CAN DEEP BRAIN STIMULATION OF THE NUCLEUS BASALIS OF MEYNERT IMPROVE THINKING AND MEMORY PROBLEMS IN LEWY BODY DEMENTIAS?

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Doctoral Thesis

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i. Declaration

I, James Gratwicke, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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ii. Abstract

The Lewy body dementias, Parkinson’s disease dementia and dementia with Lewy bodies, are two of the most common causes of dementia worldwide, and share both a common clinical phenotype and underlying pathology. Despite their growing economic and societal disease burden, there are currently only a small number of limited symptomatic therapies available, while modern approaches to develop disease modifying biologic agents have so far produced little tangible effect. There is growing recognition of the need to explore alternative treatment avenues, and the success of deep brain stimulation (DBS) in modulating aberrant neural network processing to relieve symptoms in other neuropsychiatric diseases raises the possibility that this might be achievable in Lewy body dementias.

The nucleus basalis of Meynert (NBM) provides the major source of ascending cholinergic innervation to the cortex, and is proposed to be a key node in multiple distributed cognitive networks. The nucleus degenerates significantly in Lewy body dementias, which correlates closely with the severity of cognitive decline. It is therefore proposed that deep brain stimulation to the NBM may be able to modulate cholinergic transmission to cortex, and thereby impact directly upon dementia symptoms.

In this thesis I will present preliminary evidence from two experimental clinical trials of deep brain stimulation to the NBM in Lewy body dementias. I will present data showing that this invasive neurosurgical procedure is both safe and well tolerated in patients with advanced dementia, and that low frequency stimulation may be associated with improvements in both memory functions and neuropsychiatric symptomatology. Furthermore, I will present results from the first direct electrophysiological recordings from human NBM in vivo, showing that activity in the nucleus may reflect levels of sustained attention. Finally, I evaluate the overall clinical impact of this novel therapeutic approach in Lewy body dementias, and discuss how our electrophysiological findings may relate to this, and how they contribute to our existing understanding of the physiological function of NBM.
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vi. Abbreviations

ACh – acetylcholine

AChEI – acetylcholinesterase inhibitor medication

AC-PC – anterior commissure – posterior commissure

AD – Alzheimer’s disease

CAFS – Clinician Assessment of Fluctuations Scale

CNS – central nervous system

CSF – cerebrospinal fluid

CT – computed tomography

DLB – dementia with Lewy bodies

DTI – diffusion tensor imaging

dva – degrees of visual angle

FDG-PET – [18F]-fluoro-deoxyglucose positron emission tomography

fMRI – functional magnetic resonance imaging

GPi – globus pallidus internus

Ke – cluster co-efficient

LBDs – Lewy body dementias

LED – levodopa equivalent dose

LFP – local field potential

IPG – implantable pulse generator
vii. Publications resulting from work related to this thesis


Chapter 1: Introduction

1.1. The Lewy body dementias

Dementia is a clinical syndrome comprising declining function across a number of cognitive domains including memory, attention, executive function, perception, praxis and language, which are not attributable to delirium or a psychiatric disorder. These features are accompanied by changes in personality and behaviour. The symptoms of dementia interfere with, and may preclude, occupational and social function, and cause distress to both the patient and their family (American Psychiatric Association 2000; Bouchard, 2007; McKhann et al., 2011).

The term Lewy body dementias (LBDs) refers to two distinct dementia syndromes, Parkinson’s disease dementia (PDD) and dementia with Lewy bodies (DLB), which share both a common clinical phenotype and a common pathophysiological hallmark: Clinically both dementias are characterised by prominent impairment of executive and attentional functions, accompanied by episodic memory and visuoperceptual deficits, cognitive fluctuations and neuropsychiatric disturbances, particularly visual hallucinations (Emre et al., 2007; Galvin et al., 2006; McKeith et al., 2005; Noe et al., 2004). Pathologically both are characterised by intracellular aggregates of α-synuclein protein (visualised microscopically as the eponymous ‘Lewy bodies’) across the neocortex (Lippa et al., 2007; McKeith and Mosimann, 2004). Although PDD and DLB share many common clinical and pathological features, not all are identical (Goldman, Williams-Gray, et al., 2014), nevertheless many consider separation of the two clinical syndromes to be artificial as it would imply they had differing pathologies (Aarsland et al., 2004; McKeith and Mosimann, 2004).

1.1.1. Parkinson’s disease dementia

Parkinson’s disease dementia is a late complication of the predominantly motor syndrome of Parkinson’s disease (PD), with a cumulative prevalence of 75-90% of those with a disease
duration of ten years or more (Aarsland and Kurz, 2010; Buter et al., 2008; Hely et al., 2008). It is the fourth most common cause of dementia worldwide, accounting for 2.5-6% of all cases (Aarsland et al., 2008; Aarsland, Zaccai, et al., 2005). The development of PDD negatively impacts activities of daily living (Rosenthal et al., 2010), and confers significantly increased morbidity and mortality to patients and their carers (G. Levy et al., 2002; Reid et al., 1996). It is now widely recognised that the clinical phenotype of PDD extends beyond the classical dysexecutive syndrome seen in early Parkinson’s disease to include additional deficits in recognition memory, attention processes and visual perception (Kehagia et al., 2013; Pagonabarraga and Kulisevsky, 2012), as well as visual hallucinations and cognitive fluctuations (Emre, 2003). This constellation of features has been made explicit in the diagnostic criteria for PDD (Emre et al., 2007).

1.1.2. Dementia with Lewy bodies

Dementia with Lewy bodies is the second most common neurodegenerative dementia, with a prevalence of 7.5% and annual incidence of 3.8% of dementia diagnoses (Vann Jones and O’Brien, 2013). The true disease burden is likely higher however since DLB accounts for 15-25% of neuropathologically defined cases (McKeith et al., 1992, 1996; Taylor et al., 2013). The clinical phenotype of DLB is now well-recognised, characterized by prominent deficits in attention, executive functions and visuoperceptual abilities, while mnemonic abilities are less impaired (Calderon et al., 2001; Collerton et al., 2003a; Ferman et al., 2006; I. McKeith et al., 2004). This cognitive symptomatology is accompanied by cardinal features of fluctuating cognition and complex formed visual hallucinations, which highlights the phenotypic similarity with PDD (McKeith et al., 1996, 2005). A third cardinal feature of DLB is parkinsonism, which is where distinction is drawn with PDD - current consensus criteria state that DLB can only be diagnosed clinically when motor symptoms of parkinsonism develop less than one year prior to onset of cognitive symptoms (McKeith et al., 2005). However, with increasing recognition of subclinical cognitive dysfunction in even premotor PD the validity of this arbitrary diagnostic rule is increasingly challenged by those who view both
dementia syndromes as part of a continuum of Lewy body diseases (Berg et al., 2014; Goldman, Williams-Gray, et al., 2014).

1.2. The Lewy body dementias: clinical phenotype

Both PDD and DLB display a common clinical phenotype, which is distinct from other dementia syndromes such as Alzheimer’s disease (AD, the most common cause of dementia (McKeith et al., 1992)). The cognitive profile shared by both LBDs is characterised by prominent deficits in executive functions, attention and visual perceptive abilities, with associated less prominent deficits in episodic memory (Aarsland et al., 2003; Collerton et al., 2003b; Mosimann et al., 2004; Noe et al., 2004). This profile differs from AD in that mnemonic abilities are not as badly affected, while executive functions, attention deficits and visual perceptive abilities are more impaired (Aarsland et al., 2003; Collerton et al., 2003b; Doubleday et al., 2002; Noe et al., 2004). Both LBDs also characteristically display fluctuating levels of attention and confusion, which are again not typical of AD (Ballard et al., 2002a; Ferman et al., 2004; Walker et al., 2000). Aside from cognitive symptomatology, LBDs are also associated with a number of neuropsychiatric disturbances, motor symptoms of parkinsonism, and other non-motor symptoms, which further differentiate them from other dementias (Emre, 2003; Emre et al., 2007; McKeith et al., 2005; Noe et al., 2004).

Here we describe the cognitive profile and associated clinical features of the LBDs in greater detail. Of note the fifth cognitive domain, language, is relatively preserved in LBDs (the main deficit in this area, impaired verbal fluency, is actually part of the dysexecutive syndrome (impaired self-generated search, Emre, 2003). Consequently, the language domain is not discussed.
1.2.1 Executive dysfunction

‘Executive function’ is an umbrella term encompassing several cognitive abilities, including problem solving, planning/sequencing, rule-shifting/maintenance, task switching, manipulation in working memory and response inhibition (Dubois and Pillon, 1997; Kehagia et al., 2010; Parker et al., 2013), see (Dirnberger and Jahanshahi, 2013) for review). Some also regard allocation of attention as an executive function (Kehagia et al., 2010), though here we will consider it within a separate cognitive domain. In PD executive dysfunction is often present from the point of diagnosis (Cooper et al., 1991; Foltynie, Brayne, et al., 2004; Lees and Smith, 1983; Muslimovic et al., 2005) and may even be part of a pre-motor prodromal syndrome (Goldman, Williams-Gray, et al., 2014). Executive dysfunction worsens with disease progression into PDD (Christopher et al., 2013; Pagonabarraga and Kulisevsky, 2012), being predictive of dementia onset in some series, though this remains controversial (Janvin et al., 2005; J. E. Lee et al., 2013; Gilberto Levy et al., 2002; Mahieux et al., 1998; Williams-Gray et al., 2013; Woods and Tröster, 2003). In DLB however this prolonged dysexecutive prodrome is not present and progressive executive impairment develops alongside attentional and visual perceptual difficulties from the outset (Doubleday et al., 2002; Ferman et al., 2006; I. McKeith et al., 2004; McKeith et al., 1996), which likely reflects differences in the pattern of spread of subcortical Lewy body pathology between PDD and DLB (see below). From the perspective of the patient with LBD worsening dysexecutive syndrome causes progressive difficulties with concentration, retaining information, planning and organisational skills, which interfere heavily with social and occupational function (Bronnick et al., 2006).

1.2.2 Attention

Attention is a heterogeneous construct which has been considered to comprise three different subsystems: executive control, orienting and alerting (Petersen and Posner, 2012; Posner and Petersen, 1990). It has been proposed that the executive control subsystem allocates attentional resources to tasks. It is the volitional focusing of attention and considered to
depend on ‘top-down’ signals derived from knowledge about task demands (Kastner and Ungerleider, 2000). ‘Orienting’ refers to attention being drawn to an environmental stimulus for focussed cognitive processing to the exclusion of other stimuli. It is automatic capture of attention and thought to be driven by ‘bottom-up’ signals from salient stimuli (Desimone and Duncan, 1995). Alerting is a heightened state of arousal and ‘vigilance’ is the maintenance of this aroused state over time (Parasuraman, 1998). Vigilance facilitates faster orienting and reaction time, whereas the opposite state, drowsiness, will impair these functions.

Attention deficits are detectable in both LBDs from early in their disease courses, particularly on tests sensitive to deficits in executive control of attention, such as the digit span, cognitive reaction time, Trail Making Test Part B, Stroop interference test and attentional set-shifting tasks (Ballard, O’Brien, et al., 2001; Collerton et al., 2003b; Ferman et al., 2006; Muslimovic et al., 2005; Petrova et al., 2015; Williams-Gray et al., 2008). LBD patients also develop impaired orienting of visual and auditory attention, such as on the map search task, and difficulties on tasks of sustained attention, such as digit vigilance and elevator counting (Ballard et al., 2002b; Calderon et al., 2001; Poliakoff et al., 2003; Sharpe, 1992; Wright et al., 1990). With disease progression impaired vigilance gives way to fluctuating levels of attention, demonstrated by increased standard deviations on choice reaction time measurements (Ballard et al., 2002b; Ballard, O’Brien, et al., 2001). This fluctuating attention manifests in daily life as fluctuating cognition (Walker et al., 2000), characterised by short-lived spontaneous lapses in awareness/alertness, which are clinically significant in up to 77% of patients (Bradshaw, 2004; Ferman et al., 2004).

All of these attention deficits are significantly greater in LBDs than in AD patients of matched global severity (Ballard et al., 2002b; Ballard, O’Brien, et al., 2001; Calderon et al., 2001) and worsen over the course of the disease, being the strongest predictor of both decline in activities of daily living and poorer quality of life (Ballard, Walker, et al., 2001; Bronnick et al., 2006). Such deficits are easily identifiable in the clinic: patients classically lose their
train of thought during a sentence, fail to follow the conversation, or display fluctuant alertness, and even stupor.

### 1.2.3 Memory

Memory is an all-encompassing term for the cognitive processes involved in the encoding, storage and retrieval of information. As with the other cognitive domains it is not a pure process, and is interdependent upon a person being able to direct attention to allow encoding of information, and use executive processes to allow retrieval in a particular context. As discussed above LBD patients exhibit deficits in each of these latter processes, which means that apparent memory impairments have a multifactorial basis in these disorders. For example, non-demented PD patients exhibit impaired free recall (spontaneous retrieval) but benefit substantially from cueing, demonstrating that externally triggered retrieval is intact (Costa et al., 2014; Lees and Smith, 1983). Recognition memory is also intact at this stage (Lees and Smith, 1983; Taylor et al., 1986) although there is some debate about this (Whittington et al., 2000). Overall, this indicates that in PD memories are encoded and stored, but not independently retrieved. Performance on free recall in this group is significantly predicted by scores on executive tests, indicating that executive dysfunction contributes to retrieval failure (deficient internal search strategies), and is responsible for the apparent mnemonic deficit rather than a dysfunction of storage (Costa et al., 2014; Pillon et al., 1993). This contrasts with Alzheimer’s disease where both recall and recognition are equally impaired from early on, implicating a temporal-limbic storage deficit (Helkala et al., 1988; Pillon et al., 1993).

With progression from Parkinson’s disease to PDD, however, both a cross-sectional study and a meta-analysis have shown that difficulties with recognition memory also become apparent, implicating a supervening dysfunction of temporal lobe storage mechanisms upon pre-existing executive retrieval deficits when patients convert to dementia (Whittington et al., 2000, 2006). This is supported by data showing that PDD patients exhibit significant
impairments with confrontation naming (a test requiring visual recognition memory among other processes) and greater deficits in semantic than phonemic verbal fluency (both require efficient executive retrieval but the former has a greater dependence on temporal lobe storage) (Henry and Crawford, 2004). Both confrontation naming and semantic verbal fluency are dependent on retrieval of semantic information (previously learnt general factual information, (Tulving, 1972)) and therefore these tests are relatively resistant to attentional impairments since encoding of such information would have taken place in the pre-morbid state. Therefore, it seems likely that a true mnemonic storage deficit is present in PDD in addition to the problems with deficient attention/encoding and poor executive retrieval that manifest earlier in Parkinson’s disease.

The neuropsychological profile with regard to memory is very similar in DLB: patients early in the disease course demonstrate impaired free recall compared to age matched controls, but benefit from cueing, again indicating that externally triggered retrieval remains relatively intact and therefore executive dysfunction may be primarily responsible for apparent mnemonic deficits at this stage (Ferman et al., 2006; Hamilton et al., 2004). DLB patients with more established disease demonstrate significant impairments in both recognition memory and semantic memory of similar severity to, or worse than, matched AD patients (Calderon et al., 2001; Lambon Ralph et al., 2001; Noe et al., 2004). However, many of these memory tests involve a visual component (e.g. recognition memory test for faces, Benton visual retention test), suggesting that DLB (and PDD) patients performed worse than AD patients due to the additional contribution of visual-perceptual impairments (see below) (Lambon Ralph et al., 2001; Metzler-Baddeley, 2007). Nevertheless, DLB patients perform significantly worse than healthy controls on memory tests with minimal visual demands (verbal recognition memory and confrontation naming tests)(Calderon et al., 2001; Lambon Ralph et al., 2001), suggesting that a true mnemonic storage deficit is present in DLB in addition to confounding impairments in attention, executive function and visual perception.
In the clinic, problems with memory are one of the most frequent non-motor symptoms reported by both LBD patients and their carers (Breen and Drutyte, 2013). However, the differentiation between apparent memory deficits due to attentional or executive impairments, and ‘true’ temporal-limbic storage deficits is rarely evident from the patients’ self-reported memory complaints, and this requires detailed questioning or cognitive testing to delineate.

