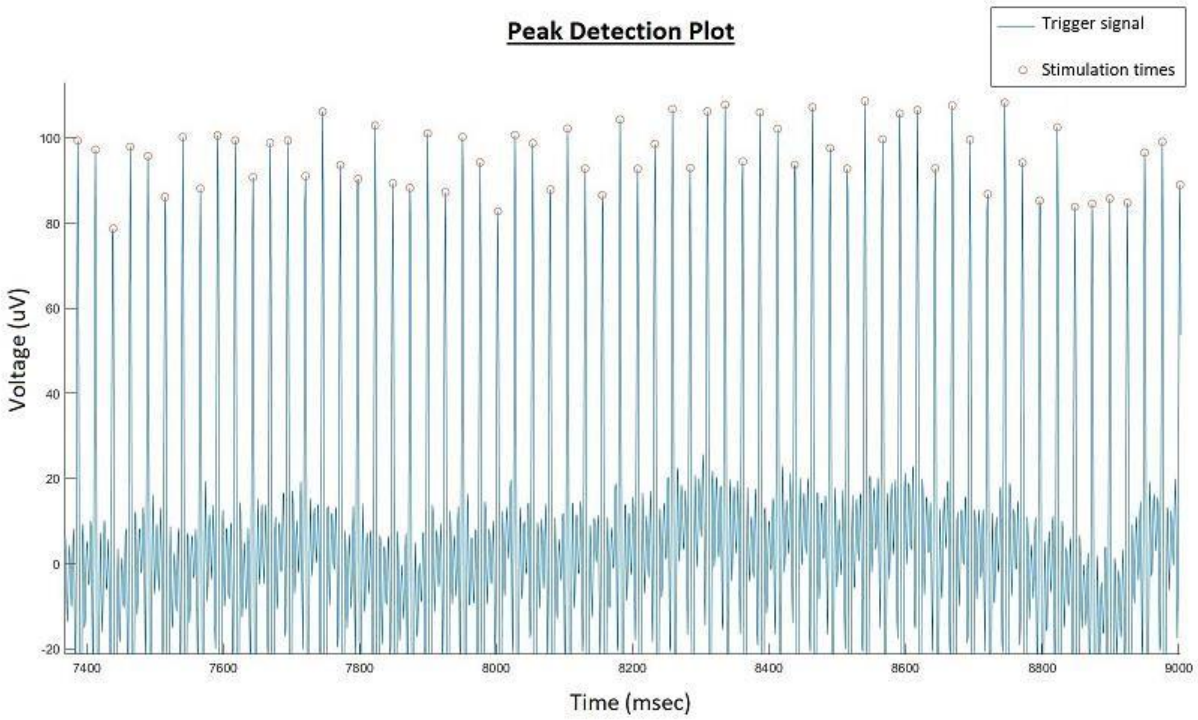


Figure 3. Plot of the stimulation artefact peak detection method. We wished to verify that, for a minimum prominence (threshold) set, stimulation times were correctly identified based on the artefact present in the 'trigger' channel signal.

Re-referenced signals were then band-pass filtered and centred around the half-width of the band of interest. Other comparative bands of interest, in addition to the aforementioned gamma ranges, included alpha and beta oscillations (at [8 12) and [12 30] Hz respectively) –the significance of these cortical frequencies in terms of wake-related context has been explained previously in this thesis. In addition, control signals of pink noise were generated, for the same duration per each trial. Pink noise trials, whose individual duration was equivalent to that of a 'real' trial, were generated in Matlab according to the principle that their frequency decomposition would be that of 1/f –therefore adjacent to the noise floor.

Subsequently, in these trials there would be no peak frequency or differences in the oscillatory activity composition across different bands of interest, as one would expect in physiological data. By comparing phase-locking of endogenous frequencies to applied stimulation with the phase-locking values calculated on pink noise trials one could therefore be able to further differentiate between physiologically-driven results (based on differences in frequency content) and outcomes driven by chance.

In each case of both trial and control (pink noise) data, the Hilbert phase (t) was obtained from the band-pass filtered signal.

To quantify synchronisation to the stimulation frequency f_{stim} (for PLV 1:1) or f_{stim}/p (for PLV 1:p), we calculated for each trial and channel

$$\text{PLV 1:1} = \frac{1}{N}\left|\sum_{k=1}^{N} e^{i\psi(t_k)}\right|,$$

where N is the number of stimulation pulses in one trial, t_k is the time of the kth stimulation pulse within the trial, and $|.|$ denotes the complex modulus. PLV 1:1 quantifies the phase concentration at the time of stimulation, considering all stimulation pulses. To quantify synchronisation to subharmonics of the stimulation frequency, we calculated for each trial, channel, and condition

$$\text{PLV 1:}\boldsymbol{p} = \frac{\mathbf{1}}{\left\lfloor \frac{\boldsymbol{N}}{\boldsymbol{p}} \right\rfloor}\left|\sum_{\boldsymbol{k=1}}^{\lfloor \boldsymbol{N/p} \rfloor} \boldsymbol{e^{i\psi(t_{pk})}}\right|,$$

with $\lfloor . \rfloor$ the floor function. PLV 1:p only considers every p stimulation pulses (t_{pk} is the pkth stimulation pulse in the trial), and therefore characterises phase concentration at the subharmonic 1:p of the stimulation frequency. For each channel and condition, PLV 1:1 and PLV 1:p were averaged across trials.

Statistical analysis

Primary trial data were organised in three main groups, with matched within-patient stimulation laterality: stimulation 'STIM' frequency at 40Hz, frequency at 100Hz and SHAM trials. After individual trial data exclusion, we first ensured that sleep state was comparable for SHAM and STIM (individual group and across both frequency groups) not only by visual scoring of stage but also in statistical comparison of slow-wave power density across pre-stim/SHAM epochs (exclusion criteria as mentioned earlier in this section). To this end we used both student's two-sample T-Test (individual stim group, SHAM) per each frequency band within group, as well as confirmed comparability through a two-way ANOVA across groups, while Bonferroni correction was deployed for multiple comparisons. Once this homogeneity was established, data from the stimulation and SHAM cohorts were further analysed.

Firstly, we used the Kolmogorov-Smyrnov method to test for normality of residuals of the power-in-band for the analysis model. Since the assumption was violated, a Box-Cox transformation was used on the data prior to ANOVA analyses examining differences between protocols in the frequency bands of interest. Again, we used a Bonferroni correction for all multiple comparisons. For connectivity result analysis,

we followed the methods described in the prior chapter and utilised the nonparametric, cluster-based permutation as specified before. With regards to the p-value calculations that this method uses, the same approach based on repetitions of the random partition that was described in the previous chapter was in use here (Maris & Oostenveld, 2007).

We also deployed the Wilcoxon Rank Sum test to compare differences in PLV between pink noise trials and stimulation trials, across different cortical regions and oscillatory frequencies, within the same stimulation frequency group (40Hz and 100Hz) and their sub-harmonics. After element-wise difference calculation of PLV at stimulation frequency of interest with endogenous oscillations minus pink noise, a stratified ANOVA analysis was used for the comparison between pooled PLV values (for both frontal and occipital EEG channels) as well as individual cortical regions, across the different stimulation frequency groups.

Results

During our stimulation experimental nights, it quickly became apparent that –despite the stimulation being delivered below sensory thresholds- PPNR stimulation trials resulted in an increased frequency of stage shifts and sleep disruption, compared to baseline nights (where DBS was not applied). Overall sleep time was truncated, since spontaneous arousal occurred earlier than during the baseline night, with patients unable to resume their usual sleep schedule (*sample case Fig. 4*). Since we had pre-specified that trials would only be included in analyses if the preceding

cortical state was comparable in terms of sleep stage and slow-wave activity, and no sleep cycle of equivalent characteristics could follow, this limited the number of trials we could collect per patient. Where length of stay (hospital bed availability) and patient tolerance rendered it feasible, this constraint was mitigated by trial collection over two subsequent nights.

Additionally, one of the patients experienced an episode of confusional arousal during their first stimulation night (with agitation and associated brief hallucinations upon abrupt post-stimulation awakening). Therefore, the recording of further trials was discontinued for the night. This was a new behaviour as per their report; subsequently no further overnight experimental high frequency (100Hz) trials were delivered during their participation, while furthermore their clinical settings were tested and adjusted so that stimulation remained OFF at night upon discharge (*a further case, where stimulation-induced fragmentation was studied under continuous settings, is discussed in more detail in the following chapter)*. Another limitation with regards to bilateral trial data collection came from the fact that one other case contracted a hospital-acquired pneumonia during their stay, with subsequent deterioration and transfer to the Intensive Care Unit. No further experimental trials were therefore feasible for this patient (save for initial unilateral trial data collection, on a night with severe sleep fragmentation), while this case was also discharged to a local hospital before the second stage of their operation with significant frailty. Therefore, there was no feasibility of performing bilateral stimulation trials for this study.

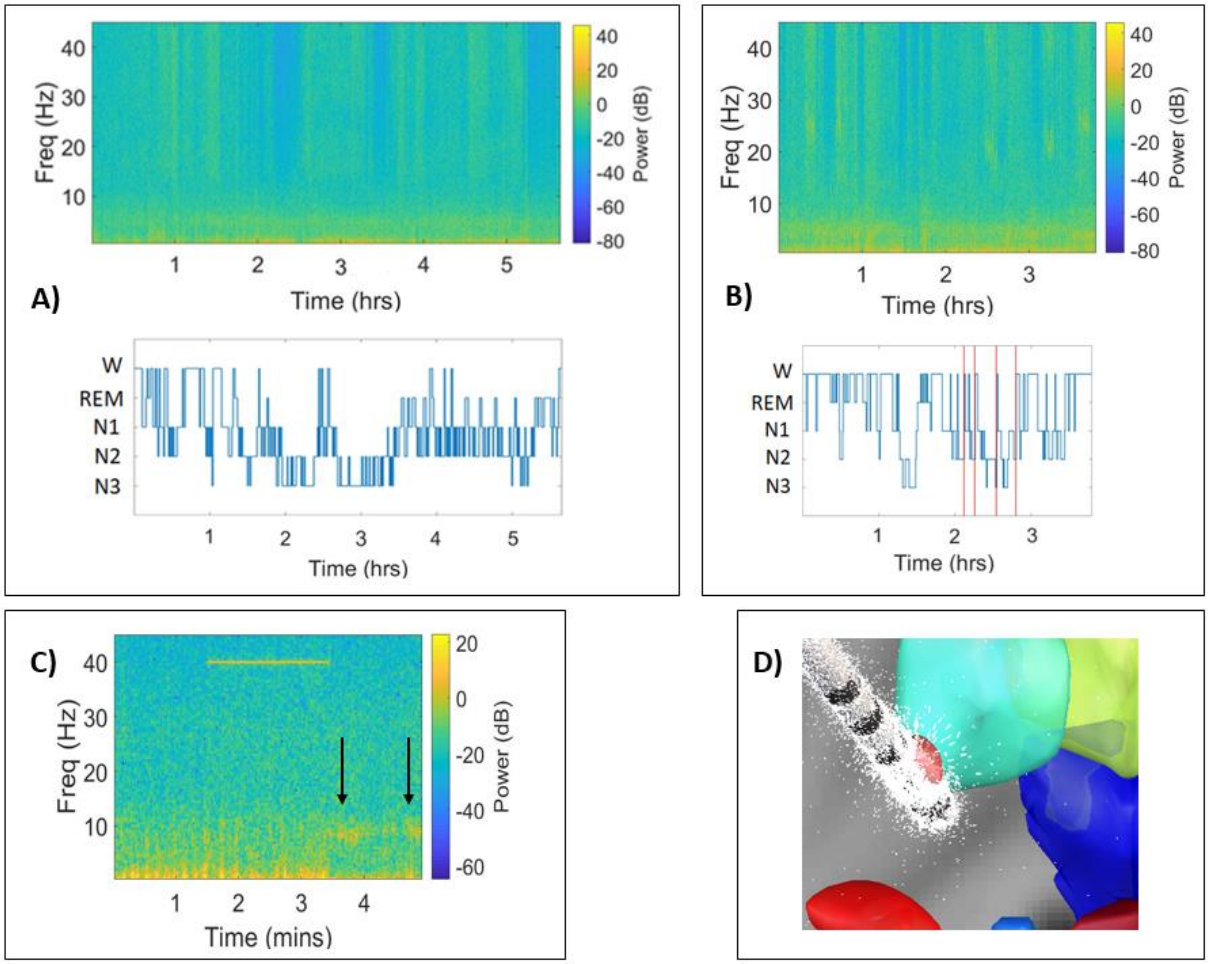


Figure 4 Sleep stage transitions and wakefulness induction through intermittent, bilateral BAAN stimulation. Baseline sleep in stimulation-naïve patient (a). Note the prolonged period of deep slow-wave sleep (NREMS2 and 3) that occurs between 2 and 3hrs 30 mins. This was targeted with stimulation in the bilateral stimulation night (b). Fragmented sleep with multiple arousals during SWS and limited time with slow wave activity during the stimulation night (b). Please also note that time until awakening differs (spontaneous in stimulation night, with behavioural hyperarousal in this patient, compared to previously timed alarm to end recording at 6hrs during the baseline night). A sample stimulation trial at 40Hz (c), where post-stimulation a decrease in slow and increase in faster frequencies can be clearly seen, consistent with an arousal shift. Electric field and volume of tissue activation, showing activation of the midbrain reticular fasciculus (MRF, shown in light blue, with maximally activated area close to the electrode in light red) (d). This was generated based on analysis of patient imaging with the LeadDBS software package.

Brainstem Intermittent Stimulation during SWS alters sleep stage and associated cortical activity

Despite the aforementioned difficulties in acquiring multiple stimulation trials, we applied DBS on 45 cases of discrete trials (total of unilateral and bilateral trials of both 40Hz and 100Hz) during live scoring. However, these were unequally distributed across cases, due to the aforementioned constraints (with considerably more skewed towards trials in PPNR2 –where, notably, lead implantation was localised with the widest margins outside the boundaries of BAAN nuclei). One could therefore argue that should we have pooled all trials that fulfilled post-delivery inclusion criteria, slight differences in implantation depth or patient characteristics might skew the result. We therefore initially sought to assess stimulation effects on a number of comparably distributed cases across our four implanted patients, with the additional clause of stage/slow wave power equivalence within- and across-subjects.

First, we performed a more conservative analysis examining trials matched within and across patients, performed during the externalisation period. Ten cases at 40Hz and ten at 100Hz were selected, with eighteen SHAM trials all delivered during SWS for comparison. With regards to pre-stimulation state equivalence, as explained in the 'Methods', in addition to state equivalence due to visual scoring per formal AASM criteria (sleep scoring, as is the norm in published animal studies) here we additionally examined the distribution of pre-stimulation slow wave power and whether the values of each trial could belong to the same distribution.

This resulted in three of these cases containing outlier values with regards to the total slow wave activity distribution when that was computed over the preceding three AASM epochs, which therefore were excluded. On these trials, when an additional three AASM epochs were subsequently examined (prior to the 90 sec pre-stimulation period of interest), sleep depth had fluctuated over this time period (f.ex. sequentially NREM2, NREM1, W, NREM1, NREM2). We ensured that the final number of SHAM trials as well as stimulation trials for each frequency was matched within and between cases (for instance four cases of SHAM trials, four unilateral cases at 100Hz and three unilateral cases at 40Hz from the same patient, all with comparable sleep depth). The residual trials (ten trials at 40Hz, sixteen SHAM trials, seven trials delivered at 100Hz) were therefore used for further comparisons of stimulation effects, with power spectra calculated as described in 'Methods'. We proceeded to formally analyse changes in slow wave power, alpha, beta and gamma power (using a two-way ANOVA with Bonferroni correction for multiple comparisons).

There were similarities in the changes that ensued after the application of either stimulation protocol (40Hz or 100Hz); changes in keeping with a change in sleep state. We noted that both stimulation protocols (40Hz and 100Hz trials) were characterised by significantly increased gamma oscillations, compared to SHAM trials (*also see Fig.5B*). This was the case for lower cortical gamma (30-45Hz), with $p<0.0001$ for both 40 Hz (CI -0.0207 to -0.0101) and 100Hz (CI -0.0187 to -0.0067) while there was no significant increase in low gamma after the SHAM trials ($p =0.3428$, CI -0.0009 to 0.0072). This was also true for higher cortical gamma (55-

70Hz), with p<0.0001 for both 40 Hz (CI -0.0228 to -0.0117) and 100Hz (CI -0.0218 to -0.00925) and no significant increase in low gamma after the SHAM trials (p =1, CI -0.0042 to 0.0043) (Fig. 5B*)*.

In addition, both stimulation protocols resulted in significant changes in cortical alpha oscillatory power, compared to SHAM trials. In more detail, the 'physiological' stimulation (40Hz) resulted in a very significant increase in alpha power during the post-stimulation period (p< 0.0001, CI -0.0995 to -0.05389). A significant increase in alpha was also noted after the 'supra-physiological' gamma protocol was delivered (p =0.0047, CI -0.0598 to -0.0061). In the case of the SHAM trials, there was no change in alpha power occurring after the 'stimulation' was applied (p =1, CI -0.0256 to 0.0108).

However, the two protocols showed differences based on the magnitude of the stimulation effects on slow-wave activity (SWA) and beta power (Fig. 5A, B). In more detail, the 40Hz ('physiological') stimulation protocol led to a significant decrease of SWA post-stimulation (p <0.0001, CI 0.14397 to 0.3052) and an increase in beta oscillatory power post-stimulation (p<0.0001, CI -0.0314 to -0.0153). In the case of the 100Hz matched stimulation trials, these effects did not survive the Bonferroni correction for multiple comparisons (p =1, CI -0.1854 to 0.3555 and p =1, CI -0.0081 to 0.0121 respectively). In the case of the SHAM group, there was no significant change in slow wave power when the pre-SHAM and post-SHAM periods were compared (p =1, CI -0.08722 to 0.0414) (Fig. 5A*)*.