1.2.4 Visual perceptual dysfunction.

Visual perception encompasses both sensory processes, i.e. the simple conscious experience associated with the physical aspects of a visual stimulus such as brightness (sensation), as well as the conscious experience of objects and object relationships (perception), i.e. how we form a conscious representation of the outside world (Metzler-Baddeley, 2007). From a cognitive point of view overall visual perceptive function can be subdivided into two components, visuospatial function (the perception of extra-personal space) and visuo perceptual function (recognising objects based on their physical aspects). Multiple studies have shown that LBD patients exhibit both marked visuospatial deficits (for example on object decision and cube analysis tasks) and visuoperceptive deficits (for example on fragmented letters and silhouette identification tasks) compared to both controls and matched AD patients (Calderon et al., 2001; Cormack, Aarsland, et al., 2004; Lambon Ralph et al., 2001; Levin et al., 1991; Mori et al., 2000; Mosimann et al., 2004; Noe et al., 2004).

Furthermore, in the case of PD patients the onset of visual perceptive dysfunction clinically has been shown to have a high sensitivity in detecting the transition to PDD (Biundo et al., 2014; Kehagia et al., 2010; Zgaljardic et al., 2004), while early subclinical impairment on the pentagon copying test of the MMSE is predictive of PDD at 5-year follow up (Williams-Gray et al., 2009).

Of course, apparent visual perceptual dysfunction in LBD patients could be confounded by concurrent deficits in selective attention. However (Cormack, Gray, et al., 2004) tested DLB patients in two visual search tasks, a parallel search condition (measuring automatic visual
search using a ‘pop-out’ effect, attention-independent) and a serial search condition (measuring selective attention-dependent search) and found that DLB patients were impaired in both conditions, performing significantly worse than AD patients in the parallel condition. This therefore indicates that LBD patients exhibit a true deficit in visual perceptive function, independent of concurrent deficits in selective attention.

Clinically, there is a strong association between worsening visuospatial and visuoperceptual impairments in LBD patients, and the presence of visual hallucinations (see next subsection), suggesting an overlapping neural basis to these symptoms (Mori et al., 2000; Mosimann et al., 2004; Ramírez-Ruiz et al., 2006).

1.2.5 Neuropsychiatric features

Patients with LBDs also suffer behavioural and neuropsychiatric symptoms, often from early in their disease course (Aarsland et al., 2007; Emre, 2003; Emre et al., 2007; McKeith et al., 2005). In particular, recurrent complex visual hallucinations are highly prevalent in both LBDs, occurring in up to 70% of patients with PDD and 80% of patients with DLB, while they are comparatively rare in AD (D Aarsland, Cummings, et al., 2001; Ballard et al., 1999; Fénelon et al., 2000; McKeith et al., 2000). Patients commonly perceive well-formed people, animals or objects, which are often unpleasant and occur daily, lasting minutes at a time (Barnes and David, 2001; Mosimann et al., 2006). Although visual hallucinations can be induced by anti-parkinsonian drugs, correlations between use of these agents in LBDs and presence of hallucinations are actually relatively weak, and instead cognitive impairment has been shown to be the major risk factor, indicating that they are a core symptom of the dementing process (Fénelon et al., 2000; Williams and Lees, 2005). Although insight is initially maintained, 81% of LBD patients will lose insight over three years (Fénelon et al., 2000; Goetz et al., 2006). The presence of visual hallucinations severely affects quality of life for both patients and caregivers, and is predictive of nursing home placement (Aarsland et al., 2000, 2007; Goetz and Stebbins, 1993; Schrag et al., 2006; Svendsboe et al., 2016).
In addition delusions, depression, apathy and anxiety all occur more commonly in LBD patients compared to those with AD (Aarsland et al., 2007; Ballard et al., 1999; Huber et al., 1989), and predict a poorer quality of life (Boström et al., 2007a; Mosimann et al., 2006; Schrag et al., 2000).

1.2.6 Motor phenotype and other clinical features

Both PDD and DLB are associated with the motor symptoms of parkinsonism, which constitutes a core diagnostic criterion for both diseases (Emre et al., 2007; McKeith et al., 2005). In both cases parkinsonism is predominantly of akinetic-rigid type with associated postural instability and gait disturbance, without prominent tremor (Lippa et al., 2007; Pagonabarraga and Kulisevsky, 2012). This is consistent with the finding that motor features mediated by non-dopaminergic pathways (speech, postural and gait impairments) are more closely associated with dementia than those motor features which are predominantly dopaminergically-mediated, such as tremor and bradykinesia (Burn et al., 2003; Foltynie et al., 2002). Extrapyramidal signs in Lewy body diseases may thus be on a continuum, with a shift towards greater non-dopaminergic motor-system involvement from PD to LBD (I. McKeith et al., 2004).

Saccadic eye movements are markedly slowed in LBDs compared to both PD and AD, possibly due to the combination of both cortical and subcortical pathology in the former (Mosimann et al., 2005). In addition, both PDD and DLB are frequently associated with dysautonomic symptoms, rapid eye movement (REM)-sleep behavioural disorder, anosima and severe hypersensitivity reactions to neuroleptic drugs, none of which are seen to an equivalent extent in AD (Aarsland, Perry, Larsen, et al., 2005; Boeve et al., 1998; McKeith et al., 1992).
1.2.7 Phenotypic differences between PDD and DLB and boundary issues

As the data above illustrate, PDD and DLB share a common clinical phenotype. However important differences between the clinical features of the two conditions exist. The timing of onset of cognitive symptoms in relation to motor symptoms is a key differentiator made explicit in consensus guidelines: the diagnosis of PDD is made when dementia develops in the context of established PD, whereas DLB is diagnosed when dementia precedes or coincides within one year of the development of motor symptoms (Emre et al., 2007; McKeith et al., 2005). This distinction appears valid since longitudinal studies indicate that the majority of PD patients develop PDD after a decade or more of motor symptoms (Aarsland and Kurz, 2010; Buter et al., 2008; Hely et al., 2008; Hughes et al., 2000). However, some patients fall into an overlapping ‘grey zone’ since DLB patients may present with simultaneous onset of cognitive and motor change (Lippa et al., 2007), while early subclinical cognitive change is now well-recognised in PD (Foltynie, Brayne, et al., 2004; Muslimovic et al., 2005). In such cases the appropriate term depends on the clinical situation, or the use of a generic term such as Lewy body dementia is appropriate (Emre et al., 2007) since the arbitrary distinction in both consensus guidelines reflects diagnostic convenience rather than a truly significant biological or clinical difference (McKeith and Mosimann, 2004).

However, subtle phenotypic differences exist between the two dementias. DLB patients display greater executive and attentional impairments than PDD patients, even after controlling for global dementia severity (Aarsland et al., 2003; Downes et al., 1999). This suggests that frontal cortical involvement might occur slightly earlier in DLB compared to PDD (Aarsland et al., 2003). Cognitive fluctuations are also more pronounced in DLB than PDD of matched severity (Bonanni et al., 2008). Psychiatric symptoms differ quantitatively more than qualitatively, with DLB patients experiencing a greater frequency of
hallucinations, delusions (including Capgras syndrome)\(^2\) and neuroleptic sensitivity reactions than PDD patients (Aarsland, Perry, Larsen, et al., 2005; Aarsland, Ballard, et al., 2001; Goldman, Williams-Gray, et al., 2014; Mosimann et al., 2006; Noe et al., 2004). In terms of motor features PDD patients display greater asymmetry of parkinsonian symptoms, while DLB patients tend to have milder parkinsonism, although eventually with disease progression these become equally severe and symmetrical in both (Dag Aarsland et al., 2001; Lippa et al., 2007). PDD patients demonstrate greater responsiveness to levodopa therapy (Molloy et al., 2005), and consequently have a higher propensity for developing dyskinesias and motor fluctuations as side-effects (Goldman, Williams-Gray, et al., 2014).

Nevertheless, these minor differences aside, PDD and DLB are two common dementia syndromes with overlapping clinical phenotypes, supporting the view that they likely represent different points on a spectrum of Lewy body disease. We now examine how closely the two syndromes share a common underlying pathophysiology.

1.3. The Lewy body dementias: Pathophysiology

1.3.1 Genetic factors

Several genes have been shown to confer increased risk for development of both PDD and DLB, including the E46K and A53T/G209A mutations in the α-Synuclein gene (SNCA) and glucocerebrosidase (GBA) mutations (Halliday et al., 2014; Morfis and Cordato, 2006; Nalls et al., 2013; Shiner et al., 2016; Zarranz et al., 2004). This highlights the genetic overlap between both dementias (see also (Meeus et al., 2012)) and further supports the view that they represent different points on a spectrum of Lewy body diseases. In addition, the

\(^{2}\) Capgras syndrome is a delusional misidentification syndrome in which the patient thinks a close family member or friend has been replaced by an identical-looking imposter (Todd et al., 1981).
apolipoprotein ε4 (APOE4) allele and the microtubule-associated protein tau (MAPT) H1 haplotype have been shown to confer increased risk for PDD (reviewed in (Halliday et al., 2014), while the P123H and V70M mutations in the β-synuclein gene (SNCB) and the A85V mutation in the presenilin 2 gene (PSEN2) confer increased risk for DLB (Ohtake et al., 2004; Piscopo et al., 2008). All these genetic factors likely contribute to cognitive decline in LBDs by different mechanisms, and recent studies have begun to investigate this; for example newly diagnosed PD patients carrying the APOE4 allele show reduced activity in the medial temporal lobe (MTL) network during memory tasks, while MAPT H1 homozygotes instead show reduced activity in the posterior visual network during visuospatial tasks (Nombela et al., 2014). In addition, parkinsonian patients with GBA1 mutations exhibit a specific resting hypometabolism in the precuneus, part of the posterior visual network, which correlates closely with severity of cognitive decline (Goker-Alpan et al., 2012). However, the exact mechanisms by which these genes influence the dementing process remain to be fully elucidated.

### 1.3.2 Molecular and cellular pathology

The histopathological hallmark of both PDD and DLB is the presence of widespread cortical Lewy bodies (Emre et al., 2007; McKeith et al., 2005), which differentiates them from other dementia subtypes (Lippa et al., 2007). Lewy bodies are the name given to eosinophilic intracytoplasmic inclusions first described by Friedrich Lewy in an early neuropathological study of Parkinson’s disease (Lewy, 1912). Lewy neurites are similar inclusions located in neural processes, which preferentially affect limbic and temporal structures (Dickson et al., 1994, 1996; Ferman and Boeve, 2007). Lewy bodies and neurites are composed of fibrillar aggregates of the insoluble form of the presynaptic protein α-synuclein (Baba et al., 1998).

Lewy bodies initially affect the brainstem and olfactory system in PD, then spread up to the substantia nigra and other midbrain nuclei, at which point the clinical manifestations of the motor disorder become apparent (Lippa et al., 2007). Further spread of Lewy bodies to
cortical regions characterises conversion to PDD, and the extent of neocortical spread correlates closely with severity of cognitive decline (Aarsland, Perry, Brown, et al., 2005; Hurtig et al., 2000). A similar relationship between cortical burden of Lewy pathology and cognitive decline is also present in DLB, however the relationship is not as robust (Gomez-Tortosa et al., 1999; Harding and Halliday, 2001). However, in specific brain regions the correlation between pathology and symptomatology in PDD and DLB is almost identical, for example visual hallucinations in both dementias are closely associated with density of Lewy bodies in the temporal cortices (Harding et al., 2002; Williams and Lees, 2005). Thus PD, PDD and DLB appear to represent different points on a continuum of Lewy body disease with motor, cognitive and psychiatric features reflecting the regional distribution and burden of Lewy body pathology (McKeith and Mosimann, 2004).

However, despite this considerable pathological heterogeneity exists both between PDD and DLB, and amongst individual cases with either syndrome. Co-existent neuronal loss, Alzheimer-type neurofibrillary tangles and plaques, microvascular disease, argyrophilic inclusions and tau pathology can all be found to varying degrees amongst individual cases (Ballard et al., 2006; Edison et al., 2008; Gungor et al., 2015; Halliday et al., 2014; Harding and Halliday, 2001; Horvath et al., 2013; Irwin et al., 2012; Jellinger and Attems, 2006; Lippa et al., 2007; I. McKeith et al., 2004; Del Tredici and Braak, 2013). These variations in underlying pathology in turn impact upon the clinical phenotype, for example in DLB co-existent AD pathology can delay onset of visual hallucinations and parkinsonism (Merdes et al., 2003; Del Ser et al., 2001). Meanwhile in PDD the presence of combined Lewy body and Alzheimer-type pathology is associated with development of dementia within ten years of PD diagnosis, whereas less pronounced morphologic changes and a greater cortical cholinergic deficit is associated with later dementia onset (Ballard et al., 2006). Indeed severity of cognitive decline appears better correlated with combined cortical Lewy body and senile plaque pathology in both LBDs (Compta et al., 2011; Samuel et al., 1996). Furthermore, the anatomical distribution of the various contributory pathologies varies between different cases.
(Colosimo et al., 2003; Galvin et al., 2006) and does not always correspond with clinical symptoms: for example, in one large neuropathological series, 55% of PD cases with Braak stage 5-6 pathology (i.e. limbic and neocortical Lewy bodies) lacked clinical evidence of dementia (Parkkinen et al., 2008).

Given this complex and varying milieu of neuropathological and genetic factors underlying the development of LBDs it is difficult to provide a generalised pathophysiological mechanism across patients from this perspective to account for the common clinical picture seen. However, diverse molecular and cellular pathologies can give rise to common patterns of dysfunction at the neural systems level, and addressing LBDs from this perspective can better bridge the gap to clinical symptomatology. Indeed, the need to understand neurodegenerative diseases at this dysfunctional systems-level is increasingly acknowledged (van Dellen et al., 2015; Pievani et al., 2011; Seeley et al., 2009; Stam, 2014).

### 1.3.3 Neural network dysfunctions

From a systems-level perspective the LBD syndromes are caused by variable and interacting dysfunction in a number of diffusely distributed, yet inter-related, neural networks which contribute to distinct cognitive and behavioural processes, including fronto-striatal, mesocortical, corticopetal cholinergic, fronto-parietal, medial temporal and noradrenergic networks. These are in turn differentially influenced by dopaminergic, cholinergic, and noradrenergic neurotransmitter deficits.

To provide conceptual order to an otherwise anarchic dataset I will approach discussion of these networks by addressing in turn each of the major cognitive domains affected by LBD (executive function, attention, memory and visual perceptual ability) and describing the major network dysfunctions underlying deficits in those areas. However, the reader must bear in mind that the division of cognitive ability into these compartmentalised domains is inherently artificial, which in turn renders the assignment of neural networks to the subservience of a constrained domain equally so. The reality is that all these cognitive networks interact and
overlap in a complex manner, and the generation of any conceptualised cognitive function such as ‘memory' is ultimately influenced by many of their individual distributed actions. Nevertheless, evidence suggests that particular neural networks are more strongly implicated in mediating certain cognitive functions than others, which gives validity to approaching the discussion in this manner.

1.1.1.1 Executive dysfunction is due to disruption of the fronto-striatal dopamine network

The prefrontal cortices are implicated in executive function (Fuster, 2008; Milner, 1982, 1995; Norman and Shallice, 1986), and distinct areas of prefrontal cortex have strong functional connections with the striatum via parallel dopamine-dependent cortico-striatal loops (Alexander et al., 1986; Middleton and Strick, 2000) (Figures 1 and 3). In line with this, healthy subjects demonstrate increased striatal dopamine neurotransmission on PET scanning while performing executive tasks (Monchi et al., 2006).