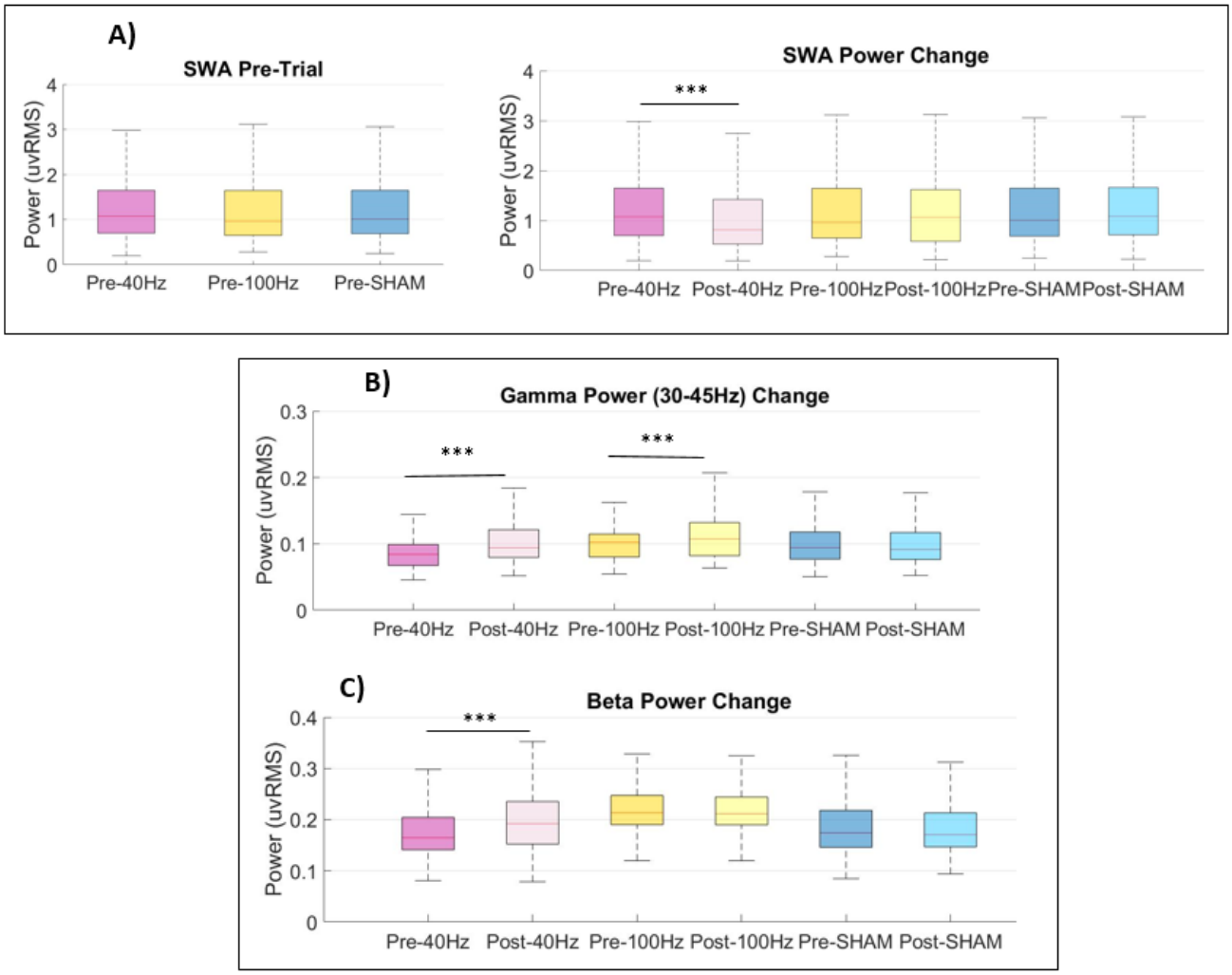


Figure 5. <u>Effects of BAAN stimulation trials on cortical activity and potential underlying mechanisms</u>. Pre-trial Slow Wave Activity (SWA) power does not differ significantly between trial groups (a, left), suggesting comparable sleep depth. Only 40Hz stimulation leads to statistically significant decreases in SWA power, compared to pre-stimulation levels (a, right) Both 40Hz and 100Hz stimulation trials lead to a significant increase in cortical (EEG) gamma activity (b). Only 40Hz stimulation leads to statistically significant increases in beta power, compared to pre-stimulation levels (c).

Given the fact that both stimulation trial types ('physiological' at 40Hz and 'supra-physiological' at 100Hz) showed similar effects with regards to oscillatory and

overall behavioural changes, we subsequently pooled bilateral and unilateral trials of comparable pre-stimulation sleep stage and appropriate distribution (judged both by visual scoring and SWA power comparisons). The subsequent number of trials (n=22 total trials across all patients) was compared with an equivalent number of SHAM trials (whose values of average pre-stimulation slow wave characteristics also belonged to the same distribution and were not outliers), in terms of post-stimulation oscillatory changes. Furthermore, sleep scoring as per AASM was undertaken for these trials, and outcomes in terms of stage transitions were calculated.

In keeping with overall impressions of sleep fragmentation during stimulation nights, during visual scoring we noted that post-stimulation, wake-associated oscillations increased and slow wave activity mainly decreased even when frank wakefulness did not immediately ensue (also reflected in trial spectrograms, *for example see Fig. 4 C*). Overall, arousal-related events (both wake epochs and ISARs) increased by 33.83%. Transitions to REM stage were increased by 16.91% following both stimulation protocols, making this the most likely transition outcome (*Table 2*).

Table 2. Stage transitions following stimulation and SHAM trials. Outcome was evaluated based on the first three AASM epochs following trial duration. Should there be further stage fluctuation post-trial, the first epoch was taken into account (for instance a REM/REM/NREM would be accounted for as REM trial outcome).

SHAM trials	
No stage transition	50%
REM	6.25%
Wake	6.25%
ISARs	18.75%
Stage Depth progression (NREM3)	12.5%
STIM trials	
No stage transition	23.53%
REM	29.41%
Wake	23.53%
ISARs	17.65%
Stage Depth progression (NREM3)	0%

The total power spectral results were in agreement with the previous findings, more limited in terms of trial number, and further corroborated the prior analyses. Specifically, we showed that while there was no statistically significant difference in terms of slow wave activity (SWA) power between the total pre-stimulation and pre-SHAM period ($p = 1$, CI -0.1630 to 0.1237), SWA significantly decreased post-stimulation ($p < 0.0001$, CI 0.2841 to 0.5333) but not post-SHAM ($p = 1$, CI -0.1791 to 0.1435). Additionally, alpha power increased during the post-stimulation period ($p < 0.0001$, CI -0.057 to 0.0306), as well as beta power ($p<0.0001$, CI -0.0185 to -0.0097) and so did low ($p<0.0001$, CI -0.0168 to -0.011) and high gamma cortical oscillatory power ($p<0.0001$, CI -0.0163 to -0.0107). However, there was no change in those frequency ranges during the post-SHAM period ($p>0.1$ in all cases).

Beta and Gamma Functional Connectivity between PPNR and cortex altered by contralateral PPNR Stimulation

We subsequently proceeded to examine the effects of the clinically-used stimulation frequency on circuit dynamics ('physiological' stimulation at 40Hz, which also showed the greatest effect on SWA reduction in matched and controlled trials). For all unilateral stimulation trials at clinical frequency of interest (delivered at subthreshold amplitudes), we compared Granger causality and imaginary coherence values between the contralateral PPNR re-referenced contacts that showed the greatest modulation between states, frontal and prefrontal EEG channels (from both hemispheres) during the pre- and post-stimulation period.

With regards to imaginary coherence values, when all frontal and prefrontal EEG channels were examined, we noted a significant increase in high gamma coherence post-stimulation for the frontal group (59-67Hz, $p = 0.004$ and $t = -4.6007$). When the channels that had exhibited greatest slow wave power per patient were examined separately (those used for investigations of PPNR power changes during slow-wave phase based on EEG, as described during in the previous chapter), we also noted a significant increase in the high gamma frequency range (62-68 Hz, $p = 0.019$ and $t = -3.0495$). We additionally saw a significant increase in coherence in the beta frequency range occurring after stimulation, present close to the half-harmonic of the contralateral stimulation for these channels (14-19Hz, $p = 0.024$ and $t = -2.7488$). We contrasted these findings to those in all pooled 'SHAM' epochs, where in most cases stage progressed (deeper sleep) or remained stable. In this group, we noted a pattern for overall decreased gamma coherence between frontal cortex and

brainstem, as evidenced for both lower (39-44Hz, p =0.014 and t= 2.9957) and higher (75-79Hz, p =0.016 and t= 2.9818) ends of that gamma spectrum (*Fig. 6*).

We then explored changes in Granger causality (GC) values that may occur after the end of the stimulation period, comparing this group to the 'SHAM' trials. This could help us highlight shifts in the activity of afferent connections from the contralateral PPNR to cortex, related to stimulation of one hemisphere. These are potentially contributing to higher fragmentation and thus lower slow wave power as observed in the power spectra analyses described above, occurring after the 40Hz stimulation trials. Post-stimulation periods were indeed characterized by an increase in GC values from the contralateral PPNR to cortex, compared to pre-stimulation trials, with frequencies of increases in flow in both the lower gamma and high gamma range. For the low gamma range, permutation clusters centered at 43Hz and 45Hz, with p =0.014 t= -2.2220 and p =0.002 t= -2.0654 accordingly, while at the high gamma range, permutation clusters centered around 65Hz (p =0.005 and t= -2.5072), 75Hz (p =0.01 and t= -2.2573) and 81Hz (p =0.012 and t= -2.1524). There was no statistically significant change in information flow in the 'SHAM' stimulation group, where sleep depth remained the same for more trials compared to stimulation trials (26.47%), or in some instances even increased.

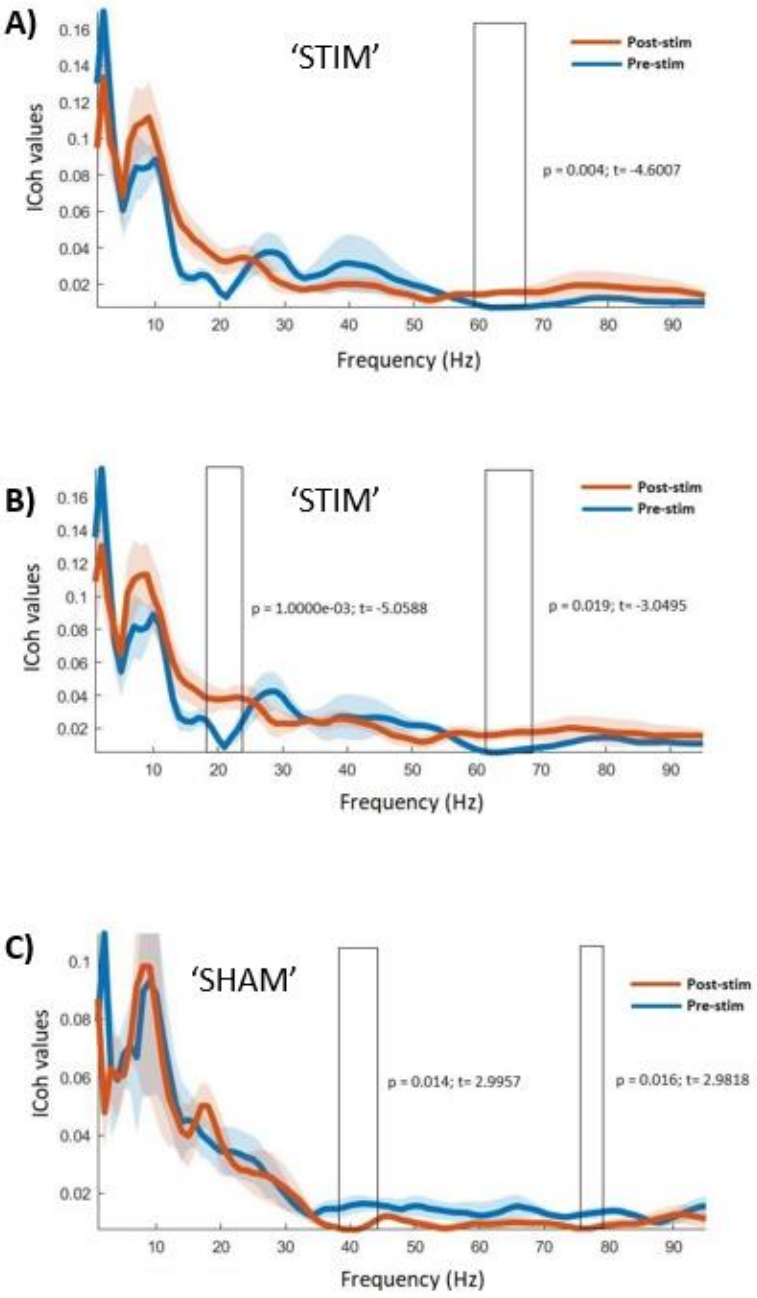


Figure 6. <u>Changes in Imaginary Coherence (ICoh) between PPNR LFPs and EEG, after stimulation (STIM) and SHAM trials</u>. A. For all frontal and prefrontal EEG channels, the post-stimulation period is characterized by a significant increase in high gamma coherence (frequency encased by rectangular shape). B. ICoh between PPNR LFPs and prefrontal channels showing maximal slow wave power. An additional finding here is increase in ICoh in the beta frequency range (close to the stimulation half-harmonic). C. ICoh between all channels (frontal/prefrontal) and PPNR LFPs, during 'SHAM' trials. Here, we note an opposite phenomenon –decreases in ICoh between brainstem and cortex, in the gamma range, as sleep stage in the majority of cases progressed in depth or stabilized. This change occurs in the low gamma (39-44Hz) and high gamma (75-79 Hz). Please refer to the text and figures for p and t values.

We additionally compared differences in Granger causality outcomes between SHAM trials and stimulation trials through calculating a direct comparison between these two groups (as opposed to within-group comparisons), based on the difference between pre-stimulation and post-stimulation values in each case. When stimulation values were directly compared with SHAM, permutation clusters of the most significant differences centered around the half-harmonic of the stimulation frequency (21-25Hz) as well as higher gamma values (65-81Hz). More specifically, significant clusters were identified in a beta range (21Hz $p = 0.001$ and $t = -2.26075$, 23Hz $p < 0.001$ and $t = -2.2803$, 25Hz $p = 0.001$ and $t = -2.21727$) and a high gamma range (65Hz $p = 0.003$ and $t = -2.82713$, 67Hz $p = 0.003$ and $t = -2.82713$, 69Hz $p = 0.009$ and $t = -2.45279$, 73Hz $p = 0.21$ and $t = -2.29470$, 81Hz $p = 0.005$ and $t = -2.61225$).

Cortical Beta and Gamma Oscillations Significantly Phase-Lock to Brainstem Stimulation Frequency

We subsequently examined phase-locking between the DBS stimulation frequency of either protocol and intrinsic cortical rhythms, as this would provide further, direct proof of synchronization at key frequencies, initiating the differences in activity that were observed once the stimulation has stopped. For this purpose, we focused on the stimulation periods of both 40Hz and 100Hz trials, as selected above. We also sought to identify any potential regional differences in this relationship, given that

SWA power and generation differs and is mainly frontal, as discussed earlier in this thesis.

As described in the ‘Methods’ section of this chapter, we examined all EEG channels to identify stimulation artefacts, both for ‘trigger signal’ identification, which involved locating the exact time of pulse delivery comparatively to endogenous cortical phase of the oscillations of interest, and for artefact rejection in channels of interest for analysis of endogenous activity. During this process, especially for the higher stimulation frequency where amplitudes were low (1mA and below) due to threshold maintenance, we had to discard trials where the peaks of pulse delivery could not be reliably identified –despite the fact that a stimulation artefact was present in the power spectra, verifying the validity of the trial (Fig. 7). We selected the re-referenced dipoles so that all stimulation artefact could be reliably removed from the signal prior to further analyses, as described and visualised earlier in this chapter.

We did not try to remedy any issues in ‘trigger’ signal identification by creating an artificial signal (stimulation frequency sinusoid) and timing it to the cortical EEG signal for two reasons. Firstly, since there is a built-in delay of milliseconds from triggering until stimulation delivery within the stimulation box or implanted stimulation generator (IPG) and secondly, since triggering was physician-operated. Although we might assume that an IPG built-in delay is relatively constant, there is a variability where any human element is introduced that could influence precision with regards to phase-relationships. This issue resulted in the exclusion of one of our patients, where stimulation artefact peaks were not reliably identified with neither

visual nor automatic detection (while feedback on this issue was also sought from Dr Benoit Duchet, PhD post-doctoral Engineering researcher, with many thanks).

In the end, we analysed all 40Hz trials from three patients, pooling all 21 frontal and pre-frontal dipoles (re-referenced EEG channels) resulting from these trials as well as analyzing all 18 occipital dipoles as a separate group. We only used trials of the same laterality within case, not combining all bilateral and unilateral trials while examining these effects due to differences in amplitude thresholding, which may potentially impact results. We proceeded in this manner in order to examine regional differences in phase-locking, suggesting a level of variance per cortical brain area in terms of phase/oscillatory synchrony, which would also be in keeping with a complex biological phenomenon.

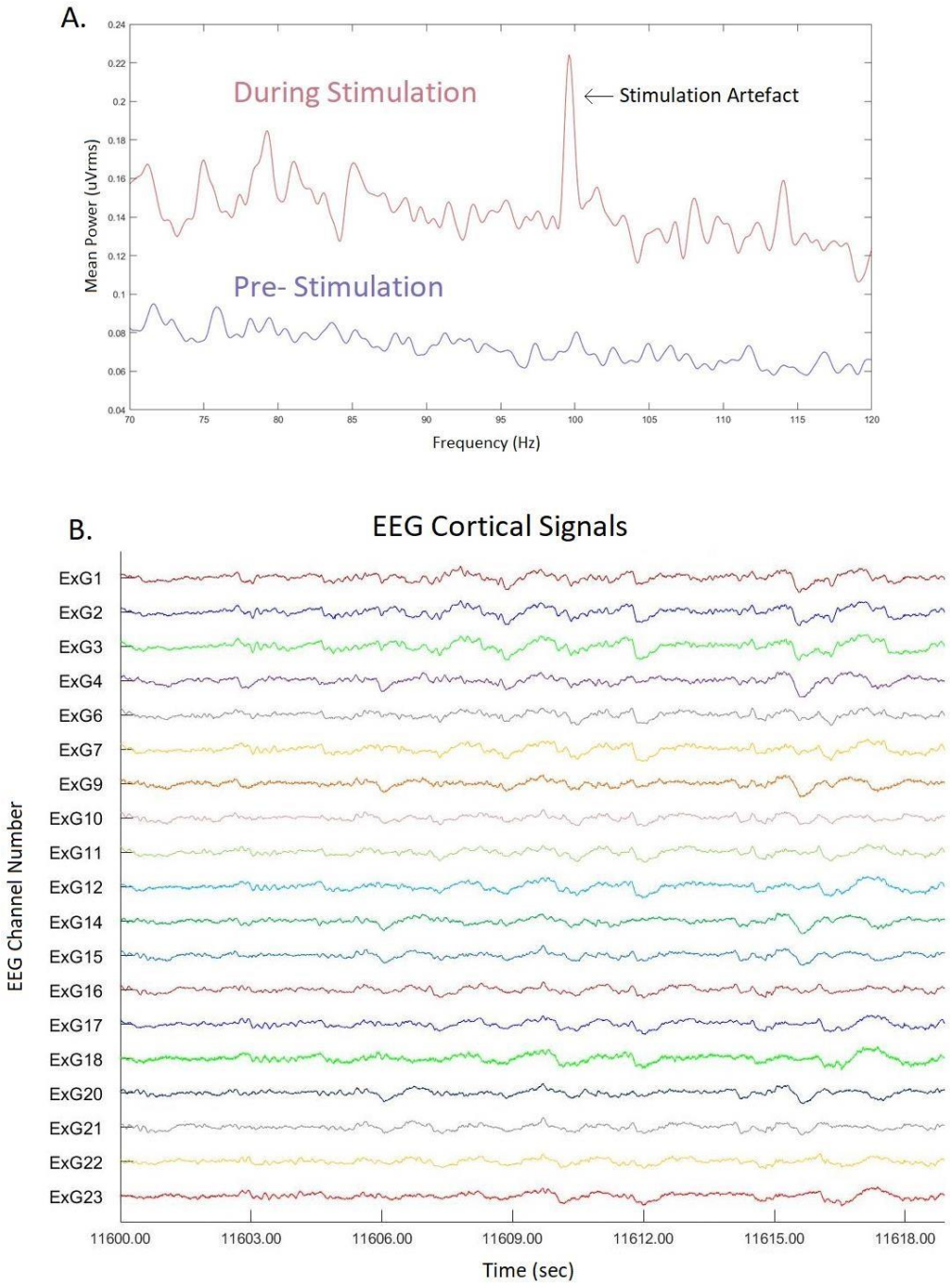


Figure 7. <u>Sample trial where, despite stimulation delivery, no artefact is present in EEG traces</u>. A. Linear plot of power spectra during stimulation delivery (unilateral at 100Hz, thresholded at 0.4mA for pulse width of 60μS). B. Due to the very low amplitudes, no stimulation artefact can be detected in the EEG traces (cortical signals). This trial had to be excluded from phase-locking analyses.

With regards to the cortical frequencies that we investigated, we focused on alpha (8-12Hz), beta (13-30Hz) as well as the part of the low gamma range where the DBS frequency is included (39-44Hz). The rationale being that the capacity of the DBS (imposed) oscillator to synchronise brainstem areas and – should PLV values support this- drive cortical areas in this frequency. We aimed to focus on lower frequency bands during the stimulation period, as opposed to very high gamma frequencies, mainly due to the fact that during DBS stimulation the EEG traces were often contaminated by high-frequency artefacts associated with muscle activity, such as frontalis in forehead areas, as also mentioned in the 'Methods' section (while we also excluded parts of the traces where significant artefacts would potentially compromise the accuracy of our analyses). We further explored coupling between the half-harmonic of the stimulation frequency and cortical beta (for a stimulation frequency of 40Hz), as well as its quarter-harmonic and cortical alpha and beta rhythms (stimulation frequencies of 40Hz and 100Hz respectively). All stimulation trials were compared to generated pink noise control trials, where 'phase-locking' values of noise to the stimulation were also examined.

We initially focused on the clinically used, 'physiological' stimulation frequency (40Hz). With regards to frontal and pre-frontal dipoles, we noted significantly higher phase locking values than noise between cortical oscillations and stimulation frequency in the beta ($p < 0.0001$, zval=4.9086) and gamma frequency ($p < 0.0001$, zval=5.5367). Cortical beta also significantly phase-locked to the half-harmonic of the stimulation, compared to the generated pink noise control signals ($p < 0.0001$, zval=4.0280). There was no statistically significant difference in beta phase-locking

with the stimulation frequency (40Hz) and its half-harmonic (p =0.5294, zval=-0.6289). In this cortical region, we noted that alpha did not significantly couple to the stimulation, neither at the exact stimulation frequency (p = 0.4569, zval=0.7440) nor at its quarterharmonic (p = 0.8013, zval=-0.2517) compared to pink noise trials.

When occipital dipoles were examined separately, we saw again that there was significantly high phase locking of cortical activity to real (40Hz effects on cortical activity) stimulation trials than in control (40Hz effects on pink noise) trials, in the beta and gamma frequency range (p <0.0001, zval=4.8634 and p <0.0001, zval=5.1149 respectively). The cortical beta oscillations occipitally also significantly phase-locked to the half-harmonic of the stimulation frequency (p =0.0325, zval=2.1386) (Fig.8A).

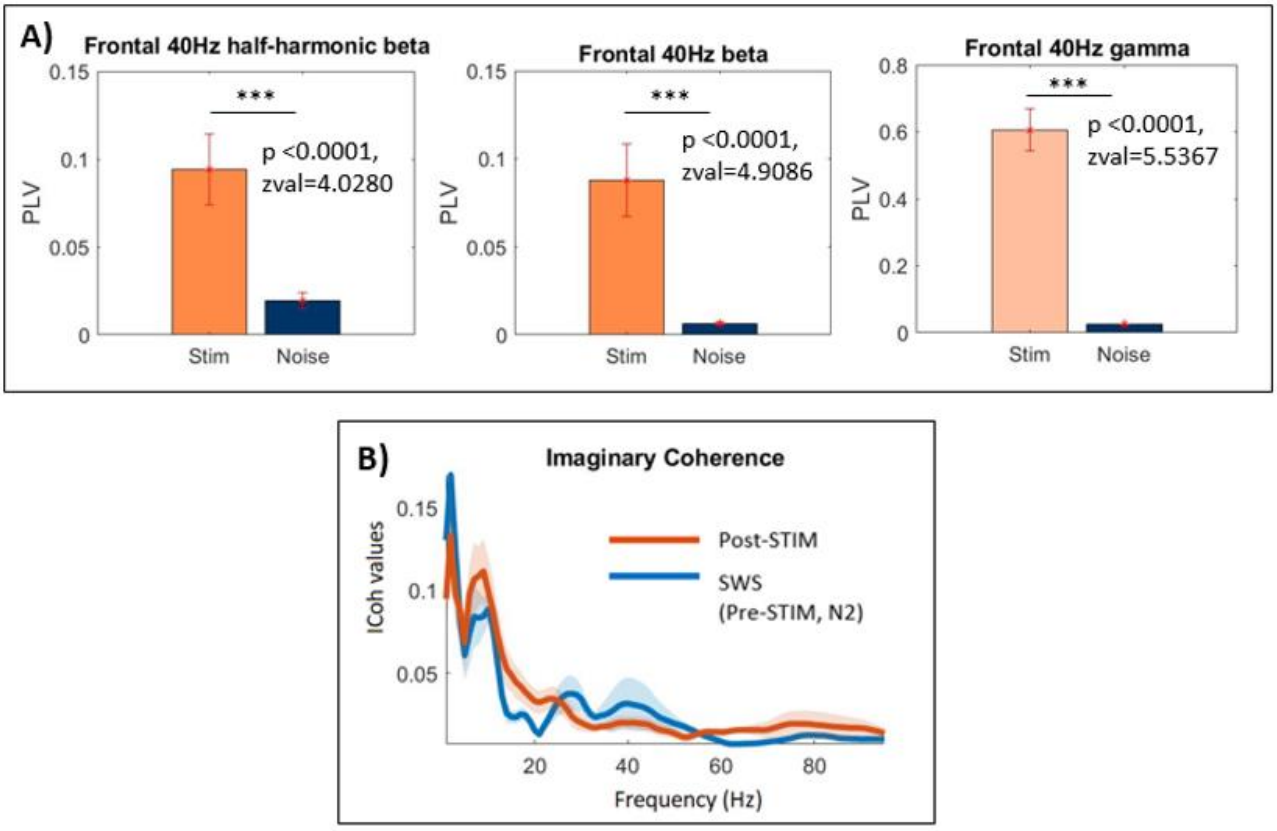


Figure 8. <u>Phase-locking of cortical frequencies to physiological PPNR stimulation frequency.</u> At 40 Hz stimulation frequency, there is significantly higher PLV between endogenous beta (for frequencies which are also close to the half-harmonic of the stimulation) and gamma and stimulation, compared to values for pink noise (a). Additionally, endogenous beta shows significant phase-locking with the half-harmonic of the stimulation (20 Hz)(a). In this context, we note the contralateral imaginary coherence increases between PPNR and cortex, adjacent to that frequency (b).

Exclusively for the occipital cortical region, endogenous cortical alpha phase-locked to the quarter-harmonic of the stimulation frequency, significantly higher than pink noise (p =0.0098, zval=-2.582), a finding that was absent from frontal regions. There was no synchrony with endogenous alpha observed at the 1:1 frequency of the stimulation (40Hz) in this region (p =0.5577, zval=0.5863). When we subsequently

compared frontal and occipital areas, an additional difference was a significantly higher phase-locking of endogenous beta oscillations, most obvious to the half-harmonic of the stimulation, occurring frontally ($p = 0.0092$, zval=2.6059).

Subsequently, we explored how pulses delivered during high ('supra-physiological') frequency stimulation trials (100Hz), during periods of comparable slow wave sleep depth, may be coupled to frontal and occipital cortical rhythms. As mentioned previously, due to the very low amplitudes delivered at this frequency to ensure that stimulation did not cause sensory/other disturbance, automatic peak identification of unilateral trials was extremely challenging. Therefore, for unilateral trials delivered at 100Hz, only data from two patients were of sufficient artefact strength to be analysed. This therefore left us with twelve frontal/pre-frontal and ten occipital dipoles of the same laterality (as well as the same laterality as the 40Hz trials), from trials delivered during sleep epochs with the same depth and SWA density as during the 40Hz trials, available for pooling in our respective analysis groups.

With regards to the frontal group, the most significant effect was with regards to phase-locking of the cortical gamma oscillation band of interest to the stimulation frequency, compared to 'synchronisation' of pink noise signals ($p < 0.0001$, zval=4.1434). Additionally, beta oscillations significantly phase-locked to the stimulation frequency, compared to noise-frequency 'synchrony' ($p = 0.0003$, zval=3.6451). Given that the half-harmonic of this stimulation frequency fell within the AC/DC component (50Hz for the UK), comparisons involving this subharmonic were not feasible. However, there were no statistically significant differences

between phase-locking between the stimulation frequency or its quarter-harmonic and cortical beta oscillations (p =0.1744, zval= -1.3583). There was no phase-locking observed between frontal cortical alpha oscillations and the stimulation frequency (p =0.2018, zval=1.2766).

We also examined the synchronisation of occipital oscillations to this 'supra-physiological' stimulation frequency, as we had for the 'physiological' stimulation frequency. Here, endogenous alpha did phase-lock to stimulation frequency to a significant degree, compared to the association between pink noise control signals and stimulation pattern (p =0.0106, zval=-2.5545). Additionally, the gamma band of interest was phase-locked to this stimulation frequency (p =0.0002, zval=3.6843). The same was true for occipital beta oscillations (p =0.0011, zval=3.2634, ranksum=148) compared to noise. The quarter-harmonic of the stimulation frequency did not exhibit this relationship (p =0.3825, zval=0.8733). When frontal and occipital effects were juxtaposed for trials in this stimulation frequency, the greatest effect was for a higher degree of phase synchrony with occipital alpha oscillations compared to frontal activity in this band (p =0.0032, zval=2.9467). The differences between frontal and occipital relationships of beta oscillations to the stimulation bordered significance (p =0.0512, zval=1.9496).

We further proceeded to examine whether phase-locking of endogenous cortical rhythms was more significant to one stimulation protocol versus the other (40Hz stimulation vs 100Hz stimulation trials, at the same pre-stimulation sleep stage). To this end, we calculated the individual trial differences with phase-locking values for

pink noise, then compared the two groups (40Hz vs 100Hz). When directly juxtaposing these values for the two protocols, both showed the same degree of efficiency of synchrony for the cortical gamma frequencies of interest frontally (no statistically significant difference, p= 0.1819 z= 4.5513). However, occipitally, phase synchrony between the 40Hz stimulation protocol and endogenous gamma at the frequency of interest was significantly higher compared to that with the 100Hz protocol ($p<0.0001$ z= 4.0704). When a two-way ANOVA (per frequency of interest, comparing stimulation frequencies and their sub-harmonics where applicable) was deployed, this result survived the Bonferroni correction for multiple comparisons.

Discussion

Overall, our results point out that in vivo stimulation of the PPNR in humans can influence NREMS by inducing sleep stage shifts and arousal events, while increasing cortical activity associated with wake states. These findings seem to be in agreement with prior evidence in rodents (Cissé et al., 2018; Van Dort et al., 2015) therefore hopefully truly translational and not exclusively related to the neurodegenerative condition present in this cohort (Multiple Systems Atrophy). In the absence of video recordings to confirm behaviour, apart from cortical spectral changes, the fact that post-stimulation state was closer to wakefulness can also be attested by the fact that the most significant increase in gamma coherence post-stimulation for all frontal average (59-67Hz, p=0.004) occurred in a comparable

frequency range to the most significant increase when quiet wakefulness and deep slow-wave sleep were juxtaposed (60-63Hz, p= 0.007).

Stage Changes and Frequency shifts post-stimulation: the concept of underlying pathology

The high amount of transitions to wakefulness and ISARs observed at such low stimulation amplitudes could, however, be at least partially attributed to a heightened sensitivity to arousal events and sleep fragmentation present in this condition, further supported by the increase in beta and gamma effective connectivity that we have reported upon in the previous chapter.

The mechanisms behind this 'brainstem hyperactivity' may influence circuit properties such as neuronal rheobase (the amount of current necessary for action potential generation and depolarisation of the target area) (Dostrovsky & Lozano, 2002; Nowak & Bullier, 1998). Therefore, the probability of induced firing in wake- and REM-related patterns after Deep Brain Stimulation (DBS) may have a much lower threshold in these conditions and result in further fragmentation such as we saw in our paradigm. Although neuronal loss of the PPNR may also diminish the population that DBS can recruit and induce firing thereof, altered local dynamics favour low sleep continuity. This is further supported by the fact that a significantly higher number of patients experiences disordered sleep in MSA compared with Parkinson's disease where –although basal ganglia are compromised- brainstem structures are relatively less affected (Ghorayeb et al., 2002).

Furthermore, with regards to clinical implications of our findings and links to pathology in this cohort, MSA patients predominantly suffer from REM sleep behaviour disorder (RBD) with a summary prevalence of 88% according to the most recent published review of the literature (Giannini, Provini, Cortelli, & Calandra-Buonaura, 2021). RBD symptoms are notoriously challenging to control and are sometimes managed with benzodiazepine additions to an existing state of polypharmacy, however symptoms remain poorly controlled in most cases (Jung & St. Louis, 2016). In a study examining REM sleep in drug-naïve RBD patients, RBD patients treated with clonazepam and matched healthy controls, it was shown that untreated RBD patients demonstrate an attenuation of slower rhythms (theta and delta) during REM sleep and have a predominant beta pattern, compared to the other groups (Ferri et al., 2017). Therefore, in this cohort prolongation of REM sleep periods and/or alteration of its characteristics in a way that could favour RBD may not be desirable and could potentially adversely affect treatment outcomes.

In keeping with previous evidence (some presented earlier in this thesis), we also found that PPNR DBS in SWS increases the rate of REM transitional outcomes based on AASM scoring, therefore favouring a prolonged REM sleep state. We also showed that suppression of slower oscillatory activity and beta dominance (with stimulation phase-locking to beta) both occur as a result of our described stimulation paradigm. When these oscillatory changes are examined in the context of the above evidence, it can be seen that they are in contrast to the effects of RBD pharmacotherapy and reverse towards the treatment-naïve phenotype.

Additionally, we could not record further trials in one patient due to a pronounced episode of parasomnia, where dream enactment was followed by a confusional arousal. Since this behaviour was –according to the patient's report- novel, we did not recommend further trials and this patient did not use PPNR DBS at a gamma frequency during the night. Additionally, there was one case from the earlier recruited cohort where nocturnal PPNR DBS caused such severe fragmentation and insomnia to the degree where the patient was switching the device off using the patient controller (*more detail in the subsequent chapter)*. Subsequently, we instructed our next patients to use very low amplitudes at night while this is a policy we have followed with patients recruited for our subsequent (MINDS) study, the first two patients of which are further discussed in the next chapter.

Brainstem Ascending Arousal Network (BAAN) Effects: nodes with similar patterns of electrophysiological activity, not one single nucleus

An additional factor that needs to be considered upon interpretation of these results is the implantation variability within the PPNR surgically defined area, explored in detail in the previous chapter. It needs to be highlighted that the effects of PPNR contact stimulation discussed in this chapter are not the results of altering the activity in one discrete nucleus, but rather reflect the impact of stimulation applied to a network of regions with diurnal patterns of behaviour present within that wider brain area.

However, despite common effects on cortical synchrony, there is evidence of site-dependent differences upon manipulation of BAAN nuclei. For instance, longer-

lasting EEG desynchronisation occurred in urethane-anaesthetised rats (of the same strain and comparable developmental age) upon carbachol injections in the pedunculopontine tegmentum, compared to equivalent doses being injected in pontine oralis (PO) (Kinney, Vogel, & Feng, 1998). Although chemical manipulations are not equivalent to electrical stimulation, which -in a sense- acts in a broader and less selective manner, variability in implantation within the same network might lead to effects of different intensity and/or state induction.

Apart from the few common sub-categories of anatomical implantation (one patient with lead in PO compared to two patients closer to the MRF for instance), the fact that patients may exhibit different levels of fragmentation-related pathology (*reflected in WASO and continuity indices, Chapter 1 of this thesis*), further limits the reliability of anatomy-related conclusions, based on individual comparisons between cohort implantation sites. Furthermore, the premise of this experimental chapter also was the examination of the effects that occur upon stimulation of maximally diurnally modulated areas in the PPNR, therefore we examine the results on a cohort level. We did however note that there were some differences in the exact behavioural effects (for instance, confusional arousal in the case of PPNR3) or number of stimulation trials tolerated per night, prior to inability to resume another sleep cycle (more trials would be feasible in the case of PPNR2, where implantation site according to LeadDBS was further away from brainstem nuclei compared to other cases).

There is published evidence in other mammals which supports both evidence of common network activation, upon stimulation of one BAAN ‘node’, as well as the fact that the effects of higher stimulation frequencies may not result in such long-lasting activation and neuronal firing. Specifically, when neurons in the cat pedunculopontine nucleus are stimulated at a frequency of 40 Hz, there was an activation of the cat pontine reticular formation, where neurons significantly increased their firing compared to pre-stimulation levels. However, stimulation at 80 Hz and 100 Hz led to transient responses in these regions (1 sec firing duration, followed by either cessation or decrease in activity (E. Garcia-Rill, Skinner, Miyazato, & Homma, 2001; Homma, Skinner, & Garcia-Rill, 2002)).

A subsequent important consideration with regards to our results is the fact that a non-continuous stimulation pattern, consisting of short periods of PPNR DBS (as opposed to continuous stimulation pattern over hours) in a homologous frequency related to REM or wake, may preferentially favour transitions. Discrete cholinergic populations of the PPNR transiently increase their firing, immediately preceding stage transitions (Kroeger et al., 2017; Mena-Segovia & Bolam, 2017). Therefore, a DBS pattern of periods of quiescence with intermittent stage-specific gamma stimulation periods may superimpose to their intrinsic patterns and interfere with this gating mechanism in a more complex way than the delivery of a constant, tonic DBS signal applied to the network.

In keeping with this observation, our earlier results of differences between PPNR gamma and beta oscillatory power between DOWN-State and UP-State transitions

seem to suggest that (although the temporal development of the gating phenomenon and a non-continuous stimulation pattern may differ), if we were to deliver selective PPNR stimulation in different excitatory phases of slow-wave sleep (SWS) we could indeed be 'tapping into' such dynamics. As shown in our analyses of passive recordings during slow-wave sleep, beta activity (that we argued may be pathological) clustered around the DOWN-state, representing a putative marker of its instability and thus failure to maintain cortical quiescence. Delivering stimulation during these short temporal windows of heightened beta activity (for instance in a closed-loop manner built within a device), could either destabilise sleep or promote maintenance of its depth, depending on the stimulation parameters and their effect on endogenous oscillations.

With regards to an increase in synchrony between endogenous PPNR oscillatory activity and stimulation parameters, since we did not have the capacity to stimulate and record ipsilaterally, phase-locking of endogenous PPNR beta with the stimulation frequency at the stimulation site was not examined. Although one could argue that a 'skewed' and unilateral stimulation pattern may induce different effects on the ipsilateral site, or that such changes are a *symptom* rather than a *cause* of the increase in PPNR activity, they do point towards an increase in beta and gamma synchrony after the region has undergone gamma stimulation. Furthermore, we found that there were direct influences of the DBS frequency on cortical activity during the stimulation period.

In this series of experiments, we noted that cortical low gamma was preferentially phase-locked to PPNR DBS at a 'physiological' frequency, while this was one of the gamma frequency bands where cortical coherence with PPNR activity decreased when sleep depth remained stable and/or progressed (in the case of SHAM trials). It could therefore be argued that the temporary enhancement of coherence between PPNR and cortex, while stimulating at this frequency range, might establish a transient pattern favouring sleep transitions (in keeping with a higher level of cortical excitability). In addition, we noted that both DBS protocols showed higher synchrony with frontal endogenous beta rhythms compared to control (pink) noise.

When the fact that (as seen in Chapter 1), the nodes of the BAAN network generally seem to have diurnal modulation around 20 Hz (as well as in the low gamma range), one could argue that there is potentially an effect of preferential entrainment of PPNR beta close to the half-harmonic of the 40Hz frequency (Duchet, Sermon, Weerasinghe, Denison, & Bogacz, 2023), leading to further cortical beta entrainment through afferent projections. Although this is an appealing hypothesis, which could further support the higher efficacy of the 40 Hz stimulation protocol, we do not have data of both stimulation and LFP recordings in the same PPNR site to test it thoroughly (*further entrainment-related investigations in the following chapter*).

In a further brief link to pathology, preferentially frontal beta modulation was found in Parkinson's disease with subthalamic nucleus (STN) DBS, albeit in an awake state, correlated with improved task performance (Swann et al., 2011). To our knowledge, no such results have been published in the case of PPNR DBS in sleep.