Functional MRI (fMRI) imaging in PD patients relates executive impairments on set shifting and working memory tasks to hypo-activation within the fronto-striatal loops connecting dorsolateral and ventrolateral prefrontal cortices, striatum and thalamus (Au et al., 2012; Lewis et al., 2003; Monchi et al., 2004, 2007). However, such hypo-activation was only present during task phases that specifically required co-activation with the striatum in controls, indicating that striatal dysfunction was the determining factor in executive impairment in PD rather than frontal dysfunction. Both the globus pallidus internus (GPi) and caudate are heavily affected by dopaminergic degeneration in PD (Taylor et al., 1986), and PET studies have specifically implicated dysfunction of these two structures in interruption of normal processing in the fronto-striatal network. For example, PD patients demonstrating executive impairments on tasks involving planning (Owen et al., 1998) or random number generation (Dirnberger et al., 2005) show significantly altered outflow activity from the pallidum to the frontal cortices. In addition, other studies have shown strong correlations
between dopamine depletion in the head of the caudate and deficits on executive tasks such as object alternation, planning and the Stroop test (Brück et al., 2001; Marié et al., 1999; Rektorova et al., 2008; Rinne et al., 2000).

Both DLB and PDD patients demonstrate similar patterns of significant atrophy of the caudate nucleus and frontal regions on voxel-based morphometric (VBM) MRI compared to both PD patients and healthy controls, indicating that degeneration of the fronto-striatal network is both specific to and comparable in both LBDs (Borroni et al., 2015). DLB patients also similarly demonstrate a significant correlation between increasing striatal dopamine deficiency on dopamine SPECT imaging and worsening executive impairments (Siepel et al., 2015). This is supported by another study in DLB patients which showed a significant correlation between density of striatal vesicular monoamine type 2 transporters (which transport dopamine into synaptic vesicles, thus an indirect measure of dopaminergic degeneration) on PET imaging and cognitive impairment on the MMSE, though executive functions were not specifically tested (Siderowf et al., 2014).

Therefore, evidence from structural and functional imaging studies support the prevailing view that executive dysfunction in LBDs is primarily due to dopaminergic depletion in the striatum disrupting transmission in the fronto-striatal network. (Dubois et al., 1994; Kehagia et al., 2013; Mortimer et al., 1982; Owen, 2004; Owen et al., 1995; Pagonabarraga and Kulisevsky, 2012; Zgaljardic et al., 2003).
Figure 1: The major subcortical neural networks affected in LBDs (according to their dominant neurotransmitters). In this 3D representation the medial surface of the right hemisphere of the human brain is closest to the viewer in both images. A = amygdala; AC = anterior commissure (lateral aspect); C = caudate; Cg = cingulate gyrus; DLPFC = dorsolateral prefrontal cortex; GPi = globus pallidus (internus); IN = insular cortex; LC = locus ceruleus; P = putamen; SN = substantia nigra; T = thalamus; VLPFC = ventrolateral prefrontal cortex; VTA = ventral tegmental area. Adapted from Gratwicke J, Jahanshahi M, Foltynie T. Parkinson’s disease dementia: a neural network perspective. Brain 2015; 138: 1454-76.

1.1.1.2 Degeneration in the mesocortical dopamine network contributes to executive dysfunction

However, dopamine-dependent neural circuitry underlying executive deficits in LBDs may not be limited to the fronto-striatal network alone. The mesocortical dopamine network originates in the midbrain VTA (ventral tegmental area, A10) and projects diffusely to neocortical areas, particularly prefrontal, insular and cingulate cortices (Oades and Halliday, 1987) (Fig. 1. Fig. 3.). Release of dopamine from this network modulates prefrontal D2 receptors and thereby facilitates cognitive flexibility, a core feature of executive processing (Floresco and Magyar, 2006). Insular cortex in particular is considered to mediate such
flexibility, acting as a hub to recruit other cognitive circuits such as the fronto-parietal network (Menon and Uddin, 2010). In support of this, insular lesions in human patients have been shown to impair performance on tasks requiring cognitive flexibility (Hodgson et al., 2007).

Post-mortem studies have shown degeneration of the mesocortical network in both PDD and DLB patients (Hall et al., 2014; Javoy-Agid and Agid, 1980; Scatton et al., 1983; Seidel et al., 2015). In vivo PET imaging studies confirm dopaminergic dysfunction in this network in PD (Ouchi et al., 1999; Yagi et al., 2010), with a specific reduction of D2 receptor availability in insular cortex occurring in cognitively impaired patients and correlating closely with impairment on executive tests (Christopher et al., 2013). Furthermore, volumetric MRI studies have shown close correlations between atrophy of insular cortex and conversion to PDD (J. E. Lee et al., 2013; Melzer et al., 2012). Therefore, evidence implicates a concurrent dysfunction in the mesocortical dopamine network in the pathophysiology of LBDs, with specific disruption of projections to insular cortex shown to contribute to worsening executive impairments, possibly by impairing the ability to recruit other cognitive networks.

1.1.1.3 Disruptions in non-dopaminergic brain networks contribute to executive dysfunction

Levodopa administration does not improve all executive deficits in LBD (Jubault et al., 2009; Pillon et al., 1989; Poewe et al., 1991), or even in early Parkinson’s disease (Lewis et al., 2005; Muslimovic et al., 2005). In fact the relationship between dopamine replacement and executive performance is complex (Cools, 2006), in that either too high or too low levels of prefrontal dopamine are associated with poor executive performance, and this may relate partly to COMT (catechol O-methyltransferase) genotype (Foltynie, Goldberg, et al., 2004; Nombela et al., 2014; Williams-Gray et al., 2007). Furthermore, levodopa does not restore dysfunctional cognitive network patterns to normal as it does motor network patterns on either fMRI (Jubault et al., 2009), or PET (Huang et al., 2007). Therefore, it seems likely that
Impairments in other brain networks and neurotransmitter systems also contribute to executive dysfunction in LBDs (Zgaljardic et al., 2004).

The noradrenergic network projecting from the locus coeruleus (LC) to the thalamus, amygdala and cortex (Fig. 1, Fig. 3.) is also compromised in LBDs (Bertrand et al., 1997; Scatton et al., 1983; Seidel et al., 2015), with the extent of neuronal loss in this system shown to correlate with development of PDD (Cash et al., 1987; Del Tredici and Braak, 2013; Zweig et al., 1993). Noradrenalin release in prefrontal cortex increases the responsiveness of neurons to diverse inputs, thereby facilitating cognitive flexibility (Vazey and Aston-Jones, 2012). Therefore, damage to this system in LBD patients may underlie deficits in executive functions reliant on cognitive flexibility, such as rule-shifting, response inhibition and working memory, and indeed administration of noradrenergic agonists reverses these deficits (Bédard et al., 1998; Riekkinen et al., 1999).

It must also be borne in mind that executive function is interdependent upon other cognitive faculties, such as the ability to maintain an alert and attentive state in order to concentrate on a task. Thus concurrent dysfunction in brain networks mediating these other functions will also contribute to the overall level of executive disability. For example, the nucleus basalis of Meynert (NBM) cholinergic network is strongly implicated in maintenance of an attentive state (discussed below), and degenerates significantly in LBDs, leading to widespread cortical cholinergic dysfunction (Bohnen et al., 2003; Gratwicke et al., 2013; Kuhl et al., 1996; Perry et al., 1985), demonstrated in vivo by a 30% reduction in cholinergic ligand binding on PET across all cortical areas, compared to only 10-12% in non-demented Parkinson’s disease (Hilker et al., 2005; Shimada et al., 2009). Close correlations have been demonstrated between this cortical cholinergic dysfunction in LBDs and worsening scores on tests of working memory, rule-switching and response inhibition (Bohnen et al., 2006a), all of which require a strong attentional component. Therefore, this suggests that damage to the NBM attention network indirectly contributes to the executive dysfunction of LBDs.
In summary, executive dysfunction in LBDs is a complex phenomenon, mediated primarily by dysfunction in fronto-striatal and mesocortical dopaminergic circuitry, but with interacting influences from dysfunctional noradrenergic and cholinergic networks too.

1.1.1.4 Dysfunction in the fronto-parietal network impairs “top-down” control of attention

A distributed fronto-parietal cortical network is thought to control attention, the key nodes of which include the frontal eye fields, dorsolateral prefrontal cortex, posterior parietal cortices and the temporoparietal junction (Petersen and Posner, 2012) (Figures 2 and 3). Synchronization of neural oscillations between these key nodes selects competing stimuli for focused cognitive processing (Miller and Buschman, 2013). This shared circuitry mediates both ‘top-down’ executive control of attention and ‘bottom-up’ orienting of attention, dependent upon whether activity is driven by prefrontal or parietal regions respectively (Buschman and Miller, 2007; Corbetta and Shulman, 2002; Kincade et al., 2005; Li et al., 2010; Rossi et al., 2009).

Imaging studies using FDG-PET have shown that both PD-MCI and PDD patients demonstrate extensive hypometabolism in frontal and parietal cortices compared to cognitively normal PD patients (Hosokai et al., 2009; Huang et al., 2007, 2008; Liepelt et al., 2009; Yong et al., 2007), while fronto-parietal cortices are particularly affected by amyloid deposition in DLB (Edison et al., 2008). In addition, VBM MRI analyses and diffusion tensor imaging (DTI) studies have shown that LBD patients demonstrate extensive grey matter atrophy and white matter microstructural alterations respectively within the above cortical regions (Borroni et al., 2015; Burton et al., 2004; Hattori et al., 2012; Lee et al., 2010; Melzer et al., 2012; Song et al., 2011; Summerfield et al., 2005). To investigate this relationship, one center co-registered MRI and FDG-PET scans in individual patients with cognitively intact PD, PD-MCI or PDD, and compared cortical metabolism and atrophy amongst these cognitive groups (González-Redondo et al., 2014). They found that cognitive decline
correlated closely with a progressive pattern of sequential hypometabolism followed by atrophy in both frontal and parietal cortices. Furthermore the spatial pattern of fronto-parietal hypometabolism has been shown to correlate closely with deficits on a test of executive control (Trail Making Test Part B), and can be reliably used to predict test scores in other cognitively impaired PD patients (Huang et al., 2007). Therefore, these studies highlight a progressive degeneration in frontal and parietal cortices in LBDs, which correlates closely with deficits in the executive control of attention.

In Alzheimer’s disease it has been shown that atrophy in specific cortical regions damages structural connections and leads to loss of functional connectivity within brain networks (He et al., 2007). Given the extensive atrophy within frontal and parietal cortices seen in LBDs then the same may hold true for the fronto-parietal network, and indeed functional imaging evidence supports this. fMRI studies show that non-demented PD activate the fronto-parietal network while performing attentional set-shifting tasks (Williams-Gray et al., 2008). However, activation of the network during such volitional shifts of attention is not as strong as in control subjects due to reduced connectivity within prefrontal cortical regions (Rowe et al., 2002). With progression to PDD, resting-state magnetonecephalography (MEG) imaging demonstrates a significant reduction in functional connectivity across cortical regions in the beta-frequency band compared to PD patients (Ponsen et al., 2012). In the healthy state it has been shown that an increase in beta band synchrony within the fronto-parietal network drives the executive control of attention (Buschman and Miller, 2007), so loss of such synchrony in PDD may represent the functional mechanism underlying impairment in this mode of attention. Similarly, DLB patients undergoing resting state electroencephalography (EEG) or MEG demonstrate reduced long-range cortical functional connectivity in the alpha-frequency band compared to control subjects (Andersson et al., 2008; van Dellen et al., 2015; Franciotti et al., 2006), which correlates closely with worsening impairments on a test of executive control of attention, the Trail Making Test (van Dellen et al., 2015).
Therefore, substantial structural and functional evidence exists to support the hypothesis that dysfunction in the fronto-parietal network impairs top-down control of attention in LBDs. However, further studies directly exploring the contribution of cortical structural changes to functional connectivity and relating this to attentional impairments in LBDs are needed to confirm these observations. Furthermore, the contribution of different neurotransmitters to fronto-parietal network dysfunction remains to be elucidated. PD patients with low activity COMT genotypes (who have higher cortical dopamine levels) appear to under-activate the fronto-parietal network with consequent poorer performance on set-shifting tasks (Williams-Gray et al., 2008), while the pattern of cortical atrophy seen within the network in LBDs correlates closely with areas showing cholinergic hypofunction on PET imaging (Hilker et al., 2005; Shimada et al., 2009) (and see section 1.3.1.6 below).

**Figure 2:** The major cortical neural networks affected in LBDs. Areas of cortical atrophy associated with visuospatial and visuoperceptual deficits in PDD (coloured green and purple, respectively) are based on the data presented in Pereira et al. (2009). Areas of cortical atrophy specifically associated with the presence of visual hallucinations in PDD (coloured blue) are based on the data presented in Goldman et al. (2014a). Functional cortical regions comprising the fronto-parietal attention network (highlighted red) are based on the data presented in Williams-Gray et al. (2008). Cortical regions are
identified according to the Allen Brain Atlas for the human brain, and manually drawn onto the corresponding 3D brain image. In this representation the same cortical regions are affected symmetrically in both hemispheres, however in the original studies above the extent of atrophy in these regions was not symmetrical between hemispheres, and varied between individual patients. In the inferior view of the cortex the cerebellum has been removed to expose the fusiform gyri more clearly. DLPFC = dorsolateral prefrontal cortex; PPC = posterior parietal cortex; VLPFC = ventrolateral prefrontal cortex. Adapted from Gratwicke et al., 2015.

1.1.1.5 Dysfunction in cholinergic and noradrenergic networks impairs “bottom-up” orienting

One view of automatic orienting of attention considers it to be mediated by “bottom-up” or stimulus-driven signals from the NBM in the basal forebrain (Sarter et al., 2005). This nucleus consists of 90% cholinergic neurons and its’ widespread projection axons provide the main cholinergic innervation to the entire cortical mantle (‘corticopetal’ innervation) (Gratwicke et al., 2013; Mesulam et al., 1983; Mufson et al., 2003) (Fig. 1. Fig. 3.). Selective activation of the NBM network causes an increase in acetylcholine levels in the cortical target field, which boosts the signal to noise ratio for salient stimuli, thereby enhancing the strength of their neural representations (Bentley et al., 2011; Goard and Dan, 2009; Pinto et al., 2013; Soma et al., 2013). In facilitating this process the NBM effectively amplifies detection of salient stimuli by posterior regions of the fronto-parietal network and ensures their attentional significance (Buschman and Miller, 2007; Sarter et al., 2006). Animal experiments have shown that this NBM-driven cortical signal enhancement is responsible for generating event-related potentials (ERPs) on the EEG (Nguyen and Lin, 2014b). These can be measured on the human EEG as negative deflections occurring 80-100 ms after an unpredictable stimulus (the N1 ERP), and have long been regarded as the electrophysiological correlate of orienting of attention (Hillyard et al., 1973).

The NBM degenerates in LBDs, with human neuropathological series showing 32% cell loss in non-demented PD patients, rising to 54-70% in both PDD and DLB, which is closely associated with increasing cortical cholinergic deficits and worsening cognitive impairment.
This is supported by both volumetric MRI and PET imaging studies which demonstrate significant NBM atrophy and cortical cholinergic binding reductions respectively in LBD patients compared to both cognitively intact PD and controls (Bohnen et al., 2006b; Choi et al., 2012; Grothe et al., 2014; Hanyu et al., 2002; Hilker et al., 2005; Shimada et al., 2009; Whitwell et al., 2007). Furthermore, in one volumetric MRI study DBL patients were shown to have up to 25% reduction in volume of the Ch4a and Ch4i subsectors of the NBM (which project to parietal cortices, see below) compared to controls, which correlated closely with worsening impairments on the Trail Making Test part A, a test incorporating visual pop-out stimuli which is sensitive to orienting deficits (Grothe et al., 2014). This is explained by the fact that disruption of NBM cholinergic input to cortex attenuates cortical signal processing (Pinto et al., 2013), demonstrated by the fact that LBD patients performing orienting of attention tasks display increased N1 ERP latencies compared to both non-demented PD patients and controls, which correlate with behavioural errors (Goodin and Aminoff, 1987; Hautecoeur et al., 1991; Stam et al., 1993). Therefore, evidence suggests that disruption of bottom-up signal enhancement in the NBM network underlies the deficits in orienting seen in LBDs.

Interestingly, direct prefrontal cortical projections to the NBM may modulate activity of its cholinergic inputs to sensory cortices and has been suggested to represent a component of the top-down fronto-parietal attention network (Sarter et al., 2005). Thus depending on the type of stimulus and task characteristics, activity in the NBM network may reflect the combined effects of top-down and bottom-up modes of attention (Bentley et al., 2004; Sarter et al., 2006), meaning that degeneration in this network in LBDs may play a key role not only in orienting deficits but in deficits in executive control of attention as well (Fig. 3.).