However, it could be argued (since PPNR is also connected to the STN and basal ganglia in general) that such a localisation of activity (as opposed to global changes) may stem from common circuits in these two cases, which enhance cortical excitability. An enhancement of frontal cortical oscillatory activity between 12-30Hz when patients are asleep could however lead to (secondary) insomnia rather than have a beneficial effect. This is further highlighted by the fact that increased frontal oscillations in this frequency band (compared to age-matched good sleepers) have been linked to insomnia, both as a primary sleep pathology (Corsi-Cabrera et al., 2012) and in association with Parkinson's disease (Mizrahi-Kliger, Feldmann, Kühn, & Bergman, 2022).

Cortical Oscillatory changes in the Beta range: 'true' beta or sigma activity?

During sleep, the frequency band that is termed beta in wakefulness overlaps with sigma and spindle activity. Sleep spindles are discrete bursts of oscillatory cortical activity generated by a thalamocortical loop between cells in the reticular nucleus thalami and thalamocortical relay neurons, with ascending input from brainstem nuclei (Steriade, McCormick, & Sejnowski, 1993; Steriade & Timofeev, 2003). Spindles have been linked to sleep preservation, gating thalamic input prior to it reaching the cortex (De Gennaro & Ferrara, 2003) as well as regulate memory and cognitive functioning (Fogel & Smith, 2011; Latreille et al., 2015; Schabus et al., 2006).

A further important point is the phase-locking of fast (12-15Hz) spindle oscillations with the excitable UP-state of the slow oscillation in humans, further hinting at a link between these activity bursts and cortical depolarisation (Klinzing et al., 2016). Although we will not thoroughly elaborate on this oscillation here, we note the published evidence showing that power in the frequency band of the spindle bursts (termed sigma power, ranging from 12-16Hz) was found to be increased in newly diagnosed patients with Parkinson's disease compared to matched controls, with further correlations to decreased time in slow-wave sleep and less sleep efficiency (H. Brunner et al., 2002; Margis, Schönwald, Carvalho, Gerhardt, & Rieder, 2015). Additionally, individual spindle burst characteristics (peak-to-peak amplitude, duration and peak frequency) were found to differ between patients with Parkinson's disease and age/sex-matched controls (Christensen et al., 2015).

Therefore, we could hypothesise that changes in sigma and spindle rhythms could also reactively occur post-stimulation, especially in keeping with their role in sleep depth preservation, therefore contributing to the increase in the 'beta' frequency band that we see post-trials in this cohort. However, fast spindle activity (overlapping with the beta band) has been described to occur with a centro-parietal as opposed to frontal predominance (early reports by (Werth, Achermann, Dijk, & Borbély, 1997; Zeitlhofer et al., 1997)), a factor which could imply that spatial dynamics observed in this cohort are a result of basal ganglia beta (as opposed to thalamic sigma) activity.

Nevertheless, there are also studies where this spatial relationship did not predominate (such as in the aforementioned work by (Klinzing et al., 2016), while it has recently been challenged in favour of a greater dimensionality in terms of spatiotemporal dynamics and specific features (Gonzalez, Jiang, Gonzalez-Martinez, & Halgren, 2022). The fact that we have showed that PPNR activity differs with regards to UP- and DOWN- state makes for a very compelling case of the investigation of its temporal dynamics with regards to spindle bursts and sigma power. Another reason why this could be pertinent is the fact that cortical low gamma was seen to be generally increased during spindles in a recent study (30-40Hz as defined in the paper) (Weber et al., 2021).The same authors also showed that both low and high gamma power (therefore the entire range from 30-100Hz in the paper) was coupled to spindle phase, albeit at a fixed phase only within an individual burst. How PPNR DBS at a gamma frequency influences cortical spindle burst morphology is another question that we are interested in investigating with additional future cases.

Post-Stimulation Changes in Cortical Alpha Frequency and Spatial Patterns

In the case of oscillations from 8-12Hz (termed alpha when predominant in quiet wakefulness and overlapping with slow spindles in sleep), we noted a preferential phase-locking between endogenous rhythms occurring occipitally and the corresponding sub-harmonic of the stimulation frequency. This seems to reflect a well-established frequency-specific biological phenomenon occurring in the wake alpha range (as opposed to slow spindles/sigma, which have a preferential frontal

distribution). Alpha has been classically termed ‘posterior dominant rhythm’ (PDR) due to its occipital occurrence during a state of quiescent eye closure, while it quickly disappears when the first stages of sleep ensue (Gloor, 1969).

In addition to its close link with a quiet wake state, stability of occipital alpha bouts during recovery from inhalational general anaesthesia have been found to correlate with reaction times and task performance (Labonte et al., 2022). Nevertheless, there may be clinical instances where the presence of such oscillations is not a clear-cut link with level of arousal, consciousness and cognitive performance, such as in the case of alpha coma, a constant state first described by Westmoreland in brainstem strokes and denoting a poor prognosis (Westmoreland, Klass, Sharbrough, & Reagan, 1975).

In our experiments however, PDR ensued as a transition rhythm during stage shifts from a deeper sleep state to light sleep, REM sleep or even wakefulness. This was consistent with the term ‘alpha intrusion’, a widespread phenomenon denoting fragmented sleep of poor quality (Cantero & Atienza, 2000; Hauri & Hawkins, 1973) and also present during non-restorative sleep in a range of conditions from fibromyalgia to depressive disorder (Branco, Atalaia, & Paiva, 1994; Jaimchariyatam, Rodriguez, & Budur, 2011; Martinez, Breitenbach, & Lenz, 2010). We found that our protocol of PPNR DBS during SWS exacerbated alpha intrusion, both in what we had assumed was ‘physiological’ gamma as well as at a supraphysiological frequency.

The fact that this result was achieved with very low amplitudes and/or unilateral stimulation carries a word of caution for continuous gamma DBS in RAS/brainstem targets over the course of the night, especially where there may be a background of predisposition to arousal events. The timing of RAS DBS may be crucial with regards to alpha-delta sleep regulation; daytime central thalamic stimulation in one patient was associated with alpha-delta sleep suppression which reversed when this diurnal driver was withdrawn (Gottshall, Adams, Forgacs, & Schiff, 2019). We will further elaborate on the beginning of our attempts to utilise PPNR DBS in such a function (diurnal driver/Zeitgeber) in the next chapter of this thesis.

Emerging Considerations: how should PPNR stimulation be scheduled according to diurnal behavioural patterns?

There seems to therefore be a valid case for taking diurnal behaviours into consideration, while scheduling PPNR clinical stimulation. This is based on the implication of these results and the entire body of published animal evidence, also showing REM prolongation and sleep fragmentation when PPNR and brainstem arousal centers are stimulated. In this stream of thought we can also add the practices of the Schiff group which pioneered central thalamic DBS (another RAS target like the PPNR) and whose 'stimulation prescriptions' for patients with disorders of consciousness and a concomitant element of dysregulated sleep patterns are exclusively delivered during daytime hours, not at night. On the other hand, there are the studies reporting improved perceived sleep quality in PD with chronic continuous PPNR DBS, as outlined in the introduction of this thesis.

However, it may well be that these results are not contradictory, but reflect a number of different convergent processes. Firstly, tonic and invariant 24/7 PPNR DBS combines the results of two underlying mechanisms. The effect of PPNR DBS on brainstem oscillations during daytime may be potentially resulting in normalisation of wake-related oscillations (and subsequently sleep pressure build-up). This is a question that we explore in the next chapter of this thesis (*as well as in the concept paper, which is available in the 'Supplementary Materials' of this thesis in its form post peer review and acceptance for publication*). On the other hand, night-time stimulation of the PPNR (or any RAS target for that matter) may be exacerbating fragmentation in susceptible cohorts. The RBD predominance in MSA patients might explain the intensity of this phenomenon in our population.

Combined, these two mechanisms may lead to different results in chronic stimulation of populations with a predominance of excessive daytime sleepiness and (as in the case of PD) sleep-disruptive freezing. Another potential mechanism could be that PPNR definition varies between neurosurgical centers; therefore, in some cases stimulation effects may also be attributed to different combinations of neuronal populations. There are many such ways where electrode positioning may result in mixed effects or even enhancement of sleep depth. For instance, serotoninergic populations are present in the dorsal raphe, rostrally adjacent to what we define as PPNR by a few millimetres, as also can be visualised in the Harvard AAN atlas that we have utilised in this thesis (also see (Edlow et al., 2012)).

Inhibition of serotoninergic activity through stimulation of related pathways could result in increased sleep depth (evidence reviewed in (Monti, 2011)). However, in our case, where the net stimulation result was sleep fragmentation, invariable tonic and 27/7 constant settings of PPNR DBS in gamma related frequencies are not deemed beneficial. We proceed to showcase our initial steps in implementing the effects of PPNR DBS that we have observed in a physiological and beneficial manner in the next chapter of this thesis.

Limitations of the study

One of the main limitations of this part of this thesis work was the low number of cases (even though in the existing literature the number of patients recruited here is far from an outlier and definitely not the lowest number). This became even more poignant when it was obvious that the number of trials per case would be limited due to increased fragmentation (and in one case exacerbation of parasomnia). Our initial mitigating strategy was that additional recordings and trials could originate from the 'MINDS' PPNR stimulation cohort, that promised to bring streaming capabilities on a longitudinal basis. However, the device associated with the trial had significant limitations with regards to streaming time (since this depletes its battery). Although the device used in MINDS is rechargeable, overnight streaming would completely drain the battery to levels where long-term battery life could be compromised. Recharge during the course of the night would mean that continuous recordings would not be feasible. Furthermore, interrupted streaming would require constant syncing with the implanted IPG, which we realised caused further artefacts

in the recording as well as sensory disturbances, since (unlike what happens when the leads are externalised) the experimenter needs to physically approach and interact with the sleeper.

Another significant limitation that precluded replication of the externalised recordings was the fact that the device cannot stream from all contacts simultaneously; it can only stream from a pre-specified dipole and should one wish to investigate multiple contacts, then another dipole is streamed as part of a sequential ‘batch’. Sleep is a dynamic process; its transitions and shifts do not wait until all such ‘batches’ have been run at a convenient time for the experimenter. The net result is a non-homogeneous mix of streamed contacts with different baseline state dynamics (which could be mitigated if the device could stream overnight so that multiple samples could be obtained but, alas, it does not).

We could expand this list of limitations further, however this is not the main aim of this section. It should perhaps be suggested that, in novel targets and/or indications in adult populations, where the baseline dynamics need to be explored and externalisation behaviourally tolerated, it remains a gold standard that should perhaps be bridged with novel technologies instead of fully discarded. Not only does it afford prolonged recordings (for as long as one desires and has recording time and patient consent for) with unparalleled signal quality and minimal disturbance of the patient overnight; externalisation can provide a full picture of LFPs of the entire region in real time, perfectly synced to cortical signals.

Moving on to another significant limitation of this work, this time related to behaviour, the lack of video monitoring overnight led to a significant gap in behavioural documentation during stimulation trials. The cause for this lack is that the STAGG trial was planned and designed prior to the beginning of this thesis (prior to 2018) by the primary investigators, whose main interests are related to motor outcomes and autonomic function. A sleep-related amendment was obtained so that the writer of this thesis could proceed with dedicated sleep research; however, there was no further funding arrangement in the clinical environment that would allow for use and reservation of video-PSG devices proprietary to the Clinical Neurophysiology department (whose main activities relate to NHS-funded clinical work as opposed to academic DBS trials).

This may also explain why respiratory sensors and periodic limb movement sensors were not used (although in the latter case, the writer of this thesis piloted the use of EMG sensors that were, however, rather impractical in the RBD context). Following the results of this work, further sleep-related funding and collaborations with clinical research teams with dedicated sleep facilities and expertise are sought so that such constraints can be mitigated.

Finally, another significant limitation is the lack of a control group. This, as mentioned in the introduction, would have been feasible with prospective recruitment of Parkinson's Disease patients with PPNR implants. However, PPNR stimulation is not routinely offered to this patient cohort in our centre (where basal ganglia DBS is preferred due to comparatively better motor outcomes). Additionally, even if we were

to compare a STN PD control group with our PPNR cohort, we would not be able to record from externalised patients unless a specific application for such an action was made and consenting was sought, since we no longer externalise ‘routine’ targets and cases in our centre.

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Chapter 3

Introduction

Following the findings described in the two prior chapters (and especially the second experimental chapter of this thesis), we wished to investigate ways of beneficially utilising the effects of gamma stimulation of diurnally modulated networks in the pedunculopontine nucleus region (PPNR). As we have briefly discussed, the effects of slow wave suppression and acceleration of the EEG, although detrimental during slow wave sleep (SWS) and fragmented night-time periods, could be beneficially utilised during undesirable daytime drowsiness and/or sleep bouts. In an ideal design and alignment with technological advances available to us, this would take place in a closed-loop manner, not only in order to target daytime decreases in wakefulness in an energy-efficient manner, but also since the body of available evidence across species seems to support a phasic (as opposed to tonic) mode of activity in the PPNR.

However, current implantable stimulator technology seems to be lagging far behind these concepts. Additionally, for the remainder of patients implanted in the PPNR during the follow-up trial to our prior cohort, externalisation of the leads was no longer implemented due to the aforementioned change in practice at this neurosurgical center, combined with specifications with regards to the novel device utilised (the company did not manufacture and had not costed for an externalisation kit).

This severely limited the capacity of assessing the potential efficacy of closed-loop sleep and vigilance modulation interventions in this cohort, since the algorithm based on the externalised lead signals and coded by the thesis author was no longer implementable. Further on to this, the alternative option of an application programming interface (API) utilising the platform of the device used in the trial was not supported by the manufacturer, since it would also require additional monitoring for its integration with the platform and a Medicines and Healthcare Products Regulatory Agency (MHRA) review application for the product.

However, the concept of diurnal neuromodulation and the effect of variable settings as per time of day on circadian rhythms is also a primary interest of our senior engineering collaborator (Prof. Denison), whose group had been working on development of the investigational device used in this follow-up trial of the prior study, mentioned in the previous two chapters. This device utilises an accelerometer and a scheduler function, to deliver different stimulation patterns depending on posture and inferred activity levels, in keeping with the trial's motor and autonomic primary endpoints (further description in the 'Methods' section of this chapter, as well as in the additional peer-reviewed material provided as a thesis supplement).

We were therefore able to proceed with his support and explore the possibility of beneficial applications of PPNR DBS stemming from our prior body of work, albeit as part of tonic stimulation and not linked to endogenous activity at the current clinical device iteration. Before we proceed with the methodology and experimental data presentation of this next chapter, we will briefly explain some of the additional

mechanisms behind how such a use of oscillatory modulation during the day may also affect subsequent night-time sleep quality, as well as conceptualise the need for such interventions in the present disorder cohort.

The hypothesis that slow wave activity is an oscillatory marker of sleep homeostasis (first described by (Borbély, Baumann, Brandeis, Strauch, & Lehmann, 1981)) has thereafter been supported by a wealth of experimental evidence. The earliest of these studies utilised sleep deprivation as an experimental paradigm and demonstrated a clear relationship between the amount of sleep restriction and the subsequent quantity of slow wave sleep (SWS), especially at the beginning of the night (see (Dijk, Hayes, & Czeisler, 1993; Werth, Dijk, Achermann, & Borbély, 1996)). More importantly, lesions of what has been described as the central 'circadian clock' of the brain, the suprachiasmatic nucleus, did not abolish this relationship between rebound slow wave activity and sleep deprivation in early seminal work by Prof Irene Tobler, implying that this relationship reflects an independent homeostatic phenomenon (Tobler, Borbély, & Groos, 1983).

These crucial early papers showcased the discovery that, if one restricts slow wave activity over prolonged periods of time (through behavioural interventions, electrical, chemogenetic or optogenetic stimulation paradigms across species), the presence of a SWS homeostasis dictates that subsequent sleep will be richer in slow wave activity as a rebound recovery mechanism. There may be neuroprotective functions unique to SWS, some of which are of particular interest in the context of Parkinson's disease and related synnucleinopathies. Recently, in a mouse model of PD,

enhancement of SWS showed a decrease in pathological protein burden compared to control and sleep deprived mice –an important finding with potential direct therapeutic implications (Morawska et al., 2021).

The manipulation of the homeostatic balance of a rising 'process S' during wakefulness (sleep pressure), that dissipates during sleep (Borb & Achermann, 1999), has already been implemented as a well-established treatment mechanism in the case of insomnia. Sleep restriction has been a mainstay treatment for insomnia for over 30 years, with its capacity to strengthen sleep homeostasis cited as one of its four key interventions, although the mechanisms behind it are being further investigated and expanded (Maurer, Espie, & Kyle, 2018). Sleep restriction is at the core of behavioural modification therapies such as cognitive behavioural therapy specifically designed to target disordered sleep (CBT-i) (Kyle et al., 2015). It is however extremely difficult to utilise methods depending on behavioural modification in the case where daytime sleep bouts are involuntary and uncontrollable, as is the case of excessive daytime sleepiness (EDS) in neurodegenerative disorders.

Directly relevant to the condition affecting our patient cohort, dysregulated breakthrough sleepiness occurs during the day in MSA (Ghorayeb et al., 2002; Santamaría & Iranzo, 2008). However, the pathophysiological correlates of EDS seem to differ compared to PD. Although in both conditions there was interestingly no strong correlation between EDS and degree of disease severity, a recent trial involving twelve tertiary referral centres reported that decreased sleep efficiency and

sleep disordered breathing were the strongest predictors of EDS in MSA, compared to restless legs syndrome and amount of dopaminergic treatment in PD (Moreno-López et al., 2011). The latter refers to paradoxical somnolence after dopaminergic treatment, which may also occur in the context of MSA (first reported by (Birgit Högl, Seppi, Brandauer, Wenning, & Poewe, 2001)) therefore complicating other EDS-related interventions.

Central stimulants such as modafinil (also an atypical dopamine reuptake inhibitor) have been trialled in PD, showing a successful reduction in EDS (B Högl et al., 2002; Knie, Mitra, Logishetty, & Chaudhuri, 2011), with modafinil also recommended as first-line treatment when non-pharmacological options fail in the case of PD as well as in MSA (also reviewed in (Klingelhoefer, Sokolov, & Chaudhuri, 2014)). However, in the case where DBS treatment is used, it may be desirable to utilise this to control symptoms and avoid (or at least delay) further add-ons in a state of polypharmacy.

As we have showcased in the first experimental chapter of this work, hyperactivity of compromised brainstem structures may be at play over night, leading to an increased number of stage shifts and lower slow wave activity (SWA) content. We showed that we can decrease slow wave power by influencing the probability of these stage shifts through intermittent stimulation of network ‘nodes’ during slow-wave sleep. Therefore, here we argue that there is perhaps a possibility of increasing sleep pressure during daytime, through selective PPNR stimulation, while avoiding potential sleep disruption secondary to tonic overnight stimulation at frequencies homologous to intrinsic oscillations and their harmonics. We can then

push slow-wave activity towards its physiological timing in SWS through a different night-time stimulation mode which, although not being informed by endogenous activity and linked to it in a closed-loop manner, can nevertheless be based on principles relevant to intrinsic oscillatory patterns of brainstem ascending arousal networks (BAAN).

Methods

We will start by describing how these concepts can be incorporated in a novel bioelectronics device, currently embodied in the Picostim-DyNeuMo research system (Bioinduction, Bristol, UK) (while a peer-reviewed publication detailing these specific concepts is also available as supplementary material in this thesis). We hoped that through utilising device platform functions in a way that incorporates our prior PPNR observations, we may re-entrain diurnal and circadian rhythmicity through the device's capacity of exerting control of Reticular Activting System (RAS) activity.

Device-related concepts and 'stimulation prescriptions'

Timing of the Stimulation Cue: Zeitgeber Plan Overview

We followed a layered approach (over different temporal timescales or layers) in order to entrain rhythms of circuits undergoing neurodegeneration towards more physiological patterns. This involved considering how RAS stimulation might

interfere with regulatory processes acting within a diurnal and circadian timescale, as well as dynamics evolving longitudinally.