Finally, the ascending noradrenergic network is also implicated in orienting of attention (Aston-Jones et al., 1999) and, as described above, this network degenerates progressively in LBDs. Administration of the selective alpha-1 noradrenergic agonist naphtoxazine to PD-MCI patients improves performance on an orienting of attention task accompanied by
improved lateralization of the N1 ERP (Bédard et al., 1998). This suggests that lack of bottom up noradrenergic input from the locus coeruleus may also play a role in orienting deficits in LBDs, however, its interaction with the cholinergic system and their relative contributions remain unclear.

Figure 3: Hypothetical model of the neural networks affected in LBDs and corresponding cognitive deficits. Solid arrows correspond to direct neural connections and colours are indicative of the primary neurotransmitter involved as shown in the key. Dashed arrows connect the relevant dysfunctional neural network to its putative cognitive effects. Purple arrows indicate that a deficit in one cognitive domain contributes to the development of impairment in another domain. Black crosses indicate damage to a neural pathway. The red dashed arrow represents direct projections from prefrontal cortex to the NBM, permitting top-down control of attention from the fronto-parietal network via recruitment of this latter structure and its cortical projections. The limbic, orbitofrontal and associative circuits in the prefrontal cortex correspond to the dissociable fronto-striatal loops of Alexander et al. (1986). Note effects of levodopa therapy at improving and worsening executive functions reliant on cognitive flexibility and learning from feedback, respectively. Electrocortical activation refers to cortical EEG desynchronization indicative of the awake/alert state as described in the text, and is driven by corticopetal cholinergic input from the NBM only. Both cholinergic input from NBM and noradrenergic input from the locus ceruleus (LC) modulate processing in sensory cortices to facilitate orienting of attention to stimuli. Cx = cortex; DLPFC = dorsolateral prefrontal cortex; fx = function; GPi = globus pallidus (internus); PPC = posterior parietal cortex; SNpc = substantia nigra pars compacta; VLPFC = ventrolateral prefrontal cortex; VTA = ventral tegmental area. Adapted from Gratwicke J, Jahanshahi M, Foltynie T. Parkinson’s disease dementia: a neural network perspective. Brain 2015; 138: 1454-76.
1.1.1.6 **Slowed cortical rhythms on the EEG reflect impaired vigilance and underlie cognitive fluctuation**

As mentioned above the onset of impaired vigilance and fluctuating attention/cognition is particularly characteristic of LBDs (Emre et al., 2007; McKeith et al., 2005). In tandem with its role in enhancing processing of salient stimuli the NBM cholinergic network also plays a key role in the ascending arousal network. The NBM receives noradrenergic afferents from the locus coeruleus (Fig. 1.) and glutamatergic afferents from the reticular formation and acts as an extra-thalamic relay to the cortex and limbic system (Jones, 2004; Szymusiak, 1995). Its cholinergic projections can directly desynchronize the neocortical EEG, replacing slow synchronised delta waves (0.1-4 Hz, indicative of the non-aroused state) with fast beta and gamma waves (15-30 and 30+ Hz respectively, indicative of arousal) (Kalmbach et al., 2012; Lee et al., 2005; Metherate et al., 1992).

Awake resting EEG studies in LBD patients consistently show an increase in slow delta wave activity across the cortex, with a progressive gradient of increasing delta wave activity seen when comparing cognitively intact PD, PD-MCI and PDD patients, or when comparing patients with AD to those with DLB (Andersson et al., 2008; Caviness et al., 2007; Kai et al., 2005; Neufeld et al., 1994; Soikkeli et al., 1991). In agreement with this, resting state MEG studies have also shown a relative increase in cortical delta oscillatory power in PDD and DLB patients compared to non-demented PD and controls respectively, alongside a relative decrease in faster beta and gamma activity (Bosboom et al., 2006; Franciotti et al., 2006; Ponsen et al., 2012). Administration of the AChEI Rivastigmine to LBD patients undergoing EEG/MEG returns these slowed cortical rhythms towards normal (Bosboom et al., 2009; Kai et al., 2005). This, therefore, supports the hypothesis that dysfunction in the NBM cholinergic network underlies the electrocortical depression characteristic of LBDs. Rodents with NBM lesions have similar slow delta activity on the EEG and concurrently display reduced arousal or coma (Buzsaki et al., 1988; Fuller et al., 2011). Therefore, NBM cholinergic dysfunction
leading to progressive electrocortical depression in LBDs may represent the pathophysiological correlate of impaired vigilance (Fig. 3.).

In addition, Bonnani and colleagues have shown that both PDD and DLB patients with significant cognitive fluctuations (measured by the CAFS [Clinician Assessment of Fluctuation Scale]) demonstrate pseudocyclic patterns of slow wave activity on the EEG in the delta-theta-pre-alpha range (1-7.9 Hz), whereas PDD patents without fluctuations do not (Bonanni et al., 2008). This, therefore, implies that development of slow EEG rhythms cycling between relatively greater and lesser states of cortical arousal represent the pathophysiological basis of cognitive fluctuation in LBDs. Furthermore, DLB patients display disrupted fronto-parietal network activations on both SPECT and fMRI functional imaging which correlate significantly with worsening CAFS scores (Peraza et al., 2014; Taylor et al., 2013). These findings were specific to DLB, since they were not seen in matched AD patients with fluctuating cognition (Taylor et al., 2013). Moreover, those DLB patients taking AChEIs displayed both significantly reduced fronto-parietal network disruption and significantly lower CAFS scores, once again implicating cholinergic dysfunction as an underlying driving factor (Taylor et al., 2013). Taken together, this evidence suggests that dysfunction in the NBM cholinergic network contributes to dysfunction in the fronto-parietal attention network, consequently allowing the generation of abnormal slow cyclical cortical rhythms in LBD patients, which are the neurobiological basis of cognitive fluctuations. However, further work is needed to establish how such rhythms are generated at a cellular and/or network level, and why some LBD patients develop them while others do not.

1.1.1.7 Atrophy within the medial temporal lobe is associated with memory deficits in LBDs

Medial temporal lobe structures (hippocampus, parahippocampus, entorhinal and perirhinal cortices and amygdala, see Fig. 3.) are involved in memory storage and retrieval (Lech and
Suchan, 2013; Squire et al., 2004). PD patients demonstrate hypoactivation of these structures during visual memory tasks from the point of diagnosis (although mnemonic deficits are subclinical at this time) (Nombela et al., 2014). However, previous volumetric MRI studies have provided conflicting results as to whether significant MTL atrophy occurs in PDD (Burton et al., 2004; Camicioli et al., 2003; Ibarretxe-Bilbao et al., 2008; Junqué et al., 2005; Tam et al., 2005). These discrepancies are likely due to the differing criteria for dementia used, and the fact that results were not co-varied by motor scores to determine atrophy specific to cognitive decline. To address these issues, a recent study used the MDS (Movement Disorders Society) Task Force Criteria for PDD (Emre et al., 2007) and recent criteria for PD-MCI (Dalrymple-Alford et al., 2011) to select representative patient groups for VBM MRI analysis (Melzer et al., 2012). Having adjusted results by individual UPDRS (Unified Parkinson’s Disease Rating Scale, part III) motor scores they showed that cognitive progression from PD to PD-MCI to PDD specifically correlated with increasing grey matter atrophy in MTL structures including the hippocampi, parahippocampi and amygdalae. A recent meta-analysis of six VBM MRI studies involving a total of 105 PDD patients and 131 controls confirms this (Pan et al., 2013).

DLB patients show either similar or greater MTL atrophy on VBM MRI compared to PDD patients matched for global cognitive decline, and the degree of MTL atrophy correlates closely with impairments in both verbal and visual memory in DLB patients (Barber et al., 1999; Burton et al., 2004; Sanchez-Castaneda et al., 2009; Tam et al., 2005).

Although these data confirm that LBDs are associated with worsening MTL atrophy, further studies are needed to specifically demonstrate a link between damage to this network and worsening memory storage deficits (assessed by decline on tests of recognition or semantic memory). At present, we can only hypothesise that this is the case based on the known functional anatomy of the MTL network (Squire et al., 2004).
1.1.1.8  Dysfunction of the NBM cholinergic network impairs encoding of memories

Aside from its role in orienting of attention the NBM cholinergic network has also been implicated in memory encoding. The release of acetylcholine from its end terminals has been shown to induce plastic re-organisation of cortical receptive field maps, representing the putative encoding of a 'physiological memory' (Bakin and Weinberger, 1996; Kilgard and Merzenich, 1998; McLin et al., 2002). Simultaneously, as described above, this transmitter release directly desynchronises the neocortical EEG by inducing fast gamma, beta and theta oscillations (Kalmbach et al., 2012; Lee et al., 2005), and evidence suggests that phase coupling of these oscillations between cortical and MTL regions is necessary for memory encoding in humans (Fell and Axmacher, 2011; Huerta and Lisman, 1993; H. Lee et al., 2013). Conversely, NBM lesions in animals have been shown to block this electro-cortical activation (Buzsaki et al., 1988; Fuller et al., 2011), and cause impairments of learning and memory (Bartus et al., 1985; Butt and Hodge, 1995; Leanza et al., 1996; Mandel et al., 1989), as well as impairments in orienting of attention (Voytko, 1996; Voytko et al., 1994).

As described in the previous section, the NBM cholinergic network degenerates significantly in LBDs with up to 70% cell loss (Perry et al., 1985; Whitehouse et al., 1983), which correlates with progressive electro-cortical depression on MEG (Bosboom et al., 2006; Franciotti et al., 2006; Ponsen et al., 2012). Therefore, we hypothesise that dysfunction in this network impairs both orienting of attention to a stimulus and (in conjunction with dysfunction in the MTL network) the induction of electrocortical synchrony necessary for the successful encoding of that stimulus into memory (Fig. 3.). Further electrophysiological studies in LBD patients are needed to investigate this further, however, it is not surprising that dysfunction of the NBM cholinergic network is implicated in both attention and memory deficits since neuroimaging and computational studies in healthy humans suggest that cholinergic enhancement of cortical signal detection (orienting of attention) facilitates formation of novel input associations (memory encoding) (Bentley et al., 2009; Hasselmo and McGaughy, 2004).
Thus these cognitive functions are inter-related and actually part of a continuous process for recording salient environmental stimuli into memory (Sarter et al., 2003).

1.1.1.9 Visual perceptual dysfunction correlates with atrophy in posterior visual cortices

Few studies have specifically looked at in vivo neuroanatomical correlates of visual perceptual dysfunction in LBDs. Using VBM MRI analysis, Pereira and colleagues showed that PD-MCI patients have greater grey matter atrophy in both occipito-temporal and dorsal parietal cortices compared to controls, and that these patterns correlated with impairments on tests of visuo perceptual and visuospatial abilities respectively (Pereira et al., 2009) (Fig. 2.). In parallel to these findings, Bozzali and colleagues used DTI imaging to demonstrate that DLB patients have significantly greater white matter microstructural damage in both temporal and occipito-parietal lobes compared to controls, and that the degree of damage to these connections significantly predicted impairments on visuo perceptual and visuospatial tests respectively (Bozzali et al., 2005). These findings correspond to VBM MRI studies in DLB patients showing focal atrophy in temporal and parietal cortices compared to AD patients matched for global cognitive decline (Watson et al., 2015). These associations agree with the dual stream hypothesis of visual processing, wherein the ventral stream from occipital lobe to temporal and limbic structures processes object recognition while the dorsal stream from the occipital to the parietal lobe processes spatial location (Ungerleider and Mishkin, 1982). Indeed functional imaging in PD patients performing visuospatial tasks shows reduced parietal activation which correlates with increasing errors (Nombela et al., 2014). The above patterns of cortical atrophy/white matter damage show spatial congruence with cortical areas showing significant hypometabolism and cholinergic deficits in LBD patients, in line with deafferentation from the NBM network (Hilker et al., 2005; Klein et al., 2010; Shimada et al., 2009). Indeed the areas of significant white matter microstructural alterations seen in the DLB patients compared to controls comprise the same fibre tracts through which cholinergic projections from NBM to visual cortices travel (Gratwicke et al., 2013), and damage to these
same tracts is also seen on DTI imaging in PDD patients compared to non-demented PD (Matsui et al., 2007). Since bottom-up NBM cholinergic input is known to enhance visual cortical responses and thereby improve visual discrimination ability (Bhattacharyya et al., 2013; Pinto et al., 2013; Soma et al., 2013), dysfunction in this network due to NBM degeneration may underlie the visual perceptual dysfunction seen in LBDs (Figure 3).

1.1.1.10  **Independent dysfunction in posterior visual processing networks underlies visual hallucinations**

The mechanisms underlying the generation of visual hallucinations in LBDs is more complex and likely represents interacting dysfunction between several different brain networks. Since the presence of visual hallucinations is closely correlated with visuospatial and visuoperceptual deficits in both PDD and DLB (Mori et al., 2000; Mosimann et al., 2004; Ramírez-Ruiz et al., 2006; Sinforiani et al., 2006), dysfunction in associative visual cortices within the dorsal and ventral processing streams has long been implicated in their generation. This is supported by neuropathological studies which have demonstrated strong correlations between Lewy body burden in parietal and temporal lobes (particularly precuneus, cuneus and limbic structures) and the presence of hallucinations in LBDs (Gallagher et al., 2011; Harding et al., 2002; Kalaitzakis et al., 2009; Papapetropoulos et al., 2006; Yamamoto et al., 2006). Nevertheless, MRI studies comparing brain atrophy patterns between PD patients with and without visual hallucinations have not consistently supported these pathological associations, differentially implicating medial temporal (Ibarretxe-Bilbao et al., 2008, 2010; Shin et al., 2012), insular (Shine et al., 2014), pedunculopontine nucleus (Janzen et al., 2012) and frontal atrophy (Ibarretxe-Bilbao et al., 2010; Sanchez-Castaneda et al., 2010). All may play a part in generation of hallucinations, however the degree of cognitive impairment between patients with and without hallucinations was not controlled for in these studies, meaning that atrophy patterns may have related to cognitive differences rather than the
presence of hallucinations per se. In addition, the use of differing classification criteria for
PDD (including MMSE < 24, Diagnostic Statistical Manual (DSM) IV-TR or MDS Task
Force criteria) further complicates interpretation of these results.

A recent study overcame these problems, by using MDS criteria to select PDD patients with
and without visual hallucinations and ensured they were matched for antiparkinsonian
medications, global cognitive decline and scores on all cognitive sub-domains, including
visuoperceptual impairments (Goldman, Stebbins, et al., 2014). Structural MRI scans from
both groups were analysed using VBM, then compared. PDD hallucinators exhibited
significant grey matter atrophy in the cuneus, lingual and fusiform gyri, middle occipital lobe
and inferior parietal lobule compared to non-hallucinators (Fig. 2, Fig. 3.). These results
confirm that discrete areas of atrophy in the posterior visual processing networks specifically
underlie the generation of hallucinations in PDD, and thereby provide an in vivo correlate to
neuropathological data. Of note, these atrophy patterns were independent of visuoperceptual
impairments, suggesting that generation of visual hallucinations in PDD does not merely
represent a progression of such impairments but is instead dependent on different
mechanisms.

Much fewer studies have so far addressed the in vivo neuroanatomical correlates of visual
hallucinations in DLB patients. However, in the couple that have, cortical thickness analysis
of MRI scans shows significant correlations between worsening atrophy of the precuneus and
superior parietal lobule and greater severity of visual hallucinations (Delli Pizzi et al., 2014;
Sanchez-Castaneda et al., 2010). These results are similar to those seen in PDD patients, and
again support the hypothesis that specific additional damage to posterior visual networks
underlies generation of visual hallucinations, beyond that responsible for visuoperceptual
cognitive impairments.

Functional neuroimaging studies provide further evidence that specific dysfunction in
posterior visual processing networks underlies generation of visual hallucinations in LBDs.
Resting state SPECT (single-positron emission computed tomography) and FDG-PET studies have shown decreased perfusion and metabolic rates respectively in posterior visual cortices in LBD patients with visual hallucinations compared to those without, particularly in the inferior parietal lobules, posterior cingulate gyrus, precuneus and cuneus (Boecker et al., 2007; Matsui et al., 2006; O’Brien et al., 2005; Oishi et al., 2005). In parallel with these findings, fMRI studies during visual stimulation paradigms confirm hypoactivation of inferior parietal lobules, posterior cingulate gyrus, precuneus, cuneus and fusiform gyri in Parkinson’s disease patients with hallucinations in comparison to those without (Meppelink et al., 2009; Stebbins et al., 2004). Importantly, administration of procholinergic medication to LBD patients in the form of AChEIs restores perfusion in these posterior visual areas, with a consequent reduction in the severity of their visual hallucinations (O’Brien et al., 2005). This, therefore, supports the hypothesis that loss of cortical cholinergic input from the NBM network in LBDs underlies the additional dysfunction in posterior visual processing networks which contributes to the generation of visual hallucinations.

Thus, recent structural and functional neuroimaging evidence supports earlier neuropathological data to implicate dysfunction in additional posterior visual processing networks in the pathogenesis of visual hallucinations in LBD patients. Furthermore, it suggests that dysfunction in the NBM cholinergic network may underlie the aberrant processing in these additional visual cortices and thereby contribute to generation of hallucinations. This is supported by clinical studies showing that treatment of LBD patients with AChEIs markedly reduces visual hallucinations (Litvinenko et al., 2008; Mori et al., 2006). Since NBM activation alters cortical acetylcholine levels and thereby enhances neuronal signal-to-noise ratios (Goard and Dan, 2009; Pinto et al., 2013; Soma et al., 2013) then damage to this network in LBDs could decrease the signal-to-noise ratio of salient stimuli. This might then allow irrelevant intrinsic and sensory information which would normally be suppressed to enter perceptual awareness in the form of hallucinations (Perry and Perry, 1995).
Concomitant dysfunction in frontal and arousal networks contributes to generation of visual hallucinations

Overlapping dysfunctions in a number of other cognitive networks are also likely to contribute to the generation of visual hallucinations in LBDs. For example, VBM MRI analysis shows that LBD patients with visual hallucinations have greater atrophy in frontal areas compared to those without (Sanchez-Castaneda et al., 2010), while fMRI studies comparing Parkinson’s disease patients with hallucinations to those without during performance of visual paradigms demonstrate not only dysfunction in visual cortical areas in the former, but also simultaneous disruption of activity in frontal areas (Meppelink et al., 2009; Shine et al., 2014; Stebbins et al., 2004). The presence of hallucinations in LBDs is closely associated with worsening impairments on tests of attentional control (Bronnick et al., 2011; I. G. McKeith et al., 2004; Meppelink et al., 2008), as well as impairments on tests of inhibitory control such as the Stroop test and go/no-go task (Barnes and Boubert, 2008), deficits attributable to dysfunctions in the fronto-parietal and noradrenergic networks respectively (as described earlier). This, therefore, suggests that breakdown in these frontal networks may play a contributory role in the generation of visual hallucinations in LBDs, perhaps by reducing attentional and inhibitory control of perceptual errors arising from dysfunction in posterior visual cortices, allowing them to enter conscious perception as hallucinations (Shine et al., 2011) (Fig. 3.). Indeed electrophysiological experiments support this view by demonstrating severe disturbances of prepulse inhibition in both PDD and DLB patients compared to AD patients and controls, the electrophysiological correlate of an organisms’ ability to filter out irrelevant sensory or cognitive information (Perriol et al., 2005).

In addition, disrupted sleep-wake cycling and REM (rapid eye movement) sleep behavioural disorder are also strongly associated with the presence of visual hallucinations in LBDs (Nomura et al., 2003; Whitehead et al., 2008). Interestingly, PET studies in healthy humans during sleep show hypoperfusion of the precuneus and posterior cingulate cortices during
REM sleep (Maquet, 2000). Given the above findings of strong correlations between relative hypoperfusion of these areas in awake LBD patients and the presence of visual hallucinations, this supports the hypothesis that intrusion of episodes of REM sleep during wakefulness may contribute to generation of hallucinations (Diederich et al., 2005). Control of both arousal and REM sleep appears to be regulated by the NBM (Lee et al., 2005, and as discussed above) and therefore dysfunction in this network may contribute to generation of visual hallucinations in LBDs not only by disrupting visual perception as above, but also by deregulating arousal mechanisms.

Overall, therefore, concomitant dysfunction in a number of brain networks involved in visual perception, inhibitory control and arousal may all play a role in the generation of visual hallucinations in LBDs, which is supported by clinical data indicating that the strongest determinants of hallucinations in PD are impairments of visuoperceptual and frontal functions combined with the presence of REM sleep behavioural disorder (Gallagher et al., 2011). However, the relative contributions of these network dysfunctions and how they interact to produce visual hallucinations remains unclear, and further studies are needed to examine this.

1.4. The global impact of Lewy body dementias

Dementia is a serious global health issue with an increasing prevalence (Ferri et al., 2005; Llibre Rodriguez et al., 2008). It is estimated that 24.3 million people suffer from dementia worldwide, with 4.6 million new cases diagnosed every year. The number of individuals affected is projected to increase to 81.1 million by 2040 (Ferri et al., 2005). After AD, DLB is the second most common type of dementia (Aarsland et al., 2008) with a prevalence of 7.5% and annual incidence of 3.8% of dementia diagnoses (Vann Jones and O’Brien, 2013). Point prevalence estimates of PDD in those with PD range up to 40% (Aarsland, Zaccai, et al., 2005; Aarsland and Kurz, 2010; Caballol et al., 2007). However, as mentioned above, the cumulative prevalence is very high at 75-90% of those with a decade or more of disease
(Aarsland and Kurz, 2010; Buter et al., 2008; Hely et al., 2008), thereby accounting for 2-5.6% of all dementia diagnoses (Aarsland et al., 2008; Aarsland, Zaccai, et al., 2005).

LBDs are associated with significantly increased morbidity, reduced quality of life and increased care giver burden compared to AD patients of similar severity (Aarsland et al., 2012; Boström et al., 2007a; McKeith et al., 2006; Reid et al., 1996; Ricci et al., 2009). They also carry a significantly increased risk of nursing home placement and increased mortality (G. Levy et al., 2002; Williams et al., 2006). Both LBDs are also associated with significant societal and economic costs; for example, in 2006, the average cost of care in Europe for a patient with DLB was €37,500 per annum, significantly greater than the cost of €18,200 per annum for the average AD patient (Boström et al., 2007b). The annual costs associated with dementia have been estimated to be between €105-160 billion in Europe (Olesen et al., 2012; Wimo et al., 2011), and $183-385 billion in the United States (Thies and Bleiler, 2011).

### 1.5. Current treatment of the Lewy body dementias

Despite their high prevalence, clinical impact and economic burden no effective treatments exist for LBDs (Aarsland et al., 2012; Ihl et al., 2011). Recognition of the key role of cholinergic network deficits in the pathophysiology of LBDs has lead to the current management strategy of using acetylcholinesterase inhibitors (ACEIs: Rivastigmine, Donepezil and Galantamine) to treat cognitive symptoms. However, these medications only provide modest general symptomatic improvements at best, and are associated with limiting side-effects, possibly due to their generalised mechanism of action (Emre et al., 2004, 2014; Litvinenko et al., 2008; Ravina et al., 2005; Rolinski et al., 2012). NMDA-receptor antagonists are the only other current mainstream treatment option, but likewise produce only modest symptomatic improvement with a high risk of debilitating side effects (Aarsland et al., 2009; Emre et al., 2010; Qaseem et al., 2008). There is, therefore, an urgent need for novel therapeutic interventions for LBDs.
Given the complex milieu of neuropathological changes, genetic influences and neurotransmitter deficits underlying LBDs, it is perhaps not surprising that attempting to treat these dementia syndromes with drugs targeting single neurotransmitter systems with generalised mechanisms of action have thus far shown only modest results. An alternative therapeutic approach could be to address cognitive deficits through targeted intervention at the neural network level. This approach has distinct advantages over the traditional model of single-ligand-targeted drug therapy. First, to compensate for deficits in all the neurotransmitter systems involved in the pathophysiology of LBDs using replacement pharmacotherapy would necessitate polypharmacy for patients, with the associated risks of multiple side effects. Second, the heterogeneity of the underlying molecular pathology means that pharmacologic agents aiming to reduce aggregation of abnormal proteins, such as α-synuclein, may be either inappropriate or insufficiently effective in a substantial number of patients. Network-targeted therapies can avoid these difficulties by attempting to modulate the disease process downstream at a systems-level to restore normal neural processing patterns and thereby relieve symptoms.

Therapies which are able to reversibly modulate neural network activity to ameliorate clinical symptoms already exist. Deep brain stimulation (DBS) has proven safety and efficacy in treating the movement symptoms of PD by altering aberrant processing patterns in motor networks (Deuschl et al., 2006; McConnell et al., 2012; Williams et al., 2010). Evidence indicates that it achieves this by altering brain functional and structural connectivity via neural plastic mechanisms to return dysfunctional motor network processing back to its natural state (Fenoy et al., 2014; van Harteveld et al., 2014; Kahan et al., 2014).

We hypothesise that this same approach could be applied to the treatment of cognitive and behavioural symptoms in LBDs, through direct modulation of neural processing in the cognitive networks described above. Several lines of evidence highlight the NBM as a potential cognitive node to target in this respect. First, animal studies strongly implicate NBM activity in cognitive and behavioural functions, including arousal, attention, perception and...
memory (discussed below), all of which are particularly impaired in LBDs. Second, as discussed above, the NBM degenerates significantly in LBDs, and dysfunction in its corticopetal cholinergic network is strongly implicated in the pathophysiology of all the major cognitive deficits which characterise these syndromes. Finally, as detailed below, the NBM is a discrete anatomical structure, and therefore an amenable cognitive node to target surgically compared to more diffuse cognitive networks, such as the fronto-parietal network, where the optimum site of network modulation is currently unclear.

I will now discuss the structure and function of the nucleus basalis of Meynert in greater detail.

1.6. The nucleus basalis of Meynert

1.6.1. Anatomy and histology of the NBM

Theodor Meynert first described a group of magnocellular hyperchromic neurones located in the human basal forebrain in 1872, naming it the nucleus of the ansa lenticularis (Meynert, 1872). Kölliker later renamed it the nucleus basalis of Meynert (Kölliker, 1896). Detailed human anatomical studies show that the NBM is a flat, nearly horizontal structure extending from the olfactory tubercle anteriorly to the level of the uncal hippocampus at its most caudal extent, spanning a distance of 13-14 mm in the sagittal plane. It reaches its greatest cross-sectional diameter under the anterior commissure in a region known as the substantia innominata, with a medio-lateral width of 16-18 mm (Mesulam and Geula, 1988). In its anterior portion the nucleus is limited inferiorly by the horizontal limb of the nucleus of the diagonal band of Broca, supero-medially by the ventral globus pallidus, and supero-laterally by the lateral extension of the anterior commissure (Figs. 4 & 5). In its posterior portion it abuts the ansa lenticularis superiorly, the putamen laterally, the posterior tip of the amygdala inferiorly, and the optic tract medially (Fig. 5) (Mesulam and Geula, 1988; Rossor et al.,
1982). There are striking interspecies differences in the anatomy of the NBM; according to comparative anatomy studies by Gorry (1963) the NBM in rodents is rudimentary and considerably interdigitated with the globus pallidus, whereas in primates and humans the nucleus attains its greatest developments in size as well as differentiation from surrounding cell groups. This may be explained by the massive expansion of the cortical mantle in higher species, which is the main innervation target of the NBM (Divac, 1975; Gorry, 1963).

Immunocytochemical analysis of the human NBM indicates the total number of neurons is approximately 210,000 per hemisphere (Gilmor et al., 1999). Histologically there is a predominance of magnocellular hyperchromic neurons containing conspicuous nucleoli and predominant lipofuscin causing displacement of the nuclei (Mesulam and Geula, 1988). These are fusiform to multipolar in shape and 40–50 × 60–70 µm in size (Mufson et al., 2003). There is no characteristic pattern of dendritic arborisation with the dendritic trees of adjacent neurons overlapping and lacking a common orientation (Mesulam and Geula, 1988). Staining shows that 90% of all NBM neurons are choline acyl-transferase (ChAT) positive, and therefore cholinergic, although smaller non-staining galaninergic and GABAergic neurons are also present (Gritti et al., 1993; Mesulam and Geula, 1988; Mufson et al., 2003). The heteromorphic shapes and isodendritic morphologies of NBM neurons have lead some to suggest that they constitute a telencephalic extension of the brainstem reticular formation (Mesulam et al., 1983) since very similar neuronal morphologies are seen there (Ramón-Moliner and Nauta, 1966). Interestingly, both areas are thought to be involved in cortical activation and alertness (see below) (Fuller et al., 2011; Saper et al., 2005). Based on cytoarchitectonics, cytochemistry and connectivity patterns Mesulam et al. (1983, 1988) designated NBM neurons as the Ch4 cell group in their classification of human basal forebrain cholinergic nuclei (Mesulam et al., 1983; Mesulam and Geula, 1988). For the remainder of this manuscript the terms NBM and Ch4 will be used interchangeably.
Intrinsic organization of the NBM

According to human anatomical studies the constituent neurons of the NBM/Ch4 can be divided into six sectors based on topographical features, which relate to their individual connectivity patterns (described below). The anterior sector, Ch4a, appears just posterior to the olfactory tubercle and extends to the crossing of the anterior commissure (Fig. 5). A rarefaction in neuronal density in the centre divides this into anteromedial (Ch4am) and anterolateral (Ch4al) subsectors. An anterointermediate sector (Ch4ai) then passes from the anterior commissure to the anterior limit of the ansa peduncularis (present in the human but...
not the primate). The passage of the ansa peduncularis (ventral amygdalofugal pathway) defines the intermediate sector (Ch4i) and at the same time divides it into superior/dorsal (Ch4id) and inferior/ventral (Ch4iv) subsectors. The most posterior sector (Ch4p) is limited by the structures described above (Mesulam et al., 1983; Mesulam and Geula, 1988). In addition anatomical studies in both humans and primates show a group of cells known as the nucleus subputaminalis (NSP), which lies supero-lateral to the main body of NBM and infero-lateral to the putamen. This has the same cytoarchitectural and cytochemical properties as the Ch4 cell group (Ayala, 1915) and it is proposed that this represents a further subsector of the NBM (Boban et al., 2006). Interestingly the human NSP has a lateral subdivision not present in the primate which appears to be more phylogenetically advanced than all other cholinergic basal forebrain regions (Simić et al., 1999).
1.6.3. Connectivity: afferent projections to the NBM

Direct axonal tracing experiments in non-human primates show that despite widespread efferent projections from the NBM to the entire neocortex, reciprocal afferent connections from cortex to NBM are not symmetrical and are restricted to limbic and paralimbic areas.

The piriform, orbitofrontal, insular, temporopolar, parahippocampal, entorhinal and cingulate regions provide its main cortical afferents (Fig. 6) (Mesulam and Geula, 1988; Mesulam and Mufson, 1984; Russchen et al., 1985). Subcortical limbic structures provide additional input,
such as the amygdala, hypothalamus, septal nuclei and nucleus accumbens (Jones et al., 1976; Mesulam and Geula, 1988; Mesulam and Mufson, 1984). Diencephalic cells projecting to the NBM have been identified in the midline thalamic nuclei and in the region between the peripeduncular and subparafascicular nuclei (Russchen et al., 1985).

Immunohistochemical analysis in rodents shows that the NBM also receives substantial projections from nuclei within the pontomesencephalic tegmentum. These include catecholaminergic projections from the ventral tegmental area, substantia nigra pars compacta, retrorubral field, raphe nuclei and the locus coeruleus, serotonergic projections from the dorsal raphe nucleus and ventral tegmentum, and cholinergic projections from pedunculopontine and laterodorsal tegmental nuclei (Fig. 6) (Jones and Cuello, 1989). In the primate NBM projections also arrive from the brainstem reticular formation and nucleus of the solitary tract, as well as many reciprocal connections with other surrounding cholinergic nuclei of the basal forebrain (Russchen et al., 1985).