On a shorter timescale, stimulation cues should discourage bouts of unplanned daytime sleep by entraining cortical rhythms towards higher frequencies and avoiding EEG slowing. Stimulation should also support healthier motor patterns by delivering more intense therapy during active times, when it is most needed. These functions create an acute bioelectronic ‘time-giver’/Zeitgeber targeting EDS, comprised of stimulation cues and reinforced by the patient’s own increased physical activity. Deployment of this Zeitgeber would result in greater build-up of homeostatic sleep pressure later in the day, priming patients for earlier sleep onset, shorter slow-wave sleep latencies and better sleep quality.

On a longer timescale, RAS stimulation cues should drive the circuit in physiological diurnal patterns of activity, for instance in keeping with increased predicted firing during wake and quiescence during slow-wave sleep. Such Zeitgebers (encoded in ‘daytime’ and ‘night-time’ settings) would also ensure that sleep fragmentation is avoided, therefore sleep pressure is at its lowest peak upon awakening and a normal 24hr (circadian) cycle is entrained. However, patients may need to mobilise from rest (for instance to tend to bodily functions, control of which may be affected by dysautonomia). Therapy delivery and safety netting should be provided by responsive stimulation delivery, sensitive to postural changes.

Personalised scheduling of the Zeitgebers in accordance with patient-specific bedtime and wake-up timepoints will ensure precise settings, with effects expected to also be consolidated through stimulation-related plasticity of the underlying RAS circuits.

Translation in the DyNeuMo Device platform

The Picostim-DyNeuMo device is capable of recording intracranial signals and facilitates remote access for monitoring and therapy refinement. In addition, embedded circadian schedulers and sensors on the device enable adaptation of stimulation based on temporal patterns and inertial signals (Toth et al., 2020; Zamora et al., 2022). The adaptive algorithm implements stimulation adjustments through integrated use of the circadian scheduler and inertial sensors on the implanted device. The embedded algorithm we tested consists of two layers of control in order to adapt stimulation parameters for daytime and night-time operation: (1) circadian adjustments to the basal stimulation frequency and amplitude, delivered through the device scheduler and (2) motion-adaptive updates to the stimulation frequency and amplitude during night-time operation, made possible via the embedded accelerometer (Fig. 1).

The inner ring of stimulation in the diagram below represents the default circadian adjustments to stimulation. For RAS nuclei preferentially active during wake in the beta and low gamma frequency range for instance, daytime/wake-related settings would be set at a representative frequency and an amplitude tolerated by the patient

and sufficiently high in order to deliver therapy. In targets with differential activity between slow-wave activity periods and rapid eye movement (REM) sleep, further modulations can be made (here showcased as different settings in the first half of the night, when -as a general rule- slow wave sleep is more common, versus the second half of the night –since REM sleep periods become more frequent towards the morning.

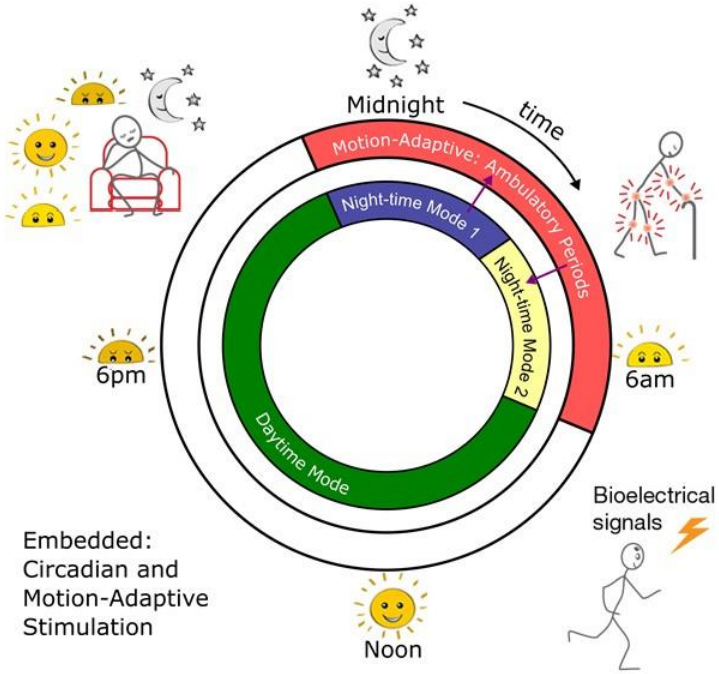


Figure 1. <u>Timing and adaptation of stimulation parameters to entrain physiological, circadian patterns</u>. A combination of motion-adaptive features with stimulation scheduled based on patient-specific rhythm timings is feasible via the DyNeuMo device. Avoidance of rhythms associated with adverse effects on sleep integrity and diurnal function (as have been defined in previous experimental chapters of this thesis) is therefore also feasible.

The outer ring of stimulation enables motion-adaptive adjustments of the stimulation amplitude and frequency during the night, to facilitate night time ambulatory periods

in the case where stimulation exerts beneficial effects on motor and/or autonomic function. When motion or postural change exceeding a set threshold is detected, the stimulation amplitude and frequency are updated to wake-related settings, especially in target nuclei where particular stimulation patterns alleviate disease-related motor symptoms. Stimulation is maintained at these parameter values until the next scheduled adjustment from the circadian scheduler, which reverts the stimulation parameters to their corresponding circadian-scheduled values.

Concept integration in trial design

These general device-related method concepts, their implementation and the examination of their efficacy were therefore incorporated in the experimental schedule of the MINDS trial, which is a feasibility study (Clinical Study Identifier: NCT05197816) investigating adaptive DBS in Multiple Systems Atrophy (MSA) for the amelioration of gait and autonomic function. Although its primary outcomes had been pre-defined as autonomic and motor (in keeping with the main interests of the Chief Investigator, Prof. Green), an amendment was made to the trial, in order to integrate outcomes relevant to the avoidance of sleep disruption and –where desirable- be able to implement an element sleep modulation through programmable tonic stimulation. Therefore, sleep-related outcomes are now an additional outcome measure, since the ethical approval of this amendment by the Regional Ethics Committee -REC.

First however, we will mention relevant modifications to our surgical implantation approach, compared to that utilised in the precursor cohort (which was studied in the prior two experimental chapters). Although our initial neurosurgical targeting of the PPNR has been previously described, there are specific device- and study-related considerations during the DyNeuMo implantation process that we will briefly touch upon here. Due to the device's cranial implantable pulse generator (IPG), compared to the larger IPG which is implanted below the clavicle in the case of the Abbott/St Jude device, a fronto-central skull pocket is created upon implantation, which houses the IPG in a cranial mount. As mentioned elsewhere in this thesis, implanted leads are connected to this cranial IPG during the same day, without a period of externalisation.

Further changes have to do with our surgical targeting protocols. Given that implantation variation within the PPNR (as discussed in the first experimental chapter) might be adding another layer of variability within the cohort in terms of underlying neural populations subjected to stimulation, an additional step was incorporated to ensure more homogeneous targeting. In addition to localization steps based on PPNR anatomical boundaries (Hyam et al., 2019), we now involve a dedicated mask, based on the location of the pedunculopontine (PPN) nucleus as predicted by the neuroimaging atlas of ascending arousal circuits in MNI space (Edlow et al., 2012). The choice of this target, rather than another BAAN node (or any of these nodes, based on results of the prior cohort), has to do with the importance of the PPN for motor and autonomic outcomes, compared to other nuclei within the surgical PPNR boundaries.

Furthermore, since there is no longer a capability of recording from all contacts of the implanted leads over prolonged hours, there is no way of reliably identifying contacts in the BAAN with maximal state-dependent modulation characteristics. The fact that the BioInduction leads also do not feature directional contact capabilities further restricts a precise localisation of any such 'hot spots' of interest. Although an attempt at streaming local field potentials (LFPs) from the device was undertaken, and there is still provision for this in the amended study protocol, its value in electrophysiological characterisation was extremely limited. The caveats of this effort (especially with regards to significant artefact presence) are described in detail in the published peer-reviewed paper that is provided in the supplement section of this thesis.

MINDS Trial: Sleep and Diurnal Electrophysiological Protocol

Sleep-related procedures, relevant to both monitoring and the programming of the 'stimulation prescription' are described here and their related events also illustrated through a patient flow diagram (Fig. 2). These procedures and reviews exist in parallel (and complement) planned events such as gait assessments, autonomic monitoring during stimulation and other data gathering that relates to the primary study outcomes.

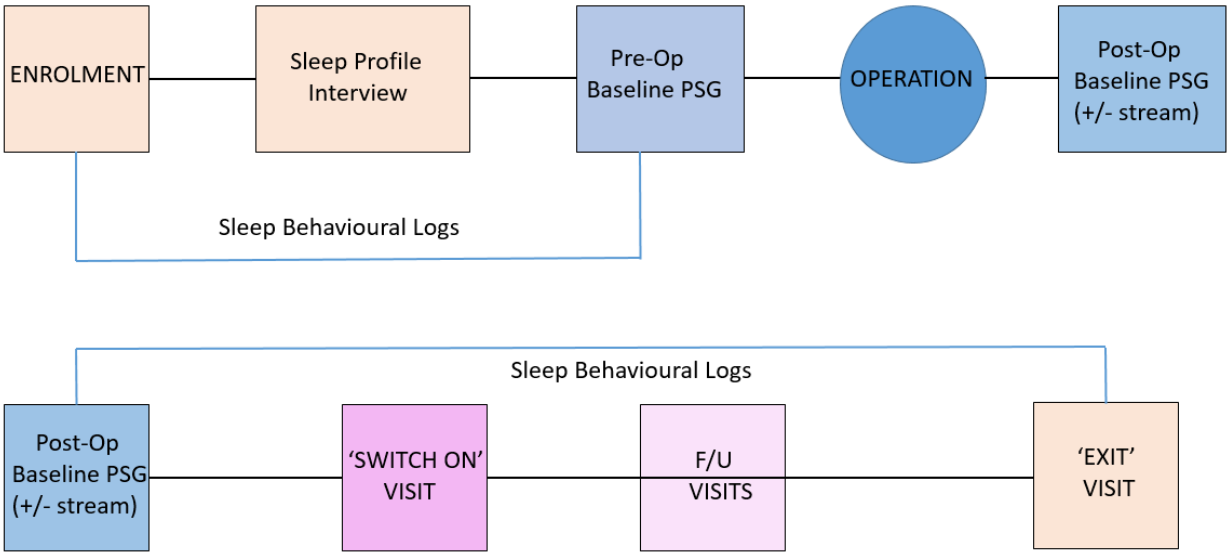


Figure 2. Events during the trial when there is either onset of capture (enrolment) or capture of sleep-related data. Further details of events and data per each are provided in the chapter text.

To capture clinically relevant dimensions of disrupted sleep/wake cycles, a set of validated questionnaires is now administered during a dedicated interview session scheduled after enrolment, in addition to other disorder-specific symptom assessments that had been described in the initial design of the trial. These questionnaires focus on EDS (captured by the Epworth Sleepiness Scale, ESS (Johns, 1991), subjective sleep quality and associated metrics (Parkinson's Disease Sleep Scale PDSS (Trenkwalder et al., 2011)), while presence of REM sleep-related pathology, common in MSA, is also now formally assessed (REM Sleep Behaviour Disorder (RBS) Screening Questionnaire, RBDSQ (Stiasny-Kolster et al., 2007)). All interviews are conducted by the author, while the bed partner is also present subject to patient consent, as per recommended practice and so that a more holistic and

subjective opinion may be obtained. Furthermore, at this time, preliminary evidence related to sleep/wake patterns and the presence of daytime napping is assessed, since patients are advised to keep a rough log of these times (bedtime, wake up time and estimated presence of naps) from the time of enrolment until interview.

Baseline wake and sleep EEG (with electromyogram (EMG) and electrooculogram (EOG) adjuncts for polysomnography -PSG), as well as continuous electrocardiogram (ECG), are obtained at a baseline pre-implantation session. They are also repeated upon recovery post-implantation (at 72hrs), with timing of recordings determined as per previous cohort practices. The plan was to combine this PSG with streaming of PPNR local field potentials (LFP) from the implanted device, while during recovery from anaesthesia we also tried to stream brief snapshots of data to examine for the presence of beta peaks. With regards to wake states captured, parameters such as eye closure, medication status and time-of-day are controlled for between sessions, to account for baseline variation effects. Following baseline post-operative assessments, patients are discharged from hospital. They are instructed to keep a diary of bedtime, wake-up time and –in the presence of specific sleep-related pathology- a daily diary events of interest, such as for instance episodes of severe sleep disruption and abnormal nocturnal behaviours or times of daily naps (the latter recorded by a carer).

After a period of six weeks, patients are invited back into hospital for the ‘switch-on’ of their stimulator and associated programming. In addition to autonomic and motor testing, wake EEG is captured during stimulation trials, in order to further assess the

impact of PPN DBS on cortical activity at different times of day. Sleep-related questionnaires are repeated, to assess for any changes in sleep pathology and patterns. Furthermore, there is an initial programming session where a daytime schedule is selected (tailored around wake up time and mean bed time, based on the patient and carer diaries). A set of night-time programmes is also selected, avoiding specific 'activating' rhythms (as identified in the previous chapters' cohort) and with consideration of patient-specific pathology. An overnight monitoring session follows and precedes discharge, to ensure the safety of the protocol and that the device functions as planned (this is of course subject to bed availability, especially in times of NHS bed crises). The patients and carers are instructed to continue with their log/diaries, so that any changes in diurnal activity patterns can be traced. Patients are further provided with information on device features and trained on battery re-charge. Follow-up involves extensive safety-netting and stimulation amplitude thresholding, to ensure both fine-tuning of personalized settings and patient safety.

During the subsequent visits for amplitude up-titration (since there is always a period between switch-on at low amplitudes and reaching amplitudes which have been used for motor and autonomic modulation), further episodes of wake EEG data are briefly captured. There is also a review of diary entries, questionnaires and patient histories with regards to progress of sleep-related symptoms and/or emergence of any night-time disruption. In the event where there is such an occurrence, programming is adjusted accordingly and re-assessed. These longitudinal subjective metrics and neurophysiological measurements are obtained during all

planned visits over a six-month course (such as daytime EEG recordings during different settings), to ensure fine-tuning of stimulation parameters and related algorithms.

This combination of objective and subjective measures was planned to ensure both potential biomarker tracking and correlation to patients' behavioural outcomes, experiences and perceived quality of life. The final settings and schedule thereof are decided based on a combination of autonomic/motor optimisation (if any) and stabilisation of sleep-related pathology. After a maximal amplitude of uptitration is reached, where autonomic activation is observed, no further changes to the settings are performed. Furthermore, prior to exit from the trial, a dedicated visit takes place where sleep-related outcomes are evaluated. In more detail, in addition to questionnaires and diary reviews, a PSG session where the current night-time stimulation programming is recorded ('baseline settings') is obtained, followed by a night of continuous daytime settings. The diurnal cycling of the device is disabled hours prior to bedtime while the patient is awake and without them being aware of this change (they are therefore blinded). A habituation night (usual settings) precedes these recordings, as well as any other PSG-related visits, to avoid any changes to sleep patterns due to novel environments.

Due to the time burden that detailed, prolonged diary entries imposed on our patient cohort, a final, most recent amendment (that is currently under consideration of the relevant Ethics Committee) was proposed. This has to do with wearable use (actigraphy), allowing for patients' patterns of activity to be tracked longitudinally

across months. This is also adding a more objective weight to any sleep/wake/nap diaries, as well as –cases where it is activated- validate the function of the device accelerometer.

MINDS: Current Enrolment Status and Available Related Data

Currently, the MINDS trial is actively recruiting for participants, with 2/5 cases having been enrolled in the study at the time of submission. Therefore, relevant results will be presented here on a case-by-case basis (also since these two patients differed in terms of sleep-related pathology), while a short section with some preliminary observations related to cortical activity changes during wake will also be provided with data from both patients. For each individual case, statistical analyses are presented within-patient, since the amount of observations performed during the longitudinal follow-up period would permit comparisons across time. Furthermore, we will include the anatomical location of each patient's implanted leads and associated area of tissue activation with final trial parameters, after analysis using the LeadDBS software.

In order to further highlight the possible adverse impact of tonic, constant 24/7 PPN stimulation on sleep architecture, while supply additional experimental observations that helped design the MINDS trial sleep arm, we will also provide a short case description of follow-up investigations in one of our prior cohort patients. This case –as mentioned previously in this thesis- experienced severe sleep disturbance when DBS settings were kept on at night. Again, analyses have be performed within-

patient, although the lead location (also provided) is comparable across all three cases.

Methods used for signal and statistical analyses have been expanded on during prior chapters. We will briefly state that we used a time window of 4sec with a 3sec overlap for the short-time fast Fourier transform (with added normalisation with regards to the noise floor within each window) for the slow-wave power spectral analyses displayed below. Additionally, we compared power spectral density statistically, using a two-way ANOVA (time of night and recording/stimulation pattern as main variable) with Bonferroni correction for multiple comparisons between nights, as well as within each night (one-way ANOVA with correction), for SWA evolution (pooled power per hour).

Results

1. Precursor case: Effects of Continuous Overnight PPNR DBS on Sleep

Following capture of both baseline and stimulation trial data, as described in the prior chapters, we additionally performed an investigation of the effect of continuous PPNR DBS at a frequency of 40 Hz, pulse width of 60 microseconds and at final clinical amplitude, on sleep architecture. This was carried out in one case where the lead location was within the PPN, as opposed to the PPNR (therefore comparable to MINDS locations and sites). This patient (PPNR1, associated clinical data provided in Chapter 1), reported severe insomnia when initially discharged on continuous (day and night) settings, while subsequently DBS was kept off at night.

On a subsequent visit, after re-habituation to the hospital environment, experimental polysomnography was performed and compared between a night ‘OFF’ stimulation and a night on 40 Hz settings. Switch-on had been performed through a gradual ramp during that evening, so that the patient was as blinded to stimulation status as possible. In addition to formal sleep scoring according to the American Academy of Sleep Medicine (AASM) criteria, slow wave activity was calculated for artefact-free periods over both nights (as per Methods) and compared between ‘ON’ and ‘OFF’ DBS settings (Fig. 3).

Additionally, a two-way ANOVA was performed for all values of slow wave power, organised across half-hour groups of bins (with factors being settings status, sequential half-hour section within each equivalent recording time). It revealed (with Bonferroni correction for multiple comparisons), that there was a significant interaction between status and recording time, with regards to the effects of settings on slow wave power (Table 1). Furthermore, there was overall a significant difference between slow wave power during the 40 Hz stimulation night and when settings were ‘OFF’, with lower power in the first case (Mean OFF: 3.9332, P=0.000, CI: -1.6548, -1.3394). With regards to comparisons in sleep scoring differences, time spent in slow-wave sleep was significantly higher when DBS was switched off compared to constant stimulation, with a significantly lower degree of wake after sleep onset (Fig. 3). Due to the fact that, during the 40 Hz stimulation night, this patient removed EMG sensors midway through the recording, REM staging is shown here amongst ‘other’ stages compared to NREM3 and wake.

Table 1. PPNR1: ANOVA results for slow wave power comparisons between 40 Hz and 'OFF' overnight settings.

PPNR1			
	df	**F**	**P**
Stim settings status	1	498.68	$1.04926e^{-108}$
Recording Interval status	5	133.13	$1.52372e^{-138}$
Stim settings status * interval status	5	310.71	$1.10392e^{-317}$

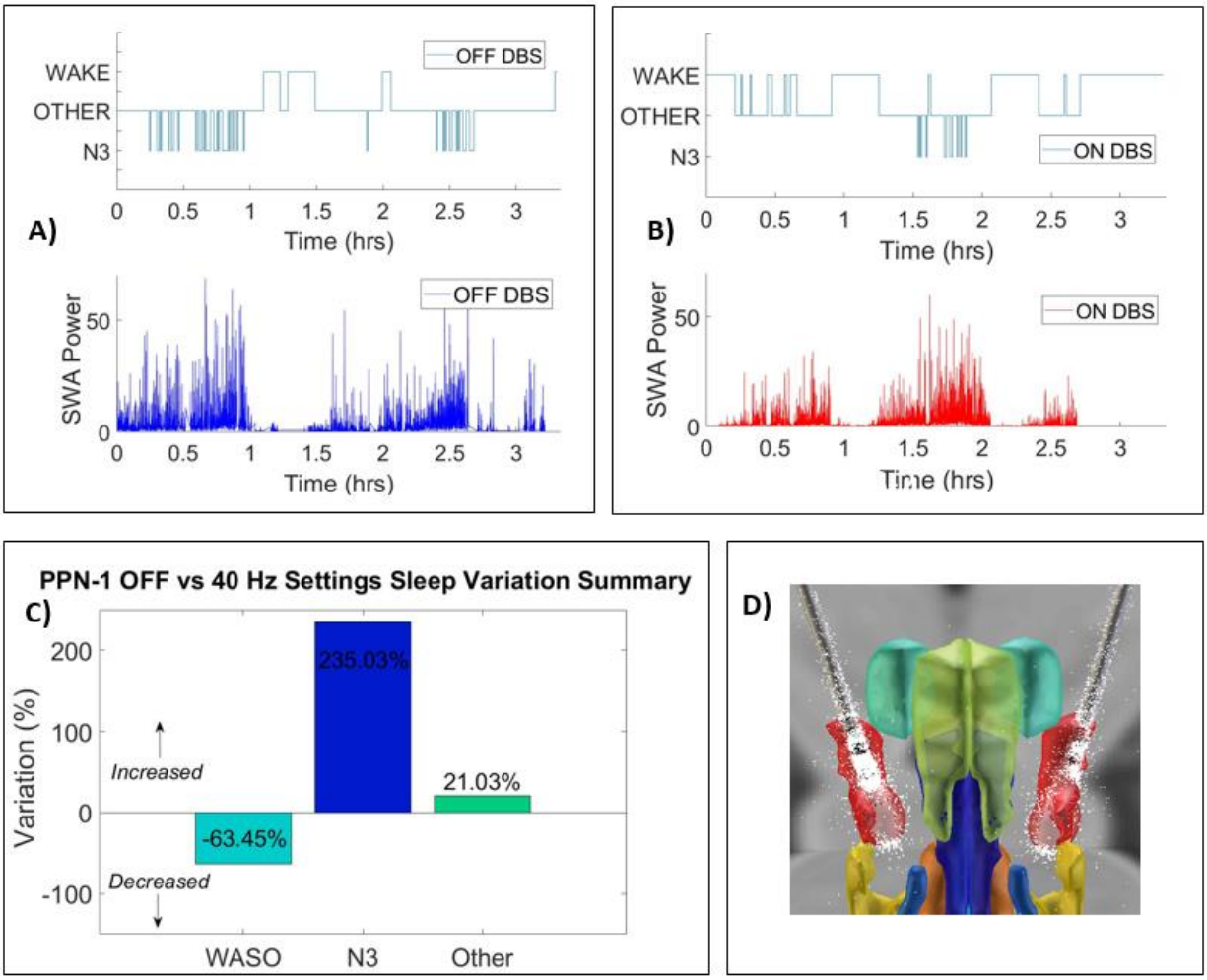


Figure 3. Effects of continuous PPN stimulation (40Hz) on N3 slow wave sleep. Sleep architecture and slow wave activity (SWA) power while stimulation is OFF (a) compared to an ON night (b). There was a significant decrease in SWA power with DBS ON, from the OFF night when SWA power was compared across nights in the Fz-Cz derivation. There is a delay in reaching peak sleep depth as well as an overall truncation of sleep duration when

ON at 40Hz. Decrease in fragmentation (wake after sleep onset –WASO) and an increase in N3 occurs while OFF compared to ON at 40Hz (c). Electric field and VTA for these settings, showing activation of the PPN and in particular the lower –most caudal- region (light red) (d).

2. MINDS-RBD case: Effects of Continuous Overnight PPNR DBS on Sleep

One patient from the MINDS cohort had a pre-existing REM sleep behaviour disorder (RBD) diagnosis, with symptoms not fully controlled on clonazepam but without excessive pathological excessive daytime sleepiness (EDS) upon enrolment (*patient-related variables in Table 3, at the end of the 'Results' section)*. In this case therefore (MINDS-RBD), we will present the results of the comparisons between programmed settings (using the DyNeuMo device) and a continuous night of stimulation at daytime frequencies and amplitudes. This case, apart from an initial perioperative period surrounding the initial interview, did not keep or provide longitudinal diaries related to sleep behaviours (such as bedtime, wake up time and naps). This was perhaps secondary to the lack of daytime pathology (not a concern in this case), in addition to the patient's attitude with regards to keeping regular diary entries.

With regards to contact selection for stimulation, we chose the deepest contacts that fit best with brainstem ascending arousal network (AAN) location, as predicted by the relevant atlas, as well as by the fact that these were the regions that had exhibited the greatest state-dependent oscillatory modulation on comparable implantation trajectories in our externalised case (PPNR1). We wished to compare streamed oscillations per contact pairs and state; however some streaming dipole

combinations exhibited significant artefact, while there were significant noise floor changes in other contacts while using the DyNeuMo streaming system (*further detail in the peer-reviewed publication provided as supplement to this thesis)*.

In the prior experimental chapters, we showed that there was potential evidence of 40Hz stimulation having higher degrees of synchrony with cortical wake-related oscillations, while it induced disruptions when administered during NREMS. Furthermore, tonic stimulation overnight in PPNR1 led to disturbances of sleep architecture. Therefore, we supplemented this frequency with a combination of slower rhythms over the documented sleep period of MINDS-RBD, when formulating the night-time prescription. This resulted in a specific sequence of frequencies, delivered across time for the subsequent recording night that we will showcase here. In particular, for the first two hours from sleep onset on that night, a frequency of 1Hz was applied with a pulse width of 60 microseconds and amplitude of 4 Hz.

During the ‘exit visit’ a set of sequential recordings was used to further investigate the effect of different DBS settings on slow wave pressure and activity. Up until then, the protective algorithm (overnight) had been followed by daytime tonic stimulation on a 40Hz frequency. As an initial step in this investigation using tonic pre-programmed settings, we sought to juxtapose the two extremes of modulation: our own ‘protective’ algorithm, that we had tailored specifically for the case of the RBD phenotype, and what could be an extreme tonic suppressor of SWA.

We chose to try a novel frequency at the high gamma range (75 Hz) due to both prior experimental chapter evidence (such as connectivity changes in that frequency and its presence during the 'UP'-state of the slow oscillation), as well as other published case report suggesting perhaps a paradoxical effect on sleep. Furthermore, the patient was naïve to this frequency. Therefore, any possibility of circuit habituation (that may have occurred with the 40Hz daytime settings the patient had been used to for five months) was eliminated.

To ensure there was a slight build-up of sleep pressure at the beginning of each night, the patient was kept awake for an additional 1.5 hrs from their reported bedtime. This partial restriction was scheduled to take place with the presence of an assistant that tried to keep the patient alert when necessary with verbal and sensory cues. Wiring up (with the PSG setup) was also timed to take place within this restriction period. Medication (clonazepam) was administered at fixed times before the onset of the recording on both nights (prior to the bed transfer and electro-occulogram and electromyogram fixation, therefore 30 mins prior to 'lights out'). This was performed to ensure soporific effects remained a constant variable.

The recording was scheduled for termination at the same time on both nights, in keeping with reported wake and sleep patterns. Specifically, the patient routinely woke up every night at 4:30am for a voiding break and levodopa medication use. This was per their report a spontaneous occurrence (as we also verified in 2/3 nights we have recorded in this session, as well as during their baseline pre-implantation recording). We therefore regarded this as an established sleep routine that the

recording timing (and frequency planning in the ‘scheduled’ settings) followed, while the patient was instructed that the hours from 5am to 7am were permissible sleep time for the remainder of the day. While an inpatient, they and their spouse were encouraged to note nap times. Lunchtime, dinner time and activity out of the ward were strictly scheduled, while no caffeine was permitted after the morning was over (drinks were restricted to one coffee only).

The patient had been utilising the ‘protective’ (scheduled) night-time settings for five months up to this point. Although they did not volunteer sleep diaries for this amount of time, they subjectively reported less difficulty with sleep maintenance and some improvement with RBD symptom frequency (although these reports would require corroboration with sleep actigraphy data at least and/or an ‘RBD attack’ diary in this phenotype). During the partial deprivation night while the scheduled settings were utilised, the patient became progressively drowsy, less engaging and requiring constant verbal engagement and sensory cues (light touch and tapping) to maintain wakefulness for the prescribed amount of time. The recording started at 23:53:36 while the patient had been unable to follow instructions for biocalibrations and fell asleep straight away.

On the control tonic gamma night, stimulation was titrated around the time when the scheduled ‘wind-down’ process would have started in the scheduler. Instead, we started stimulation at 75Hz at low amplitudes, slowly increasing with incremental pauses of five minutes between 0.5mA steps. There was no drowsiness (or sleep attacks as had been described in the previously cited case report), however once

4mA were reached the deprivation assistant noted an increase in facial flushing. When the experimenter (wiring at that stage) was notified, pupil diameter was assessed and was found to be dilated bilaterally, with a diffuse flush on the cheeks and neck. The patient did not complain of palpitations –however they were monitored for heart rhythm irregularities and clinically observed for fifteen minutes. Wiring was resumed when these symptoms subsided and (since visible sympathetic activation had been achieved) the patient remained at this stimulation amplitude for the remainder of the experiment.

No overt drowsiness requiring sensory input occurred for yet another hour, when the deprivation assistant was instructed to gradually cease providing verbal input, the patient was instructed to lie in bed and medicate as per the 'scheduled settings' night. We started the recording at 23:52:57 and (as can be verified in the traces) the patient was able to follow through with biocalibration instructions until their completion. Sleep onset was not immediate as per the prior recording night, but the first sleep epoch occurring was one of REM sleep at second 1020 (17 minutes in the recording). Therefore, this was a first sleep period starting with REM sleep (REM sleep onset night) which lasted very briefly until wake ensued again at 1500 seconds (25 minutes in the recording). This was in contrast with the prior 'scheduled settings' night where there was a NREM onset and progression to deeper slow wave sleep at the beginning of the night. While final formal sleep scoring of both nights is pending, based on first pass and live scoring the majority of the second night seems to consist of REM sleep bouts.

With regards to the 4:30am awakening, on the control night of ‘scheduled settings’ there was a spontaneous arousal for the patient’s usual voiding/medication routine. They went back to bed after being unwired and reportedly slept soundly until 7am. On the tonic high gamma night, the patient was on a bout of slow wave sleep where there would be expected to be REM and light stages. Sleep depth was evolving therefore the patient was not woken up and unwired until after 5am. They were still experiencing slow wave activity, which was interrupted and were therefore very sleepy. Although they were instructed that sleep was permitted until 7am, when they were visited for an expected ‘lights on’ they were found awake and sat up in bed. They were overall pleasant about this and stated that they had not been able to sleep after they were woken up, but did not feel overtly tired. They were subsequently enquiring about how the new settings would be incorporated in their final diurnal programming.

With regards to a first step in analysing these two experimental nights, visual artefact identification was performed (to exclude inadvertent increases in slow wave power due to motion, orofacial, blinking and other artefacts). The ‘time without artefact’ was then processed, with regards to calculation of slow wave activity (SWA) power (0.5-4Hz) and its plotting for a common, relatively artefact-free, derivation across both nights (Fz-Cz) with normalisation for the respective noise floor. We noted that the 75Hz stimulation night ‘pushed back’ bouts of slow wave activity, away from their expected place at the beginning of the night and towards its expected end (when REM would normally predominate) (Fig.4B). This became even more obvious when we computed average SWA power over 5-minute intervals, with two main SWA

bouts occurring early in the 'scheduled settings' night while three smaller and later peaks of SWA were observed while on tonic high gamma settings (at amplitudes that had sufficiently caused prior signs of sympathetic activation) (Fig. 5).

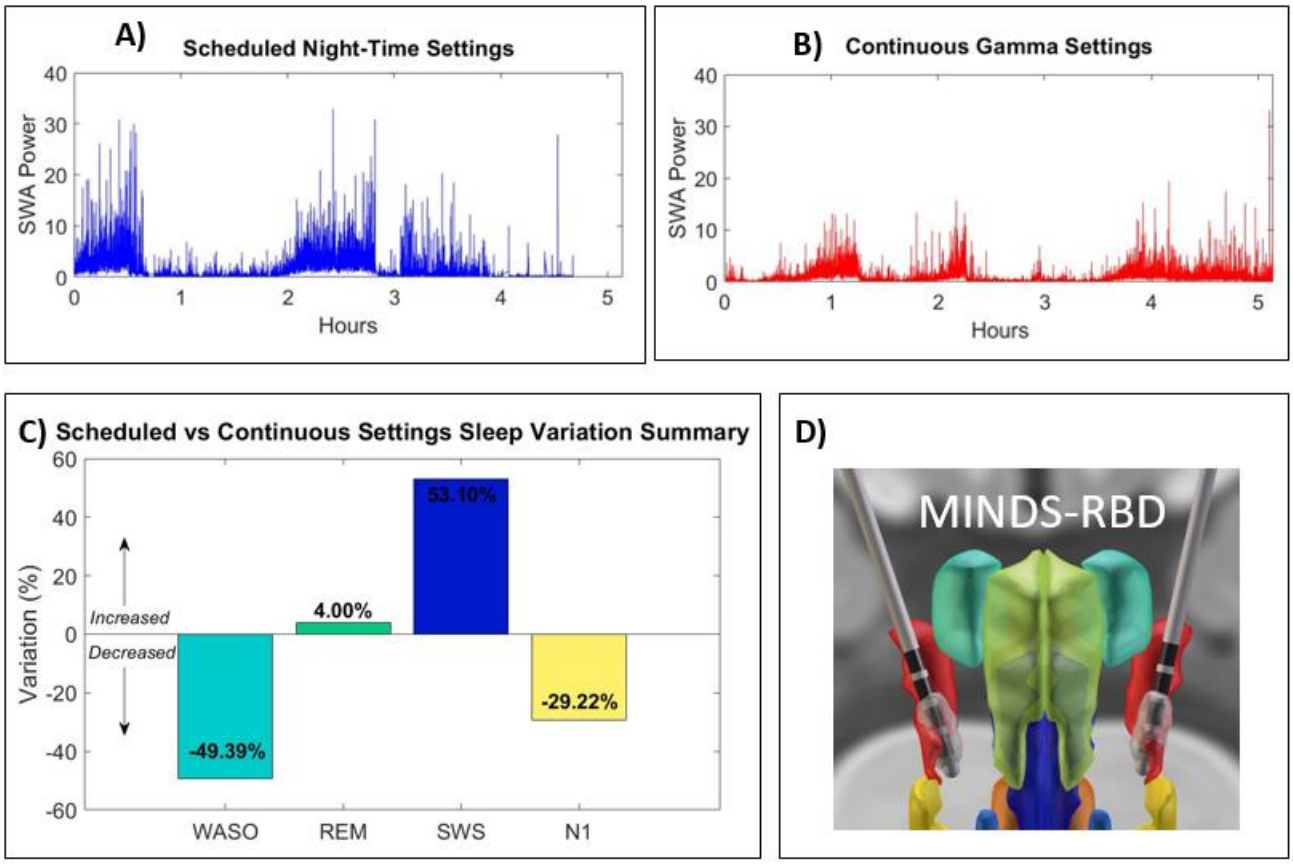


Figure 4. <u>Effects of continuous PPN stimulation (75 Hz) on slow wave sleep</u>. Sleep fragmentation decreases and total time spent in slow-wave sleep increases with scheduled night-time settings, compared to continuous gamma stimulation (a, b). Decrease in fragmentation (wake after sleep onset –WASO) and an increase in SWS occurs while scheduled are compared to continuous settings, with a decrease in light sleep (N1) (c). SWA power changes across both nights are also shown (a, b) with lower SWA power and more delayed peaks while on continuous gamma for the same time period. Daytime VTA for contacts used is shown in grey (d) also targeting the lower-most caudal portion of the PPN as in the previous case (PPNR1).

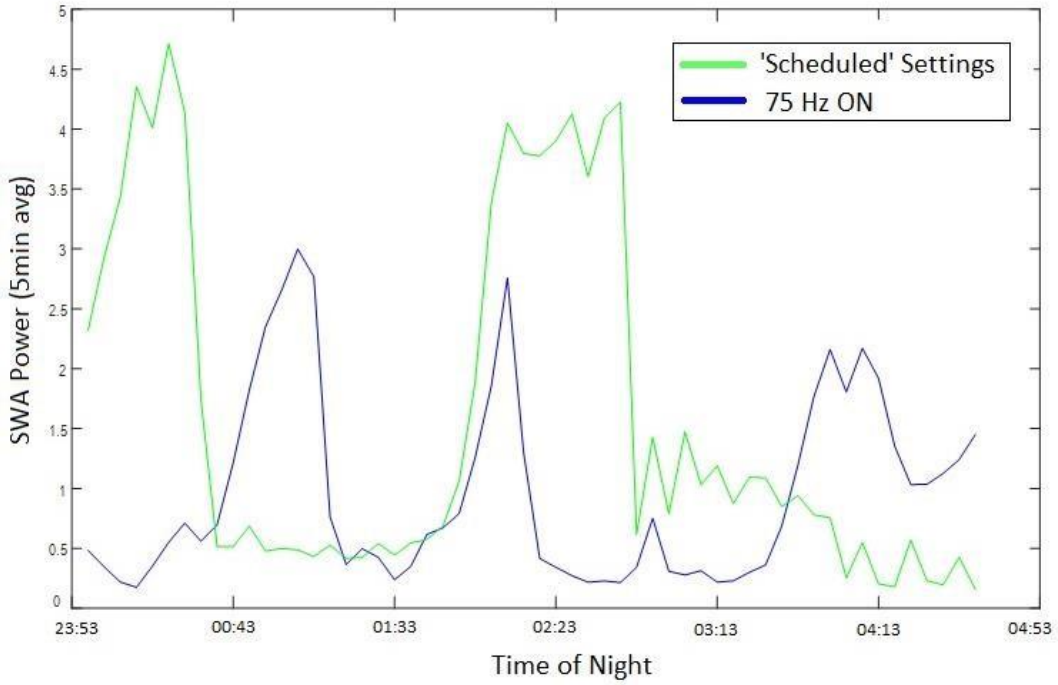


Figure 5. Modulation of SWA bouts across the stimulation nights. On the high gamma stimulation settings, SWA is delayed and 'erupts' in peaks throughout the night, without a clear early main period. At the end of the comparable part of the night (prior to where the patient woke up spontaneously as per their usual habit in the other recording), SWA is still building up. In contrast, the 'tailored' settings with scheduled PPNR 'release' frequencies and very low amplitudes of stimulation (that the patient had also habituated to for five months) show a profile more comparable to physiological night-time activity.

As in the case of PPNR1, a two-way ANOVA was performed for all values of slow wave power, organised across half-hour groups of bins (with factors being settings status, sequential half-hour section within each equivalent recording time). It revealed (with Bonferroni correction for multiple comparisons), that there was a significant interaction between status and recording time, with regards to the effects of settings on slow wave power (Table 2).

Table 2. MINDS-RBD: ANOVA results for slow wave power comparisons between 75 Hz and 'scheduled' overnight settings.