Retrograde axonal tracing experiments cannot be performed in human subjects, therefore at the present time we assume that the afferent and efferent (described below) connections of the human NBM are very similar to those demonstrated in the primate. The cytoarchitecture of the NBM, its size and its degree of differentiation from the globus pallidus are similar in both species (Gorry, 1963; Mesulam et al., 1983; Mesulam and Geula, 1988), and the only minor anatomical differences are the presence of the Ch4ai subsector and the lateral subdivision of the NSP in the human nucleus (Mesulam and Geula, 1988; Simić et al., 1999). Therefore, drawing inferences on human NBM connectivity from that in the primate appears valid, and indeed human pathological data gives indirect support to this assumption (Mesulam and Geula, 1988). The ongoing development of Diffusion Tensor Tractography techniques will allow confirmation of these anatomical relations from in vivo imaging of human subjects in the near future.
Figure 6: The major proposed connections of the human NBM with cortical and subcortical structures. Major afferent projections (bright green arrows) are inputs to NBM as a whole: catecholaminergic projections from VTA, SNpc, retrorubral field, raphe nuclei and locus coeruleus; serotonergic projections from dorsal raphe nuclei and VTA; cholinergic projections from pedunculopontine nucleus. Major efferent projections (bright red arrows) are shown according to their principle NBM subsector of origin. Most NBM subsectors have additional minor projections which overlap with the cortical target fields of other subsectors, creating redundancy in the topographical arrangement of projections (these are not shown but are detailed in the text). The major efferent projections to the caudate/putamen and thalamus are from NBM as a whole (dark red arrow). All efferent projections are cholinergic. The diagram is based on axonal tracing experiments in primates (Mesulam et al., 1983; Russchen et al., 1985), immunohistochemical studies in rodents (Jones and Cuello, 1989) and pathological observations in human tissue (Mesulam and Geula, 1988). Cx = abbreviation for ‘cortex’; NBM = nucleus basalis of Meynert (subsectors of NBM as described in main text); retic. formation = brainstem reticular formation; SNpc = substantia nigra pars compacta; VTA = ventral tegmental area. Adapted from Gratwicke J, et al. The nucleus basalis of Meynert: A new target for deep brain stimulation in dementia? Neurosci. Biobehav. Rev. 2013; 37:2676-88.

1.6.4. Connectivity: efferent projections from the NBM

The human NBM provides the single major source of cholinergic innervation to the entire cortical mantle (Mesulam et al., 1983). Indeed, release of ACh in neocortex can be directly evoked by electrical stimulation of the rodent NBM or optogenetic stimulation of its projection axons (Kalmbach et al., 2012; Kurosawa et al., 1989). The efferent connectivity between individual subsectors of NBM and cortical areas displays a topographic specificity.
according to both retrograde tracer experiments in the primate and neuropathological studies in human AD patients (Fig. 6): Ch4am provides the major cholinergic projection to frontal, parietal and cingulate cortices situated along the medial wall of the hemisphere. Lesser projections are directed to the hypothalamus, hippocampal formation, ventral somatosensory cortex, amygdala, ventrolateral orbital, middle insular, periarluate, peristriate, parahippocampal regions and the inferior parietal lobule. The Ch4al subsector is the principal source of cholinergic projections to frontoparietal opercular regions and the amygdala. Additional projections are directed to the olfactory bulb, medial frontal pole, dorsomedial motor cortex, ventrolateral orbital cortex, insular, inferotemporal area and parahippocampal regions. The Ch4id and Ch4iv subsectors have similar projection patterns: they give prominent projections to ventrolateral orbital, insular, periarluate, peristriate, inferotemporal, and parahippocampal areas as well as to the inferior parietal lobule. Minor projections occur to the medial frontal pole, dorsomedial motor cortex, frontoparietal opercular areas, the amygdala, anterior auditory cortex, and the temporal pole. Lastly the Ch4p subsector has a more restricted major projection to the superior temporal gyrus and the temporal pole. Its lesser projections are confined to adjacent inferotemporal and posterior insular regions (Jones et al., 1976; Mesulam and Geula, 1988; Mesulam et al., 1983). Efferent cholinergic fibres from the human NSP course in the external capsule towards the inferior frontal gyrus, which lead Simic et al. to propose that it projects to the cortical speech area in man (Simić et al., 1999).

The complex topographical arrangement of Ch4 efferent connectivity also contains considerable overlap between individual subsectors according to primate tracing studies. Some cortical areas, such as the ventrolateral orbital, insular, parahippocampal and peristriate cortices, receive projections of comparable size from many different Ch4 subsectors (Mesulam et al., 1983). This may allow for some redundancy in the system, which could prevent these cortical areas from substantial cholinergic denervation should one Ch4 subsector be preferentially affected by disease. On the other hand, other cortical regions such
as medial frontoparietal, superior temporal and temporopolar regions receive Ch4 projections from a much more restricted number of subsectors, and could therefore be much more vulnerable to cholinergic denervation following limited NBM cell loss in those areas. This is supported by observations in human post-mortem brain tissue which show that there is secondary degeneration in the nucleus basalis following temporal lobe lesions, but not after frontal or parietal lobe lesions (Kodama, 1929).

Immunohistochemical mapping in post-mortem brain tissue from healthy human subjects shows that efferent cholinergic projections from the NBM leave the nucleus in two highly discrete organized fibre bundles which form the medial and lateral cholinergic pathways (Fig. 4) (Selden et al., 1998). The cholinergic axons in these bundles are mostly unmyelinated (Wainer and Mesulam, 1990). Both the human post-mortem studies and MRI Diffusion Tensor Tractography in healthy volunteers demonstrate that the medial pathway leaves the NBM anteriorly and joins the white matter of the gyrus rectus. It curves round the rostrum of the corpus callosum to enter the cingulum, travels posteriorly to the splenium and enters the retrosplenial white matter to merge with fibres of the lateral pathway in the occipital lobe (Hong and Jang, 2010; Selden et al., 1998). Individual axons radiate from this pathway to supply the medial orbitofrontal, subcallosal, cingulate, pericingulate and retrosplenial cortices. The lateral pathway subdivides into a capsular division, travelling within the external capsule, and a perisylvian division, travelling within the claustrum (Selden et al., 1998). On leaving the lateral aspect of NBM the capsular division gives off a bundle of fibres ventrally which travel in the white matter of the uncinate fasciculus to supply the amygdala and temporal lobe cortices. The rest of the capsular division ascends in the external capsule adjacent to the putamen and its individual fibres radiate out to supply the dorsal frontoparietal cortex, middle and inferior temporal gyri, inferotemporal cortex and the parahippocampal gyrus. The perisylvian division courses within the claustrum into the white matter of the inferior frontal and superior temporal gyri. From here its fibres radiate out to supply the frontoparietal opercular cortices, superior temporal gyrus and the insula. The medial and
lateral cholinergic pathways merge anteriorly in the white matter of the orbitofrontal gyri. These cortical projections from the NBM also have a weak contralateral component (Mesulam et al., 1983).

These cholinergic projection fibres form a dense plexus in all regions of the human neocortex, displaying numerous end-terminal swellings which likely represent synaptic specializations as they are often in intimate contact with cortical cholinceptive neurons (Mesulam and Geula, 1988). There are differences in the regional densities of NBM cortical innervations: limbic and paralimbic areas (particularly hippocampal, amygdala and piriform regions) receive substantially higher levels of cholinergic input than adjacent neocortical association areas. Apart from the cortex and amygdala both primate and human pathological studies show that the NBM also sends substantial efferent projections to a number of diencephalic structures, including the caudate nucleus, putamen, thalamus (Fig. 6) and habenular nucleus (via the stria medullaris)(Jones et al., 1976; Mesulam and Geula, 1988; Mesulam et al., 1992).

Overall, the heterogeneous neural input to NBM from predominantly limbic structures combined with its dominant cholinergic output to the entire neocortex places it in a unique position in the brain where it can influence all aspects of complex behaviour according to the prevailing emotional or motivational state (Mesulam, 1987).

1.6.5. Pharmacology of the NBM

As mentioned above 90% of neurons in the human NBM are cholinergic, staining intensely for both acetylcholinesterase (AChE) and choline acyl-transferase (ChAT) (Mesulam and Geula, 1988; Mufson et al., 2003). Their cortical projections provide the main source of these two enzymes in neocortical areas, with loss of NBM neurons in dementia correlating with decreased cortical enzyme activity (Etienne et al., 1986; Gaspar and Gray, 1984; Perry et al., 1985). However only 6% of human NBM neurons co-express the m2 muscarinic acetylcholine receptor, and they are therefore not the major cortical source of this receptor, which also shows reduced cortical levels in dementia (Mufson et al., 1998).
Cholinergic NBM neurons in both rodent and primate also express significant levels of both high (trkA) and low (p75NTR) affinity nerve growth factor (NGF) receptors (Bothwell, 1995; Sobreviela et al., 1994), as well as retrograde transported NGF (Mufson et al., 1999), which promotes cell survival and upregulates ChAT activity (Hefti, 1986). Studies on post-mortem human brain tissue show that NBM neurons are the only CNS neurons that continue to express high levels of p75NTR during adulthood (Mesulam et al., 1992), and it is hypothesized that disruption of NGF signalling could underlie the selective degeneration of NBM neurons in AD (Counts and Mufson, 2005). In addition, human NBM cholinergic neurons express both the calcium binding protein calbindin, and the glutamate receptor subunit GluR2, both of which are involved with intracellular calcium homeostasis (Geula et al., 2003; Ikonomovic et al., 2000). Interestingly, the expression of each of these receptors in NBM neurons undergoes a selective reduction in elderly humans and in AD: the combined effects may cause deregulation of intracellular calcium and resultant excitotoxic cell death (Geula et al., 2003; Ikonomovic et al., 2000; Mufson et al., 2003).

Aside from magnocellular cholinergic neurons, immunocytochemical studies in rodents and humans show that the NBM also contains a mixture of smaller non-cholinergic perikarya: gamma amino butyric acid (GABA) interneurons, which may potentially exert inhibitory influences on neighbouring cholinergic projection neurons, and small interneurons expressing the inhibitory neuropeptide galanin (GAL) (Gritti et al., 1993; Mufson et al., 2003). In AD there is a hyper-innervation of galaninergic fibres on remaining NBM cholinergic neurons (Mufson et al., 2003), and it is therefore proposed that GAL down-regulates NBM cholinergic function, particularly in dementia (Bowser et al., 1997). Finally, other fibres immunoreactive for somatostatin, neuropeptide Y, neotensin, pro-opiomelanocortic peptides, substance P, vasoactive intestinal polypeptide (VIP) and oxytocin interdigitate with NBM cholinergic neurons in primates and humans, suggesting that they all contribute to the regulation of cholinergic function within the nucleus (Mufson et al., 2003).
1.6.6. Function: the role of the NBM in memory

The function of the NBM has been investigated extensively over the last forty years and the most widely held view is that it plays a key role in the formation of memory. Indeed, as mentioned above, the NBM is uniquely positioned to provide the cortex with information about the behavioural importance of stimuli in order to affect learning, and it provides the main cholinergic projection to the amygdala (Nagai et al., 1982; Selden et al., 1998), which mediates adversely motivated learning and is known to modulate memory formation in other regions (McGaugh, 2002).

Experiments in rodents, primates and humans have established that cortical cholinergic function is essential to the acquisition of new memories (Croxson et al., 2011; Fisher et al., 1998; Murray and Fibiger, 1985; Petersen et al., 1977). Lesions of the NBM cholinergic system in rodents and primates reduce cortical cholinergic function and thereby impair learning and memory on a variety of tasks (Bartus et al., 1985; Butt and Hodge, 1995; Irle and Markowitsch, 1987; Leanza et al., 1996; Mandel et al., 1989; Roberts et al., 1992).

In addition, stimulation of the rodent NBM directly induces cortical plasticity and re-organizes receptive field maps (the region of auditory frequencies detected by a group of cortical sensory neurons) in relation to stimuli (Bakin and Weinberger, 1996; Kilgard and Merzenich, 1998), representing a “physiological memory” (McLin et al., 2002). This phenomenon is dependent on cholinergic function as it is blocked by anti-cholinergic agents (Bakin and Weinberger, 1996) or by selective lesions of the NBM (Baskerville et al., 1997; Webster et al., 1991). Moreover, induction of these plastic changes is associated with cortical EEG desynchronisation (change from slow synchronized delta waves to fast gamma and theta waves) (Bakin and Weinberger, 1996), which is itself associated with plasticity and learning (Huerta and Lisman, 1993; Lee et al., 2005; Raghavachari et al., 2001). Therefore, the NBM has been shown to directly induce cortical plasticity and electrophysiological correlates of learning and memory via the release of ACh from its cortical projections.
Miasnikov et al. (2009) take this further, demonstrating that the physiological changes induced in the cortex of rats by NBM stimulation do not represent simply plastic re-organisation but actually have all the attributes of true natural associative memory: associativity, specificity, rapid acquisition, consolidation, long term retention and extinction (McLin et al., 2002; Miasnikov et al., 2009). Thus, compelling evidence exists that the NBM plays an integral role in the formation of memories.

1.6.7. Function: the role of the NBM in attention

A parallel line of investigation into the functions of the NBM hypothesizes a role in the mediation of attention. Pharmacological manipulations in humans show that the central cholinergic system is intimately involved in the mediation of attention (Bentley et al., 2004; Dunne and Hartley, 1985) and selective basal forebrain lesions in animal studies provide extensive evidence that the NBM and its cholinergic projections are key in mediating a range of attention functions (McGaughy et al., 2002; Muir et al., 1992; Robbins et al., 1989; Voytko, 1996; Voytko et al., 1994).

Both rodent and primate experiments show that increasing cortical ACh levels, either by NBM stimulation or by iontophoretic application, dynamically modulates cortical coding of sensory inputs, producing more reliable coding of stimuli associated with a background suppression of contextual information (Goard and Dan, 2009; Pinto et al., 2013; Roberts et al., 2005; Soma et al., 2013). In agreement with this, electrophysiological recordings from parietal and sensory cortices in rodents and primates performing attention tasks show that cholinergic input disproportionately increases weighting of task-relevant versus task-irrelevant inputs (Broussard et al., 2009; Herrero et al., 2008). Therefore, this modulation of cortical sensory processing by the NBM cholinergic system serves to increase the signal to noise ratio for salient stimuli, thereby enhancing the strength of their neural representations (Bentley et al., 2011). As discussed earlier, in facilitating this process the NBM effectively amplifies perception of salient stimuli by posterior regions of the fronto-parietal network and
ensures their attentional significance, the neurobiological correlate of ‘bottom-up’ orienting of attention (Buschman and Miller, 2007; Sarter et al., 2006).

Attention and memory functions are not mutually exclusive, especially as every step in the process of learning (input selection, manipulation in working memory, construction of associations for recall) is dependent upon attention (Sarter et al., 2003). Indeed, both human neuroimaging studies and computational modeling suggest that cholinergic influences on sensory cortex serve both to enhance signal detection (and thus attentional significance) and by doing so facilitate the formation of novel input associations (memory formation) (Bentley et al., 2009; Hasselmo and McGaughy, 2004).

1.6.8. Function: the role of the NBM in modulating the behavioural state

It has long been known that the cholinergic projections of the NBM to the cortex are intimately involved in the regulation of cortical activation and arousal. Electrical stimulation of the rodent NBM, or optogenetic stimulation of its projection axons, can directly desynchronize the neocortical EEG and induce fast gamma oscillations indicative of the awake and alert state (Kalmbach et al., 2012; Metherate et al., 1992). Conversely lesions in the rodent NBM prevent cortical EEG desynchronisation and instead produce slow synchronized delta waves (typical of the sleep state) with corresponding behavioural unresponsiveness/coma (Buzsaki et al., 1988; Fuller et al., 2011).