MINDS-RBD			
	df	**F**	**P**
Stim settings status	1	677.12	0
interval status	9	387.06	0
Stim settings status * interval status	9	689.38	0

We found that the highest mean slow wave power per hour occurred during the last hour of recorded sleep during the 75 Hz stimulation night, with nearly double the mean power compared to what occurred in the first hour, while comparisons with all prior hours were highly significant ($p<0.0001$). Meanwhile, SWA power occurred in two bouts for the 'scheduled' settings: the first during the first hour while the second one occurring in the third hour of sleep. In the non-averaged values, this second sleep bout was characterised by the highest SWA power ($p<0.0001$ and CI -0.79 to -0.47 for the comparison between the first and second bout). The lowest SWA power occurred during the last hour of the recording (the polar opposite to what happens in the 75Hz stimulation night), which also differs highly significantly in that regard with all prior hours ($p<0.0001$).

With regards to sleep scoring of both nights based on AASM criteria, we noted that in the MINDS-RBD case, the 'scheduled' settings (protective programming) were related to a relatively lower level of fragmentation (wake after sleep onset –WASO) and longer overall time of SWS occurs compared to continuous 75 Hz settings (Fig.4C).

3. MINDS-EDS case: Effect of Daytime PPNR DBS on Excessive Daytime Sleepiness

The second case enrolled in the MINDS trial did not have an RBD diagnosis and was not on benzodiazepines, but exhibited a pathological degree of excessive daytime sleepiness (EDS, as assessed during interview and documented by the ESS values) upon enrolment (*patient-related variables in Table 3, at the end of the 'Results' section)*. In this case as well, contact selection for stimulation was based on AAN atlas contacts and the prior findings in PPNR1. We proceeded to slowly titrate the stimulation amplitudes for our first selection of contact pairs (over an average period of 30 days), assessing for side effects and responses.

Furthermore, in this case we devised a cross-over experimental design to examine whether any improvement (objective or subjective) could be contact-dependent, in a stable medication status. After a period of stabilisation on the first (AAN/putative arousal modulating contacts) we swapped to what we predicted could be a contact combination more relevant to gait and motor symptoms. We selected this pair during a follow-up clinic visit, where the patient was instructed to step whilst sitting and compared beta activity between stepping in situ and sitting, for all available streaming dipoles. We then proceeded to titrate stimulation amplitudes for these contacts, assessing for witnessed nap number and duration, as well as subjective reports of drowsiness during the day.

We collected and analysed over 100 days' worth of diaries (consisting of nap records as well as bedtime/ wake up time). These diary entries were provided by both the

patient with EDS (bedtime, wake up time) as well as their spouse, who acted as observer/recorded all nap times. In the context of this chapter, nap data will be presented to examine the question of whether diurnal patterns of PPNR DBS can modify EDS. Diary entries were analysed using two sample t-tests as well as ANOVA (we corrected for multiple comparisons). Variables that were explored were total nap duration, number of naps per day/presence of days with multiple nap episodes, as well as time-of-day when naps occurred.

We first examined the effect of tonic daytime PPNR DBS at a gamma (40Hz) frequency on subjective sleepiness and observed behavioural reports in this case. Based on the initial ESS response of subjective sleepiness, we noted an improvement (Fig.6A) which took place after switch-on (not post-implantation, therefore not attributed to a stun effect). We also examined the pattern of naps (in terms of number per day, total time spent napping per day, as well as what time the naps took place). We computed weekly averages of nap activity and proceeded to compare all combinations of weeks (to identify when naps were decreasing) correcting for the multiple comparisons we had made (Bonferroni correction).

There seemed to be a decrease in overall time spent asleep during the day (napping) acutely after switch on. This was confirmed through our analyses, with the second week compared to the first having a significant reduction ($p=0.0022$), with a mean reduction of a bit over an hour of daytime sleep (61.9 minutes, CI 11.75 to 112.06 minutes). Despite an overall deterioration in terms of disease progression, this reduction was longitudinally maintained (week 15 had an average of 51.2

minutes spent in daytime sleep compared to week 1, with p= 0.0386 and wider CI however, ranging from 1.04 to 101.34 minutes). We also noted that, compared to the first week of stimulation, time of the last nap occurred significantly earlier (by around 5 hrs) close to the end of the data collection time (p=0.0036 with mean difference of 5.29 hours and CI from 0.89 to 9.69 hours, between weeks 1 and 12).

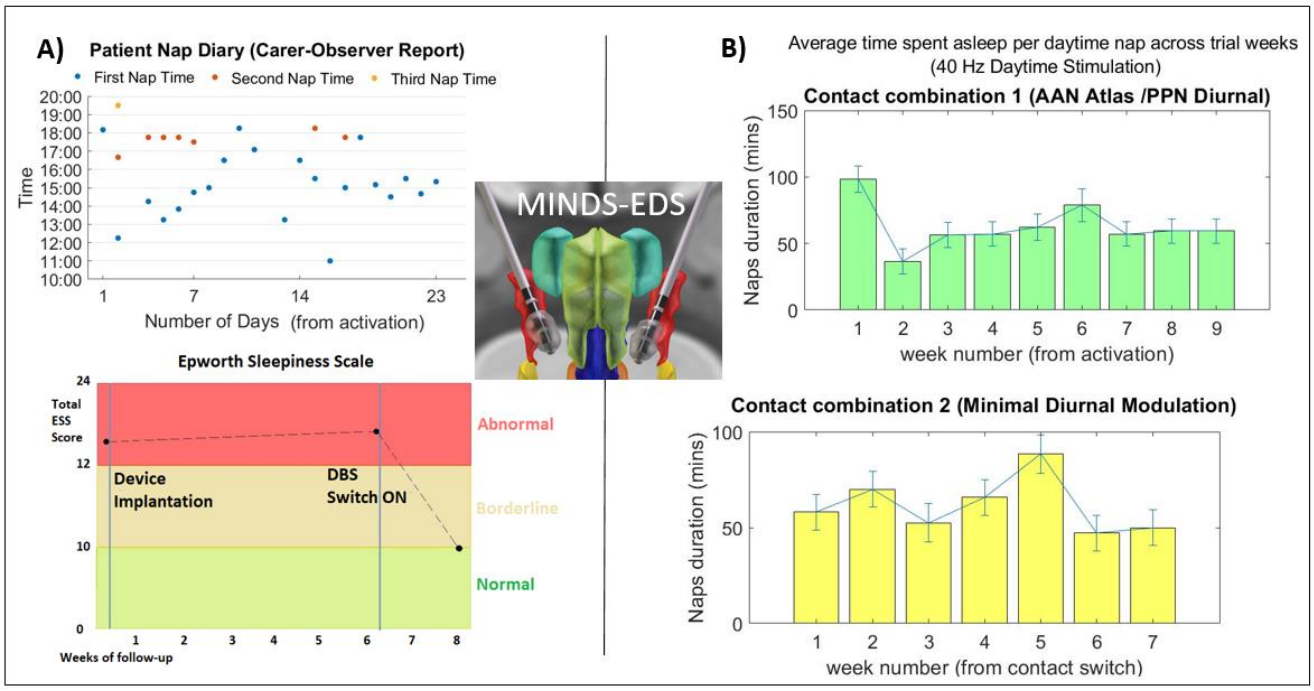


Figure 6. Acute modulation of excessive daytime sleepiness (EDS) by PPNR DBS. A. Subjective daytime sleepiness scores (quantified by the ESS) are acutely modulated by PPNR DBS. We note that the ESS score decreases (less subjective sleepiness) acutely after the device is switched on. B. Time and number of naps per day, after PPNR DBS switch-on. We see that acutely the number of naps per day and their dispersion in the time domain decreases.

We also saw an element of longitudinal reduction in nap number, from 2 naps as a daily average during the first week to 0.86 naps per day in week 15 (p= 0.0279 for a reduction in 1.14 nap and CI between 0.05 and 2.35). While the first period/contact

combination however had culminated in a stable state where one short daily nap was the norm, the period of the second contact combination was characterised by fluctuations, as well as the fact that compared to weeks spent in both the titration of the first contact combination (week 2 after the initial response) and during its stable settings (weeks 8 and 9), nap frequency increased (p=0.0036 mean increase by 1.31 naps with CI 2.40 to 0.22, and p= 0.0211 mean increase by 1.17 naps with CI between -2.26 and 0.07 for both of these last weeks, respectively).

With regards to nap duration in more detail, aside from the aforementioned overall decrease in about an hour of time spent in daytime sleep from week 1 to week 15, we also saw that the initial steep response with nap duration reduction was not maintained. More specifically, week 2 had an average of 51.9 less minutes spent in sleep during the day compared to week 14, with p=0.0322 and CI ranging from 102.06 to 1.75.

These comparisons seemed to match the overall trend of reports during the duration of the first contact combination: overall, naps were less and the patient felt less drowsy than before. However (as also verified by the weekly comparisons) there was no further steep improvement but rather a longitudinal plateau. Observer reports reflected this fact ‘about the same’ while we noted that –at this stage severe motor deterioration and bulbar symptoms started appearing- the patient became increasingly withdrawn.

The one big difference in both observer and patient complaints started occurring a few weeks after the ‘motor’ contact selection. The patient was complaining of ‘feeling

out of it, all the time' and the carer also reported that things were off their prior established routine (at least with regards to daytime loss of vigilance and lack of engagement). We therefore sought to examine if any periods stood out with regards to higher nap durations and/or occurrence (grand pooling of all daily averages across the time periods spanning duration of titration and periods up to contact swap).

There was only one comparison that stood out of the total; that was an increase in nap number between the time of stable settings in contact combination 1 (AAN) and the titration period of contact combination 2 ('motor' settings) ($p = 0.0187$ and CI from 0.70 to 0.067). (Fig. 6B). All other big pools of comparisons were not statistically significant, while when at the end of the 'motor settings' trial we reverted to the original combination, both subjective (and observer) reports do not indicate any improvement.

4. Acute Effects of Daytime Gamma PPNR DBS on Cortical Activity: Preliminary Data from both MINDS cases

We also examined the effect of PPNR DBS on cortical oscillatory activity during quiet wakefulness in both patients. Wake data collection sessions were conducted in a state of complete eye closure in a quiet room, with the patient sitting in a comfortable chair and instructed to relax but refrain from falling asleep. Recordings were compared only between the same time-of-day (and patient schedule) as well as with specific medication considerations. Given the aforementioned recorded prevalence of levodopa-induced hypersomnolence in the MSA cohort, we also regularly

monitored for post-dosing somnolence in both cases. At the current doses utilised by patients, such an overt effect was not observed. However, we controlled for all medication status changes between recordings, since this could lead to subtle oscillatory changes that could influence subsequent analyses.

While we are still collecting data in this trial to determine effects of daytime PPNR stimulation, so far we have noticed that PPNR DBS has the potential to entrain cortical activity at the half-harmonic of the stimulation frequency, in the beta oscillatory range (13-30Hz). For trials matched for re-referencing dipole, medication status, patient and time of day, where there is sufficient frequency content close to the sub-harmonic of the stimulation frequency, we note modulation of intrinsic beta suggestive of 1:2 entrainment, with content 'pulled' towards this frequency.

This phenomenon can be observed across patients (both MINDS-RBD and MINDS-EDS) (Fig.7) at a gamma (40Hz) stimulation frequency. However, we only saw global effects at the 20Hz beta peak (the 40Hz half-harmonic) for the patient without EDS. We additionally observed overall lower beta amplitudes in the EDS case throughout sessions and recordings (although this needs to be confirmed quantitatively when all planned trials are collected, over the two different contact combinations). In the case of MINDS-EDS, entrainment at the half-harmonic of the PPNR DBS frequency was localised frontally, with a slower beta peak at the occipital region (where alpha waves predominate) around 15Hz. As a second experiment, we modulated the stimulation frequency to assess if we could successfully entrain this lower beta peak in MINDS-EDS. While we succeeded in doing so (Fig.7A), the

patient complained of worsening oscillopsia, therefore we decided not to incorporate a long-term trial of these settings.

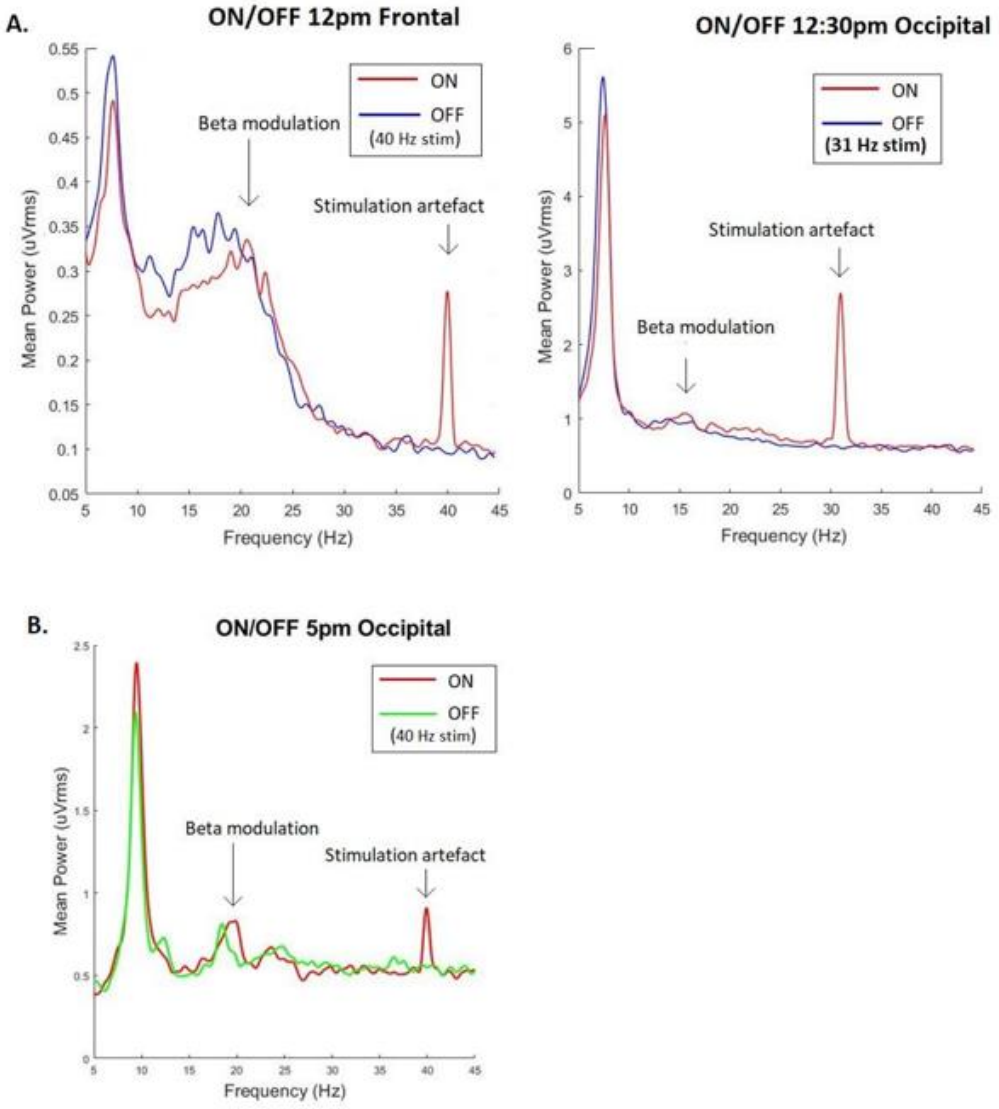


Figure 7. <u>Acute modulation of cortical oscillatory activity by PPNR DBS</u>. A. In recordings from the EDS case (MINDS-EDS) we note how two different gamma frequencies modulate the beta peak around the stimulation half-harmonic in two different cortical regions. Oscillatory content at half of the stimulation frequency is enhanced (first case at 20Hz and second case 15.5 Hz). B. Modulation of the beta peak at the MINDS-RBD case (without EDS) using the 40Hz stimulation frequency range. The 20Hz beta peak is enhanced and more sharply delineated. Note that for the same region and albeit at a later time of day, mean power in band is higher in the beta range for the case without EDS (which also showed global entrainment).

To further confirm the hypothesis that beta peak modulation was correlated with wakefulness, we examined the 'naturalistic' patterns of beta activity in the non-EDS case, while they were a resident inpatient for a follow-up session. We noted that when DBS is OFF (no stimulation delivered), beta activity decreases as the day progresses (Fig.8, left). When we switched the stimulation on, we saw that in the same dipole beta activity increased, while the 40Hz stimulation protocol seemed more effective in its entrainment compared to an 80Hz stimulation frequency that we briefly investigated. Although we included washout periods of five minutes between trials, there could perhaps still be an add-on effect with beta being enhanced due to priming from this previous protocol (Fig.8, right).

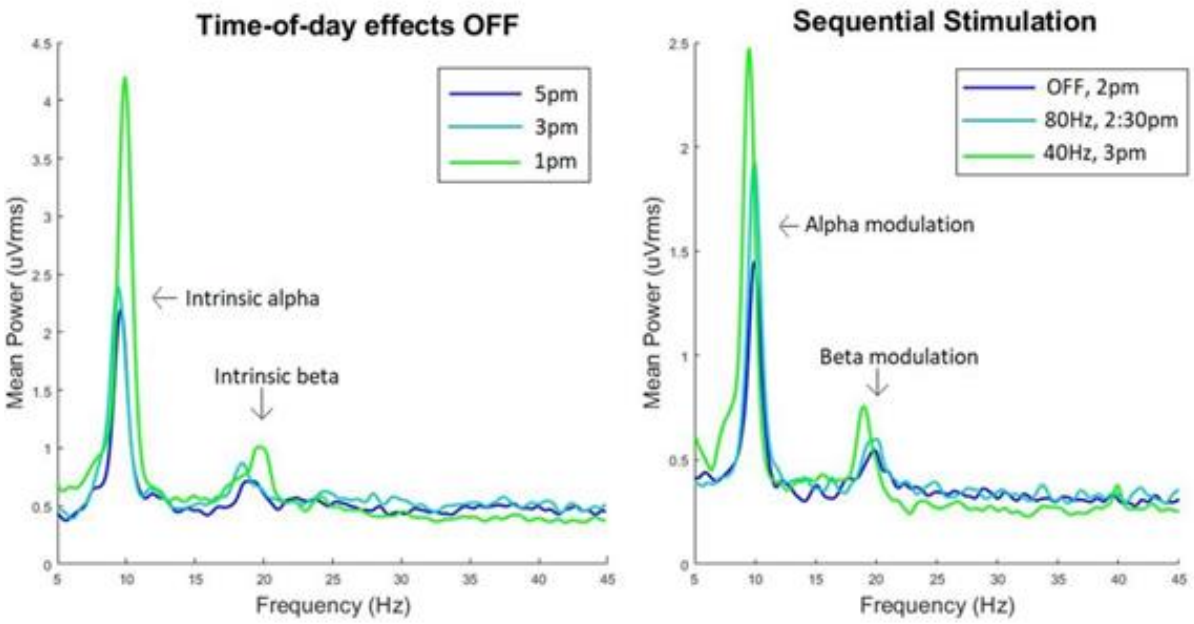


Figure 8. <u>Reversal of 'time-of-day' or tiring effects by PPNR DBS</u>. Intrinsic beta activity decreases as time of day progresses, in recordings from the non-EDS phenotype while an inpatient (left). We note that application of gamma protocols reverses this, suggesting an opposing modulatory force at play.

Table 3: Enrolment variables for the two MINDS study participants.