This has led many (Buzsaki et al., 1988; Freund et al., 2009) to propose that the NBM may be the structural basis for the concept of generalized ascending activation to the cortex as originally proposed by Moruzzi and Magoun (1949). The NBM occupies a key position in the arousal network to fulfill such a role, receiving noradrenergic projections from the locus coeruleus (Gaspar et al., 1985) and appearing to have a reciprocal relationship with the orexinergic neurons of the hypothalamus, with an interplay between the two appearing to control the wakeful/aroused state (Jones, 2008).
Lee et al. demonstrate that basal forebrain cholinergic neurons in the rat discharge maximally in awake states (Lee et al., 2005), and that their activity strongly correlates with both cortical gamma activity (which reflects cortical arousal (Maloney et al., 1997)), and cortical theta oscillations (which can promote synaptic plasticity (Huerta and Lisman, 1993)). These results suggest that the NBM can indeed drive cortical activation via gamma rhythms and, in conjunction with the discussion above, simultaneously induce cortical plasticity via theta rhythms during attentive waking periods.

Drawing all these lines of evidence together, one can hypothesize that the function of the NBM is to modulate the overall behavioural state of the animal to one of activation or “readiness”, during which there is perceptual enhancement and a lowered threshold to induce memory for salient stimuli (Hasselmo and Sarter, 2011). The behavioural correlate of this would be a state of enhanced cognitive function, with improved attention, perception and an improved ability to process and learn new information. It follows that degeneration of the NBM would impair the induction of this activated state, making orienting of attention, accurate perception of stimuli and forming new memories more difficult, as is the case in patients with Lewy body dementias.

1.7. Deep brain stimulation

1.7.1. Overview of deep brain stimulation and current clinical applications

Deep brain stimulation emerged as a therapy for PD at the end of the twentieth century, following the demonstration that electrical stimulation of the basal ganglia-thalamo-cortical motor network could markedly improve motor symptoms (Benabid et al., 1987; Limousin et al., 1995). DBS to either the globus pallidus internus (GPi), or more commonly the subthalamic nucleus (STN) are now well established as safe and effective therapies for the
motor symptoms associated with PD, and their long-term efficacy and associated improvement in quality of life is well documented (Deuschl et al., 2006; Follett et al., 2010; Krack et al., 2003; Limousin et al., 1998; Williams et al., 2010). Modulation of the motor network using DBS is also an effective therapy for other movement disorders including essential tremor (Deuschl et al., 2011) and dystonia (Vidailhet et al., 2012), and has also shown encouraging results in the treatment of Gilles de la Tourette syndrome (Ackermans et al., 2011; Kefalopoulou et al., 2015).

More recently DBS has shown promise in treating the symptoms of several psychiatric disorders, including obsessive-compulsive disorder and depression, through neuromodulation of cognitive-motor circuits within the fronto-striatal network and affective circuits within a limbic-cortical network respectively (Blomstedt et al., 2012; Lozano et al., 2012). This highlights the fact that invasive neuromodulation of different large scale brain networks can be effectively applied to attenuate different clinical symptoms.

The surgical procedure for DBS involves permanent implantation of multi-contact titanium electrodes (usually bilaterally) within the target anatomical structure(s) and subsequently connecting these subcutaneously to a neurostimulator/pulse generator (IPG) implanted in the chest wall (Foltynie and Hariz, 2010) (see Fig. 7). Given its invasive neurosurgical nature DBS implantation to any target carries generic risks for patients, including possible intracerebral haemorrhage (0-10%), infection (0-15%) and seizures (0-1%), but when performed in experienced units these are rare, and the procedure is generally regarded as very safe (Foltynie et al., 2011; Foltynie and Hariz, 2010). Nevertheless, when considering the case for DBS in an individual patient the potential beneficial effects must be weighed against all potential risks. Once a DBS system is implanted, physicians with expertise in the area can program the IPG (using an external programmer device) to deliver electrical stimulation to the target brain structure at a specific amplitude, pulsewidth and frequency in order to modulate its neural activity (see (Volkmann et al., 2006) for practical detail on common programming parameters). Which of the four contacts on each DBS electrode is active, and
whether the stimulation is delivered in a monopolar (IPG as anode, contact as cathode) or bipolar (individual contacts on each electrode assigned as anode and cathode) configuration is also customisable, and guided by clinical response to stimulation (Foltynie and Hariz, 2010; Volkmann et al., 2006).

Figure 7: A diagram illustrating the hardware components for deep brain stimulation. A multi-contact titanium electrode is implanted into a target deep brain nucleus (the NBM in the case of the trials detailed in this manuscript) and is connected by subcutaneous wiring to a stimulator (implantable pulse generator, IPG) implanted in the left chest wall.

### 1.7.2. Mechanisms of action of DBS

The mechanism by which DBS relieves symptoms in PD and other conditions is not well understood. It is currently thought that high frequency stimulation (HFS, 100-180 Hz) of subcortical nuclei inhibits pathologically synchronized low frequency network oscillations (McConnell et al., 2012; McIntyre and Hahn, 2010; Rosin et al., 2011). This may represent an “informational lesion”, disrupting errant signals from being propagated downstream in a neural network as a result of upstream disease processes such as dopaminergic depletion (Grill et al., 2004).
In contrast to the inhibition of neural signals caused by HFS, converging evidence suggests that low frequency stimulation (LFS, 5-40 Hz) has the opposite effect and can excite neural elements. For example, Nandi et al. (2008) show that LFS to the pedunculopontine nucleus in parkinsonian monkeys can overcome afferent inhibition of the nucleus from the basal ganglia (Jenkinson et al., 2004; Nandi et al., 2008) while Wu et al. (2008) demonstrate that LFS of the tuberomammillary nucleus in the rat can potentiate seizures induced by amygdaloid-kindling (Wu et al., 2008). Most relevant to the current manuscript, Kurosawa et al. (1989) demonstrate that LFS in the range of 20-50 Hz in the rat NBM produces large direct increases in cortical ACh release from its cholinergic terminals (Kurosawa et al., 1989). This corresponds with the fact that neuronal discharge rates of around 20 Hz are normally seen in the NBM of rats during active behaviour in the aroused/awake state (Buzsaki et al., 1988).

However, conceptualising the effects of DBS on different neural networks as simply inhibitory or excitatory is too simplistic a view, especially since electrical stimulation at any particular frequency has been shown to have simultaneous differential effects on different neural tissues (e.g. inhibiting soma and exciting axons) (McIntyre et al., 2004). Instead, a recent study using dynamic causal modelling of neuroimaging data shows that acute application of DBS differentially modulates the strength of functional connections between distributed nodes within a brain network (i.e. the ‘effective’ connectivity within the network is altered), which in turn predicts clinical response to therapy (Kahan et al., 2014). Furthermore, preliminary evidence suggests that long-term application of DBS may have superadded effects on network function by inducing structural changes to network connectivity through neural plastic mechanisms, which shifts network dynamics back towards a healthy state (van Harteveld et al., 2014). Thus the general mechanism of action of DBS appears to be its ability to modulate the functional and structural dynamics of a brain network as a whole, though its specific effects on different target neural networks is still being elucidated (McIntyre and Hahn, 2010).
With specific regard to the NBM cholinergic network, the experiments by Kurosawa et al. (1989) and others clearly indicates an overall activating effect of LFS on corticopetal cholinergic projections from NBM, which could potentially be harnessed for therapeutic benefit (Kalmbach et al., 2012; Kurosawa et al., 1989).

1.8. Deep brain stimulation of the nucleus basalis of Meynert for cognitive neuromodulation

1.8.1. Effects of NBM DBS on cognitive processes in animal studies

Investigating potential therapeutic effects of NBM DBS in Lewy body dementias has been limited by a lack of good animal models of the cholinergic deficits in LBDs. Transgenic mouse models using α-synuclein overexpression have well characterized nigro-striatal dopaminergic deficits, but NBM cholinergic deficits have not been examined in sufficient detail (Chesselet and Richter, 2011; Nuber et al., 2008). However, new transgenic models of LBDs have recently been developed which better approximate the human disease in terms of both cholinergic pathology and cognitive deficits (Laursen et al., 2013; Magen et al., 2012), which will open up new possibilities for investigating the effects of NBM DBS in LBDs.

On the other hand, evidence that low frequency NBM DBS can enhance cognition in healthy animals does exist. McLin et al. show that pairing brief NBM stimulation (unilateral, bipolar, 50-100 μA, single pulse, 0.2s pulsewidth) with 6kHz tones in freely moving rats induces a cardio-respiratory behavioural response, which is reproducible when the 6kHz tone is presented alone afterwards. This response is specific to the conditioned tone only, not to others, thus indicating that NBM DBS induces an associative memory in the animal (McLin et al., 2002). Additionally, Montero-Pastor et al. implanted DBS systems in the NBM of rats and stimulated them at low frequency (unilateral, monopolar, 60-100 μA, 1Hz, 0.5ms) during
acquisition and testing on a two-way active avoidance task (a test of associative memory) (Montero-Pastor et al., 2001, 2004). At pre-training, NBM DBS resulted in significantly faster learning of the task, indicating enhancement of memory acquisition. Post-training DBS improved performance on a test of retention of information in memory, indicating enhancement of memory consolidation, and was sensitive to both the duration and amplitude of NBM DBS. Overall, the group found that the greatest improvements in performance with NBM DBS were in those rats that had been identified as poor learners prior to the trial rather than those who were designated as cognitively normal.

Thus, although these animal models are not representative of the neurodegenerative processes occurring in LBDs, these data do support the concept that low frequency NBM DBS may modulate cognitive functioning.

1.8.2. Studies of NBM DBS for dementia symptoms in humans to date

The first case report describing NBM DBS for dementia in a human was performed over thirty years ago. In 1984, Turnbull et al. implanted a single electrode into the left NBM (via a frontal approach) in a 74-year old man with clinically moderate AD. Stimulation was delivered at relatively low frequency (bipolar, 3V, 50Hz, pulse width 210ms) in cycles of 15 seconds on followed by 12 minutes off (Turnbull et al., 1985). After nine months of stimulation no clinical improvement in cognition was observed. However, a PET scan six months post-implant demonstrated preserved cortical metabolic activity in the stimulated hemisphere compared to the unstimulated side. The relevance of these metabolic changes remains questionable, however, given the lack of clinical improvement. Methodological factors possibly limiting the clinical effect included the unilateral short-lasting and intermittent stimulation, and the fact that NBM targeting was neither image nor pathologically verified, and therefore, whether the electrode was accurately placed in NBM cannot be certain. In addition, the particular stimulus cycle chosen was unusual by today’s
standards given that stimulation was only delivered for a total of 30 minutes in every twenty-four hours.

NBM DBS was recently revisited by Freund and colleagues, this time to treat LBDs. Their patient was a 71-year-old man suffering from severe PDD with marked executive dysfunction, memory impairment and visual perceptual difficulties. At baseline, he could only recall 12 words in the Rey Auditory Verbal Learning Test (AVLT(sum) - a test of immediate episodic verbal memory and learning) and was unable to perform the delayed conditions of the test at all (AVLT(recall) and (recog) – tests of long term episodic and recognition memory respectively) (Freund et al., 2009). He scored 4 points on the Clock Drawing Task (CDT – a test of visuoperceptual ability) and took 5.5 minutes to complete the Trail Making Test (TMT-A – a test of executive control of attention, visual scanning and executive function (working memory and sequencing)). Qualitatively, he displayed poor attention span, rigid thinking, bradyphrenia and marked ideational apraxia. He underwent implantation of bilateral DBS electrodes into the Ch4i subsector of NBM, chosen as it is the largest subsector (Fig. 5) (Mesulam and Geula, 1988), therefore, giving the highest possibility of successful electrode placement. Moreover, it has the most widespread cortical projections, giving the potential to modulate more cortical regions (Fig. 6) (Mesulam et al., 1983). Both the surgical procedure and stimulation were safe and well tolerated. With initiation of bilateral low frequency NBM DBS (monopolar, 1.0V, 20 Hz, 120 µs) score on the AVLT(sum) doubled to 25, indicative of a marked improvement in immediate episodic memory and learning. The patient was also able to perform AVLT(recog) for the first time, recognizing six words, demonstrating some improvement in retention of information in memory. The CDT score rose to 9, and TMT-A completion time halved to 2.5 minutes, indicating improvements in visuoperceptual and executive/attentional functions respectively. Performance also improved on tests of processing speed and praxis, with additional subjective benefits observed in attention, alertness, drive and spontaneity (Barnikol et al., 2010; Freund et al., 2009). All these cognitive benefits were sustained for two months during constant stimulation and were shown
to be time-locked to the cessation and re-introduction of NBM stimulation, and thus dependent upon it. The authors also reported that the patient demonstrated an overall qualitative improvement in personality and social communication with NBM DBS, which improved his overall quality of life, however this was not formally measured.

This case report provides preliminary evidence that low frequency NBM DBS can be performed safely in individuals with advanced LBDs, and may improve cognitive functioning across a number of domains. However, the results must be regarded with caution as this was only performed in a single patient.

The only formal clinical trial of NBM DBS to date has been conducted in AD patients; Kuhn and colleagues performed a double-blind randomised crossover trial of bilateral low frequency NBM DBS in six patients with AD (J Kuhn et al., 2015). Patients with mild to moderate AD (MMSE range 18-26) were selected and inclusion criteria included a stable dose of acetylcholinesterase medication for at least three months, typical cerebrospinal fluid changes of tau protein and amyloid beta42 levels and retained capacity to give informed consent to surgery. Electrodes were implanted bilaterally into NBM, though due to surgical planning constraints subsector targeting varied across patients from Ch4im anteriorly to Ch4p posteriorly. All patients received the same NBM stimulation parameters (monopolar contacts 0-8, 2.5V, 90 µs, 20Hz) during an initial one month randomised double-blind cross-over phase (two weeks with NBM DBS on followed by two weeks off, or vice versa), then had individual parameter adjustments made (all still at 20Hz) during a subsequent eleven month open label phase. Both the surgical procedure and stimulation were safe and well tolerated. Results showed that global cognitive functioning (as measured by the MMSE and the Alzheimer’s Disease Assessment Scale (ADAS-Cog)) remained stable in all patients over the four-week double-blind period, and stable in four of six patients after one year of subsequent open-label stimulation. At one year the group’s average scores on the MMSE and ADAS-Cog worsened by 0.3 points and 3 points respectively, which is a slower rate of decline than that seen in comparable pharmacologically treated AD patients (2.4 points and 4.5 points per year.
respectively), although direct comparison is difficult since the latter figures were averaged over much larger sample populations. FDG-PET imaging at one year compared to baseline also showed a 2-5% increase in cortical glucose metabolism with low frequency NBM DBS, deviating from the average 5.2% decrease per year seen in untreated AD patients. However, a detailed neuropsychological battery did not show any changes in specific cognitive functions throughout the trial period.

This study provides further evidence that low frequency NBM DBS appears safe and well tolerated in dementia patients, and also preliminary evidence that it may slow the rate of cognitive decline in AD. However, again the data should be interpreted cautiously given that no changes in specific cognitive functions were seen. This may have been due to several limitations, including the relatively short double-blind period (due to ethical constraints) and the variation in NBM subsector target amongst patients (due to constraints on surgical implant trajectories due to concomitant brain pathologies).

Overall, human clinical trials to date have provided preliminary evidence to show that low frequency NBM DBS is both technically feasible and safe in demented patients, and that the therapy may be associated with a slowing in the rate of cognitive decline in AD patients. However, there is currently a paucity of evidence regarding the use of NBM DBS for the treatment of dementia, particularly with regard to the specific treatment of LBDs. There is, therefore, a need for randomised controlled trials, utilising double-blind periods of substantial length and detailed assessments of all cognitive sub-domains and behavioural disturbances in LBD patients. Such trials will help address major unresolved issues, including the technical feasibility and safety of NBM DBS in LBDs, the efficacy of this treatment for specific cognitive and/or behavioural symptoms, the optimal target site(s) within the NBM and stimulation settings.
1.9. **Aims of this thesis**

In this thesis, I will explore the safety and efficacy of bilateral nucleus basalis of Meynert deep brain stimulation as a potential therapy for patients with Parkinson’s disease dementia and dementia with Lewy bodies (from Chapter 4 onwards both dementia syndromes are collectively referred to as Lewy body dementias). More specifically I aim to:

1) Evaluate the safety and tolerability of low frequency NBM DBS in patients with PDD and evaluate the effects of stimulation on specific cognitive impairments and behavioural symptoms (Chapter 2).

2) Evaluate the safety and tolerability of low frequency NBM DBS in patients with DLB and evaluate the effects of stimulation on specific cognitive impairments and behavioural symptoms (Chapter 3).