Score/Variable	MINDS-RBD Enrolment Scores	MINDS-EDS Enrolment Scores
Age	64	68
Sex and Gender	Male	Male
Disease Duration	3 years 11 months	6 years
Disease Subtype	MSA-P	MSA-P
UMSARS Hx	21	26
UMSARS Ex	24	24
COMPASS-31	58.5	27
RBD Dx	Yes (clonazepam 5-10mg)	No
RBDSQ	6	1
PDSS	7 (item 1) 69 (items 2 to 15)	8 (item 1) 123 (items 2 to 15)
ESS	7	15
Stimulants	No	No
GFQ	11	49
FOGQ	5	22.0
PDQ-8	18	17
EQ-5D VAS	40	40
Zarit Burden	15	24
ACEI-III	93	94
HAD Scores	<7 (HADS-A and HADS-D)	HADS-A: 8, HADS-D: 7
L-Dopa post-surgically	Yes	Yes
L Dopa cumulative dose (if yes)	600 mg DA	1200 mg DA (self-titrated up to 1600mg)

Abbreviations: MSA-P: Multiple system atrophy- parkinsonian type, UMSARS: Unified Multiple System Atrophy Rating Scale, COMPASS-31: Composite Autonomic Symptom Score-31, RBD: REM-Sleep Behaviour disorder, RBDSQ: REM Sleep Behavior Disorder Screening Questionnaire, PDSS: Parkinson's Disease Sleep Scale, ESS: Epworth Sleepiness Scale, GFQ: Gait and Falls Questionnaire, FOGQ: Freezing of Gait Questionnaire, PDQ-8: Parkinson's Disease Questionnaire, ADL: Activities of Daily Life, EQ-5D VAS: EuroQuol-5D Visual Analogue Scale, ACE I-III: Addenbrooke's Cognitive Examination I-III, MOCA: Montreal Cognitive Assessment, HADS, DA: Dopamine, Dx: Diagnosis.

Discussion

At the time of submission of this thesis, the patient with an EDS phenotype has undergone a steep deterioration with regards to all motor milestones (requiring carers and additional aids in order to mobilise), exhibiting further symptoms of disease progression (such as severe hypophonia, drooling and oscillopsia) as brainstem degeneration progresses. They are additionally showing increasing signs of apathy and amimia, with the patient requiring significant motivation to engage with family and peers. In this context of extreme decline, the author wonders about the amount of quality of life that an extra hour of sustained wakefulness may have to offer. After an acute response to stimulation, over the longer term, habituation seemed to ensue with regards to wake maintenance. With the initial (possible 'diurnal modulation') contacts, a steady state had been achieved, that may have been briefly destabilised during the settings' change.

It is however apparent that in the context of near complete loss of bodily autonomy a small stable improvement in one domain may not be enough. We must also consider further factors –although the carer-observer seems as vigilant as ever, noting and reporting all changes at the same regular pace, could it be that some of this stability is an inherent bias as disease progresses? This thesis does not focus (and will not report) the motor and other sequelae of this case, therefore the description and quantification thereof as trial outcomes is not something that will be covered here.

Further interventions that could be explored in the case with EDS (with an aim for an exclusive benefit to vigilance at this stage) would be a novel frequency change, especially after the results obtained during the high gamma trial in the MINDS-RBD follow-up session with regards to homeostatic sleep pressure. Since the number of naps and their duration were destabilised mainly during the titration of the second contact combination, it could well be that there is an amplitude (rather than location) effect at play. Therefore, we propose that contact selection be further explored and optimised in future cases (hopefully with LFP indicators, albeit not as extensive as those in the prior cohort), while –if the patient so wishes- we could proceed with frequency explorations in this case of EDS.

However, there was a higher number of side effects when novel settings were tried out in the MINDS-EDS patient, putting to question whether at a repeat session a titration of 75Hz up to the level of sympathetic activation is achievable. Other factors that may be at play, such as the presence of sleep disordered breathing (despite no reports of excessive or new overnight disturbance) as well as the need for treatment of a secondary mood disorder and/or their abulic state are all things to consider. However, the differential diagnostics and treatment approaches to this case will not be further expanded upon in this research thesis setting.

On a note closer to experimental neurophysiological objectives, acute effects of the daytime parameters on the daytime power spectra from both patients involve nonlinear acceleration of peak frequency in response to stimulation, as well as an increase in higher frequency content, when compared to an off-stimulation state

within the testing session. Should these results persist across time and over future trial participants, they could provide evidence that targeted neuromodulation of the RAS using bioelectronic Zeitgebers could effectively influence established biomarkers (such as wake EEG slowing), while any longitudinal baseline frequency shifts could be attributed to the underlying plasticity changes in RAS-cortical circuits.

We noted however that the EDS case was characterised by lower cortical daytime beta peaks and did not entrain at the respective half-harmonic (at least frontally) of the stimulation frequency until late after the steady-settings state of the first contact combination. Comparatively, the patient with an RBD phenotype showed early occipital entrainment at the stimulation half-harmonic. The patient with an RBD phenotype also had a comparatively higher level of cognitive reserve and (amongst other differences) a lower carer burden at enrolment (Table 3), while their overall progression is considerably less steep. This result is reminiscent of early reports of differences in photic stimulation entrainment at the half-harmonic between healthy older adults and patients with mild Alzheimer's dementia (Kikuchi et al., 2002) as well as later work with multimodal non-invasive stimulation at a 40Hz frequency by the Tsai research group (as per their feasibility trial reports' preliminary upload, (Chan et al., 2021).

When non-invasive 40Hz stimulation protocols were implemented at early stages of Alzheimer's in two mouse models of the disease, resulting neuroprotective effects were reported in terms of transcriptomics, synaptic density and microglial response, as well as enhancements in cognitive performance compared to control animals

(Adaikkan et al., 2019). In the preliminary report of their feasibility trial (*NCT04042922*), the group state enhanced delayed recall and functional connectivity at 3 months as well as other structural and functional benefits.

Neuroprotective mechanisms of DBS are one of the potential therapeutic benefits that are proposed for this treatment, potentially mediated by a decrease in disease-mediated neurotoxicity (reviewed by (McKinnon et al., 2018)).

However, there are studies suggesting that there is a threshold effect, after which neuronal loss can no longer be prevented. For instance, neuronal rescue was limited to primates with milder 1-methyl-4-phenyl-1, 2, 3, 6- tetrahydropyridine (MPTP) lesions, suggesting that, where disease burden exceeds a critical capacity, the protective and circuit-modifying effects of DBS may be limited (Wallace et al., 2007). The potential need for careful staging and early intervention where DBS treatment is considered in order to achieve a protective benefit has been voiced before, by prominent clinician researchers (Charles, Gill, Davis, Konrad, & Benabid, 2008).

However, there is no current knowledge with regards to the parameters favouring an earlier intervention for EDS reduction (such as to facilitate a progressive improvement rather than a sustained plateau), through PPNR or other arousal nucleus DBS. The fact that circadian pattern and sleep normalisation is a secondary endpoint of this five-patient study, therefore patients are not selected on the basis of EDS or parasomnias associated with MSA, may also mean that we will not have enough numbers of cases with an EDS phenotype to compare to this early case. Based on this preliminary evidence presented here, there is perhaps a need for

earlier intervention given the fact that there was an initial substantial response followed by a period of stability and modest gains, as opposed to no effect at all and/or even a paradoxical response.

With regards to the night-time experimental results in the case with an RBD phenotype, the picture seems to be more encouraging. From a sleep viewpoint, experimental therapeutic aims were the creation of a safe ‘sleep stimulation prescription’ in the presence of parasomnia, as well as its validation against other more traditional settings. Based on subjective patient and carer reports (as well as the RBDSQ longitudinal values that have been collected so far), there has not been a deterioration in episode frequency since switch-on of the DBS and programming of the nocturnal settings. The patient reports an overall impression of less frequent events, however this has not been formally quantified. For future cases, the addition of actigraphy may be really helpful in tracking RBD episodes longitudinally as an objective metric, especially where overall patient reporting may be infrequent.

When examining how a tailored stimulation prescription would fare in comparison to other approaches in the RBD phenotype, compared to tonic 75Hz stimulation settings, the novel algorithm was considerably superior in terms of physiological sleep architecture. We also did not see any sleep attack effects as reported in the 2011 case description that has been mentioned in the prior experimental chapter. Inversely, we saw sympathetic activation and enhancement of arousal, while additionally, the patient has now been on a trial of daytime 75Hz stimulation and both him and his carer are denying any such ‘sleep attacks’. At a first glace outside

the SWA analyses, these cases may have one similarity though: while uncontrollable sleep attacks were not witnessed in this case, the REM-onset sleep period is slightly reminiscent of sleep onset in narcolepsy.

Sleep Onset REM Periods (SOREMPs) have long-term been considered as hallmarks of narcolepsy and are part of its classic diagnostic criteria, incorporated in classifications shortly after their discovery (Rechtschaffen, Wolpert, dament, Mitchell, & Fisher, 1963; Vogel, 1960). This phenomenon is not only present in humans; it is also a feature of other naturally occurring narcolepsy variants, such as those occurring in Doberman dogs (Nishino et al., 2000). However, heavy reliance on a short REM sleep latency (<15 minutes) is often criticised by experienced sleep clinicians, due to a low sensitivity across different disease subtypes (for instance see (Baumann et al., 2014)). In our case REM sleep latency was 17 minutes, above the current SOREMP criteria but way below what we generally consider normal human variant, which is around 90 minutes after sleep onset for the first REM period, based on the classic hallmark paper by Dement and Kleitman (Dement & Kleitman, 1957). Furthermore, based on formal AASM sleep scoring of the data, differences between total time spent in REM between 'scheduled settings' and 75 Hz stimulation are not that large (around 4%, with actually slightly longer times spent in REM during the 'scheduled settings' night).

However exciting this dataset may be, it needs to be stressed that this is a single night trial of 75Hz settings in a patient naïve to this stimulation frequency. In a clinical concept, the tailored scheduled night-time settings would need to be compared to

an OFF night as well as a control 40Hz night, for additional validation against other more ‘traditional’ setting combinations as well as our prior case of tonic night-time settings (PPNR1). The author is currently exploring funding and collaborative avenues so that this preliminary data can be obtained in a dedicated investigational setting, without further extensive investigations impinging on the MINDS trial primary objectives (autonomic and motor endpoints).

With regards to effects of this high gamma frequency on sleep homeostasis, as an exploratory question from a more fundamental perspective, we noted results in keeping with suggestions from our prior experimental chapters and the continuous overnight trial in PPNR1. There were behaviourally different outcomes between the two stimulation schedules early on, with the high gamma stimulation aiding resilience during the short period of sleep restriction that was aimed at priming the patient to an early sleep onset. While video evidence was not available, we luckily were able to obtain the timestamped sleep latency, which inhibited the patient from being able to follow bio-calibration commands. Upon tonic stimulation with the high gamma frequency, slow wave activity seemed to occur in short ‘breakthrough’ bouts, much like water leaks from a dam constantly under pressure.

The author proposes that tonic PPNR DBS at a high gamma frequency, which as has been shown and discussed in prior experimental chapters seems to be directly related to cortical excitability as opposed to SWS, acts as a constant suppressor of endogenous SWA pressure in the stimulation naïve patient. Overall, these settings do seem to act as suppressors of SWS, which –as the night progresses and its

homeostatic pressure increases- competes with the stimulation cue, eventually getting larger and larger break-throughs of activity.

How diurnal PPNR DBS may act synergistically to reduce daytime slow wave sleep and increase sleep pressure that can then be permitted to be physiologically released, resulting in more normalised patterns of activity, remains to be determined with further cases and/or dedicated experimental approaches in the Parkinsonian diseases cohort. An additional avenue given the scarcity of such existing data could also be dedicated mathematical modelling of this process, that could then be further validated across conditions and its predicted outcomes compared between surgical and non-surgical interventions.

Limitations of the Study

As stated earlier throughout this thesis, these are preliminary reports on trial results while both recruitment and analyses are ongoing. Further work on these datasets (also as new experiments are performed and reports come in) is needed to confirm many assumptions made in this chapter.

With regards to limiting factors in the experiments, most of these have been mentioned in this chapter as well as in prior experimental chapters. An additional factor with regards to assessing titration effects is pupillometry; this could offer additional objective metrics with regards to desired sympathetic activation. However, due to the unavailability of lab-specific equipment, there are barriers with

regards to how this could be arranged even if a formal protocol amendment were in place. Relevant considerations could be beneficial in further settings optimisation for future patients.

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Conclusions

It may be argued that a hallmark of subversive (or creative) thought is the will to reshape the current gestalt in a given field. Yet, inevitably, as a practice becomes established, it in turn becomes the current gestalt –the prior subversive hypothesis becomes dogma. The field of functional neurosurgery is not immune to this phenomenon (some would say that no field is), despite the arguable fact that patient observation and the trickle-down effect of translational discovery could perhaps make the re-evaluation of established practice more acceptable. As a first remark upon the conclusion of this thesis, I would therefore like to thank the main supervisor for his acceptance of a 'left field' idea as a proposed research topic and his unwavering support of my notorious sleep-related enthusiasm. This support was even more valuable given that the topic was outside his very well-established career in the field of motor and autonomic neuromodulation, with no previous primary sleep interest or foray in this often obscure topic.

Undoubtedly, surgical neuromodulation is also a field where unrestrained and subversive experimentation aimed at deciphering fundamental biological questions is not wholly feasible, since there are valid ethical constraints limiting experimental and trial design. One cannot just sleep deprive and/or plan restriction of all access to external circadian cues in the case of patients with a lethal condition and complex physical and psychological vulnerabilities, while (on a far more prosaic level) neither can one enforce compliance with note keeping and subjective descriptions, as

disease progresses and hope becomes increasingly difficult to find. Another subsequent question is the differentiation in terms of experimentation between what is necessary for patients' well-being and quality of life and what is necessary for the understanding of integral physiological functioning. The latter, however crucial, may require more extensive testing that might not immediately translate to clinical benefit and could result to patient fatigue or even discomfort.

As the author repeatedly realised over the course of this work, the solution is establishing an honest and empathetic dialogue between the experimenter and the patient-participant (never considered a mere experimental 'subject'). Over the course of such an aggressive disorder as the one that was examined during this work, patients slide down an ever-steeper slope of disempowerment. The freedom to choose and be informed about what they will take part in during a research trial may offer some comfort, as does the fact that –instead of being peddlers of vaguely miraculous cures- the clinical research team will approach any potential benefits moderately and appropriately while evidence is still being obtained. We can then proceed to discuss as part of a team, rather than in an imbalanced (academic experimenter and 'subject') power dynamic, why we are proceeding with what we are doing and what makes us passionate about it. Patients are then often keen to contribute in ways that may surprise us; feeling that such an effort will transcend their finite experience and contribute to knowledge that may one day lead to this 'miraculous' cure that they had been hoping for.

After establishing such an alliance, the experimenter can proceed with challenging current dogma and expanding the scope of practice (re-examining participant understanding and consent at constant intervals during the process). This was particularly vital in the case of this thesis, since –although intracranial recordings and clinical paradigms were previously used as a means of understanding function- deliberately setting out to examine if surgical modulation of sleep is feasible in humans was a novel concept in the field. Additionally, this work posed the question whether the field of surgical neuromodulation has been generally ignoring the capabilities offered by timing of stimulation cues in synchrony with endogenous diurnal and circadian rhythms. This is in relation to both therapeutic benefit in conditions where DBS is utilised, and in order to preserve battery life and device functionality (where more efficient stimulation patterns may be used).

Taken at face value and outside the relevant clinical concepts, sleep and arousal modulation as the first aim of this work may seem too far removed from neurosurgical practice or even be treated with a suspicion of goals of 'enhancement' and transhumanism. However, it is vital to emphasise once again that the field of sleep disorders (especially narcolepsy and parasomnias) contains treatment-resistant variants that could perhaps benefit from a trial of non-pharmacological approaches. With this initial body of work, we showed that we may selectively be able to successfully suppress slow wave sleep and shift towards higher states of cortical excitability, therefore be able to target sleep bouts where this is undesirable.

During the course of this thesis, the author conceptualised a device capable of detecting undesirable sleep periods and targeting them with stimulation (with support from the main supervisor of this thesis on surgically relevant aspects, as well as senior overview from our crucial engineering collaborator with device development expertise). A relevant patent has therefore been filed (N419679GB) with the Oxford University Innovation team.

While the patented device was mainly referring to responsive stimulation triggered upon sleep detection (in a similar mechanism to our stimulation trials in the second chapter of this thesis) we have also started to explore the avenue of variation of tonic stimulation to combat excessive daytime sleepiness. Although this research is currently at a preliminary stage, we hope to see that excessive daytime sleepiness can improve to some degree after the implementation of diurnally variable stimulation algorithms that result in a beneficial balance of sleep pressure. However, in order to fully capitalise from intrinsic patterns of reticular activating system (RAS) activity (that as we have explained earlier in this work are phasic rather than tonic and gate arousal states) this author believes that intermittent burst patterns need to be utilised. Currently, closed-loop algorithm delivery is not feasible with the first version of the DyNeuMo device, which is being used in our clinical trials. Therefore, new algorithm development and implementation (as also described in the N419679GB patent) is required for optimal treatment benefit.

In the specifications of N419679GB, the implications of this work on disorders of consciousness (DoC) are also taken into account. It will be further stated here that,

although there is an overlap between DoC and sleep disorders with hypersomnolence, these –and the states of arousal encountered therein- are not equivalent. Furthermore, also relevant to a more general behavioural concept overview, a level of processing of environmental stimuli and their salience/threat attribution is still present during deep sleep, as discussed during the first chapter of this thesis. This evolutionarily-preserved function means that there exists the possibility to rouse someone from deep sleep, upon which point they can return to previous levels of normal wake-associated cognitive functioning. This is not possible in the presence of DoC, where decreases in arousal threshold are pathological and secondary to an insult in brain networks, which regulate the balance between excitation and inhibition. A similar remark can be made for states of anaesthesia, where an external, artificial pharmacological suppressor is imposed on circuits. In this context, recovery to normal wake behaviour does not occur due to an internal, naturalistic process reflecting complex computations and environmental valuations, but due to the weaning off of such pharmacological agents.

However, due to the general decrease in arousal tone and circuit dysregulation present in DoC, there are common behavioural and electrophysiological parallels with sleep and circadian disorders. Decreased arousal during the day and increases in arousal at night are also seen in disorders of consciousness, such as vegetative and minimally conscious state. This disruption is a consequence of impaired circadian rhythms, whose level of impairment correlates to disease severity (vegetative patients were significantly more impaired than minimally conscious patients (Cruse et al., 2013)). In addition, several studies have shown that brain

activity patterns associated with drowsiness and sleep (such as slow wave activity) are increasingly present in patients with a disorder of consciousness outside of sleep or sleep-like behaviours (at resting-state, for example (Sitt et al., 2014)).

Therefore, our exploration of intrinsic activity of the PPNR during states of slow wave sleep and wakefulness, as well as our finding that slow wave activity can be suppressed with specific stimulation protocols, might be of particular importance in the DoC field. During the course of this work the author, as part of a team led by the main supervisor of this thesis and also containing a senior engineer collaborator and a consultant neurologist, summarised these findings and worked on a successful grant proposal aimed at applying PPNR stimulation in the concept of DoC.

The initial next steps of research stemming from this thesis could therefore be broadly described as follows. Firstly, trying to expand neurosurgical indications of PPNR stimulation to DoC and exploring whether suppression of daytime bouts of slow wave oscillations could lead to neurophysiological activity closer to healthier patterns, as well as if such a change can meaningfully impact behaviour. Secondly, continuing the exploration of the role of PPNR stimulation in sleep per se; especially in the context of parasomnias and conditions with excessive daytime sleepiness. Since this two-pronged follow-up will not be feasible without collaborative efforts with experts in these two respective fields (sleep and consciousness) in addition to academic neurosurgeons, I have already started building links with national and international groups working in the field, as well as actively looking for further collaborations on additional projects.

Finally, this work has direct implications on device development, evidenced not only by the patent it has resulted in or the fact that it has explored the relatively novel concept of diurnal algorithms. The findings of state-dependent activity and the feasibility of its manipulation in the concept of sleep and arousal state make an additional case for closed-loop adaptive stimulation based on discrete fluctuations of cortical and subcortical activity. Since after the first four cases of this work externalisation was no longer an option, a custom-developed stimulation algorithm that the author had developed and tested in laboratory mice was never tested in humans (as mentioned elsewhere in this thesis). I sincerely hope that future technological advancements and collaborations might enable a constructive dialogue, bridging areas of expertise to develop novel device capabilities and treatment modalities.

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