3) Record local field potentials from the NBM in these patient groups, both in the resting state and during two attention tasks, to investigate the physiological function of the NBM in vivo (Chapter 4).

4) Perform combined resting state NBM LFP and magnetoencephalography recordings in these patient groups to investigate the functional connectivity of NBM at the brain network level, and thereby gain further insight into its physiological function in vivo (Chapter 5).
Chapter 2: Deep brain stimulation of the nucleus basalis of Meynert for Parkinson’s disease dementia

2.1 Patients and methods

2.1.1 Experimental design

We conducted a randomised, double-blind, crossover trial of bilateral NBM DBS to compare deficits on a short battery of cognitive tests after six weeks of active stimulation and six weeks of sham stimulation. NBM DBS was achieved using electrodes which straddled the GPi, thus allowing the potential for subsequent conventional DBS for co-existing motor impairments (Follett et al., 2010). The study was sponsored by University College London, and was performed at the National Hospital for Neurology and Neurosurgery, London, UK.

2.1.2 Patients

Participants were recruited from the population of Parkinson’s disease patients referred to our clinic. Patients were eligible for inclusion if they: met Queen Square Brain Bank Criteria for the diagnosis of Parkinson’s disease (Hughes et al., 1992); had motor fluctuations (off periods and/or levodopa-induced dyskinesias) known to improve with GPi DBS (so that if they did not experience subjective benefit from NBM DBS at trial end they could opt to switch to GPi DBS for co-existing motor symptoms (Follett et al., 2010); were appropriate candidates for GPi DBS aside from the co-existence of dementia; were aged 35-80 years; were able to give informed consent; met diagnostic criteria for PDD (Emre et al., 2007); had an MMSE score between 21-26 (in order to select those with moderate dementia severity with retained capacity to give informed consent); had minimal atrophy on MRI brain scans (to ensure technical feasibility of electrode implantation); were living at home with a carer-informant; were willing to comply with the trial protocol and attend necessary clinic visits. In addition,
all patients were already receiving a stable dose of AChEI medication at the time of recruitment (with either an established suboptimal response to this medication or intolerance of higher dosage) and this was continued throughout the trial. Changes in AChEI dose were not permitted during the trial period to avoid confounding any cognitive effects of NBM DBS.

Exclusion criteria were: diagnosis or suspicion of other cause for parkinsonism or dementia; known abnormality on CT or MRI brain imaging considered likely to compromise compliance with the trial protocol; prior intra-cerebral surgical intervention for Parkinson’s disease.

2.1.3 Ethics and consent

The trial conformed to the Seoul revision of the Declaration of Helsinki (2008) and Good Clinical Practice guidelines, and was approved by the East of England Research Ethics Committee. All potential participants were assessed for their capacity to provide written informed consent by an experienced neuropsychologist independent from the trial team. This trial is registered with ClinicalTrials.gov, Identifier: NCT01701544.

2.1.4 Randomisation and blinding

We randomly assigned participants to either the stimulation off-first group (sham stimulation for six weeks, followed by active stimulation for six weeks) or the stimulation on-first group (vice versa). We used computer-generated pairwise randomisation according to order of enrolment, so that equal numbers of patients were recruited to each group and the order of those receiving on- followed by off- stimulation and vice-versa was counterbalanced. The randomisation sequence was held by an unblinded clinician who was also responsible for programming the stimulation. Participants and assessing clinicians were blinded to the stimulation condition. The unblinded clinician spent the same time adjusting each patient’s stimulator at the start of both active and sham stimulation periods. The stimulation parameters
were selected to avoid any immediate or long-lasting side effect that could be perceived by the patient or blinded clinicians, and thus have the potential to unblind them.

### 2.1.5 Baseline procedures

All enrolled participants underwent a baseline assessment (Fig. 8) which included a detailed neuropsychological battery, and motor, non-motor and psychiatric symptom scales (discussed in detail below). During baseline assessment the participants also completed two separate measures of IQ; the two subtest form of the Wechsler Abbreviated Scale of Intelligence gives a brief estimate of current IQ by combining scores on a measure of crystallised verbal intelligence, the vocabulary subtest for measuring word knowledge, and a measure of fluid performance intelligence, the matrix reasoning subtest for measurement of visual information processing and abstract reasoning skills (Wechsler, 1999). In comparison, the National Adult Reading Test estimates pre-morbid intelligence in English speaking patients with dementia (Nelson and Wilson, 1991). It is an untimed measure consisting of 50 words with atypical phonemic pronunciation, thereby testing the patient’s vocabulary rather than their ability to apply regular pronunciation rules. The pronunciation of previously learnt words is thought to be spared in cognitive decline, allowing it to be used as a proxy for estimating pre-morbid crystallised verbal intelligence. Comparing the scores on these two IQ tests at baseline allowed us to estimate the degree of cognitive decline in each individual participant relative to their pre-morbid state.

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3 In psychology, crystallised intelligence is the ability to use knowledge and experience gained across one’s lifetime. It does not equate to memory, but may rely on accessing information from long term memory (Catell, 1971).

4 Fluid intelligence is the capacity to solve novel problems independent of knowledge from the past. These terms, crystallised and fluid intelligence, are conceptualised as separate mental systems and are interdependent, and thus highly correlated with one another (Catell, 1971).
2.1.6 Neurosurgical procedure

Within one month of completing baseline assessments participants underwent stereotactic implantation of bilateral DBS electrodes. All patients were operated under general anaesthesia (average time anaesthetised was 3-4 hours) using a Leksell stereotactic frame (Elekta Instrument AB, Stockholm), without microelectrode recording. Following attachment of the frame the NBM was visualised in each patient using pre-operative stereotactic axial and coronal proton-density MRI scans on which the pallidum, optic tract, anterior commissure and the adjacent NBM were visible (1.5T Siemens Espree, PDw Turbo Spin-echo; 1.0 x 1.0 x 2.0 mm; TR 4000ms TE 13ms). Target selection for placement of the deepest contact/s was at the level of maximal NBM diameter, the Ch4i subsector (around 5mm below the AC-PC plane), with more dorsal contacts positioned in the overlying GPi. Ch4i was chosen as it is both the largest subsector of NBM, giving highest probability of successful electrode placement, and has the most widespread cortical projections, thus potentially influencing...
more cortical areas (see Section 1.6.4. above and (Gratwicke et al., 2013)). However, targeting this subsector of NBM meant that some compromise had to be made with regard to the optimal anteroposterior location of contacts within overlying GPi. Planning of the surgical trajectory in each individual patient was undertaken using commercially available software (Framelink, Medtronic, Minneapolis, USA). The NBM was visualised as the hyperintense signal lateral to the hypointense optic tract and medial to the hypointense anterior commissure (lateral extension) on proton density MRI. The entry point was chosen to ensure a trajectory which avoided both sulci and the ventricular system, while maximising the length of the trajectory within the visible NBM hyperintensity.

During surgery several methods were employed to avoid brain shift: minimal CSF loss was achieved by placing the 14 mm burr hole and 3-4 mm dural opening on a gyrus rather than a sulcus; the burr hole was flooded with saline irrigation after opening the dura; the time from dural opening to final DBS electrode implantation was limited by sealing the dural defect with fibrin glue as soon as the electrode was in situ; surgery was performed in a similar position to that adopted during image acquisition in order to minimise postural movement of intracranial structures (though a slight head-up tilt was employed during surgery to encourage venous drainage).

A 1.5 mm diameter blunt-tip radiofrequency electrode (Leksell RF electrodes, Elekta, Stockholm) was introduced to the target while performing dynamic impedance monitoring. Care was taken to avoid electrode deviation by contact with either the burr hole or the dural edges. After withdrawal of the radiofrequency electrode a quadripolar DBS electrode (model 3387 [Patient A] or 3389 [Patients B-F], Medtronic, Minneapolis, MN, USA) was soft-passed down the same track. This has four platinum-iridium cylindrical surfaces of diameter 1.27mm, length 1.5mm, and centre-to-centre separation of 2mm. The contacts were numbered 0 (lowermost/most ventral, which was intended to lie in the body of NBM) to 3 (uppermost/most dorsal, which was intended to lie in the GPi). Depth of implantation was controlled by a depth stop positioned a defined length along the electrode shaft. The
electrodes were then secured in place using a Stimloc skull fixation device. The accuracy of DBS lead contact location was confirmed immediately with postoperative stereotactic MRI (Fig. 9): The distance between the intended MRI target and the actual position of the implanted electrode was calculated and surgery was not considered complete until acceptable placement of the electrodes had been image-confirmed. In all six cases (twelve electrode implants) actual position of the most ventral electrode contact was within 1mm of intended MRI target location.

![Figure 9: Determining DBS lead contact location from stereotactic, proton density MR images (Siemens Espree, 1.5T): First column (above: axial image; below: coronal reconstruction along lead trajectory, magnified): Stereotactic MR images obtained after lead implantation were imported into a dedicated software platform (FrameLink, Medtronic). Images were reconstructed along the axis of the lead and a template placed on the lead artefact to determine the stereotactic coordinates of each contact. Second column (above: axial; below: coronal): The stereotactic coordinates for each contact were then transposed onto preoperative stereotactic images, allowing an accurate assessment of lead location (white dot). Third column (above: axial; below: coronal): The heavily myelinated optic tract and anterior commissure (lateral extension), hypointense on proton density MR images, are coloured light blue. The intervening hyperintense nucleus basalis of Meynert (NBM) lies between these two structures and is coloured yellow. In the coronal image the NBM is seen to lie superior to the temporal lobe (amygdala / supra amygdala complex) and inferior to the internal (green) and external (red) segments of the globus pallidus. The active contact (black dot) is seen to lie within the NBM.]

Disposable extension connectors were attached to the end terminals of both subcutaneous leads, and these extensions were externalised though the scalp. Patient’s leads were externalised in this manner for a five-seven day period in order to allow trials of external
stimulation to take place, as well as direct recording of LFPs from NBM and GPi, and also combined LFP-MEG recordings (see Chapters 4 and 5 below). Prophylactic systemic antibiotics (Cefuroxime 1.5 g) were administered intra-operatively, and three more doses administered within the following 24 hours. Patients were reviewed in the recovery suite both by the operating neurosurgeon and a neurologist (myself) to confirm that there was no new neurological deficit following surgery. Once awake the patients were transferred back to the main ward to recover.

Following recovery on the ward over the interval five-seven day period each patient underwent a second shorter operation (average time under general anaesthesia two hours): the existing scalp wounds were re-opened, and the disposable extension connectors removed and discarded. Two 60 cm extension cables were connected to the subcutaneous end-terminals of the DBS leads, and tunnelled down through the subcutaneous soft tissue on the left side of the neck. A 5-6 cm incision was made in the left pectoral area and an Activa PC IPG (Medtronic, Minneapolis, MN, USA) was implanted sub-muscularly. The distal terminals of the extension cables were connected to the IPG ports. Following thorough irrigation and careful haemostasis all wounds were closed (vicryl sutures to subcutaneous tissues, galea and nylon sutures to skin). Prophylactic systemic antibiotics (Cefuroxime 1.5 g) were again administered intra-operatively, and three more doses were administered over the subsequent 24 hours. Patients were reviewed in the recovery suite both by the operating neurosurgeon and a neurologist (myself) to confirm that there was no new neurological deficit following surgery. Once awake the patients were transferred back to the main ward to recover over the following few days. Patients were only discharged home once they had fully recovered to their baseline pre-operative level of functioning (on average 3-4 days post-operative). DBS systems remained switched off during this period, and on discharge.

All adverse events were recorded immediately post-operatively, throughout the rest of the trial period, and beyond into the open label follow up period.
2.1.7 Externalised assessments

As mentioned above, following electrode implantation all patients had their electrodes externalised on the ward for five to seven days before IPG implantation. During this time three types of assessment were performed on each patient:

1. Trial simulation: this was performed both to assess for any side effects of acute simulation (which might have the potential to unblind the patient later on at the randomisation stage), and also to ascertain that stimulation would be both safe and tolerable for each patient during MEG recordings performed during subsequent days. Two days after electrode implantation, when the patient was fully awake and had largely recovered to baseline level of function on the ward, the externalised electrodes were each connected to an external stimulator in turn. Frequency was kept constant at 20 Hz and pulsewidth constant at 60 µs during all assessments. With the left electrode connected first, monopolar stimulation was tested first using each of the two deepest contacts in turn (0-, then 1-) with escalating amplitudes up to a maximum of 3.0 V. Bipolar stimulation was then tested using the same electrode, first using the deepest contact pair (0-1+), and then the second deepest pair (1-2+) with escalating amplitudes up to a maximum of 3.0 V. The same procedure was then repeated for the right electrode. Any subjective symptoms reported by the patient or observed by the assessing neurologist at a particular stimulation setting were meticulously recorded.

2. Local field potential (LFP) recordings: the patients attended our research laboratory during the daytime having taken their usual medications. Their externalised electrodes were connected to an amplifier and we recorded bilateral LFPs simultaneously from NBM and GPi in each patient, first at rest, then while they performed customised versions of Posner’s covert attention test (a test of orienting of visual attention) and the sustained attention to response task (SART, a test of sustained attention or vigilance). These LFP recordings are described in detail in Chapter 4.
3. Magnetoencephalography (MEG) recordings: patients attended our MEG scanning suite during the daytime having taken their usual medications. Their externalised electrodes were connected to an amplifier, while the patient was seated in a 275 channel CFT MEG system (VSM Medtech Ltd., Vancouver, Canada). We simultaneously recorded bilateral NBM and GPi LFPs and cortical MEG at rest. We then connected each electrode in turn to a custom-built stimulation-record amplifier and simultaneously recorded contralateral NBM and GPi LFPs and cortical MEG during ipsilateral monopolar NBM stimulation at 20Hz. Finally, we simultaneously recorded bilateral NBM and GPi LFPs and cortical MEG during ipsilateral bipolar NBM stimulation at 20Hz, for each hemisphere in turn. These combined LFP and MEG recordings are described in detail in Chapter 5.

2.1.8 Post-operative procedures

All participants underwent their first post-operative assessment one week after pulse generator implantation (Fig. 8). An abbreviated cognitive battery was performed, consisting of: California Verbal Learning Test-II (CVLT-II), the Wechsler Adult Intelligence Scale-III (WAIS-III) digit span, verbal fluency, Posner’s covert orienting of attention test and Simple and Choice Reaction Times (these tests are discussed in more detail below in 2.1.9 Primary outcome measures). These selected tests from the detailed neuropsychological battery are amenable to repeated administration due to tests being either less susceptible to practice effects or parallel versions being available.

Three weeks later patients attended for 24 hours and were screened for the effects of stimulation in an open label manner, using the WAIS-III digit span (a test of attention and executive function) as an objective measure. Only low frequency stimulation at 20Hz was used (for the reasons discussed above in Chapter 1). Only monopolar stimulation was used at a pulsewidth of 60 µs. Optimum stimulation voltages were determined as those producing highest digit spans with the lowest energy, without side-effects, and these were adopted for the blinded phase (Table 1). DBS was subsequently turned off for two weeks, and then
patients were randomised into the simulation off-first or on-first group for the subsequent six weeks. Following this there was a two-week washout period (DBS off), then patients were switched over to the opposite condition for a further six weeks. All assessments performed at baseline were repeated at the end of each six-week period, except for measures of IQ (Fig. 8). The abbreviated cognitive battery was performed immediately prior to each change in DBS condition, as well as 24 hours afterwards. All trial assessments were performed by a clinician blinded to stimulation status. Minor adjustments to concomitant licenced medications were permitted throughout the blinded trial period, aside from changes to the doses of any AChEIs being taken.

After the blinded crossover phase patients were given the option of having NBM stimulation permanently switched on, or receiving conventional GPi DBS (through the superior electrode contacts) for motor symptoms. Patients were invited to routine follow-up with neuropsychological assessments and open-label DBS adjustments at least every six months.

2.1.9 Primary outcome measures

The pre-specified primary outcomes were the differences in scores on each item of the abbreviated cognitive battery between the two blinded stimulation conditions (after six weeks on-stimulation vs after six weeks off-stimulation). These included:

- **CVLT-II**: A list of 16 nouns, with four items drawn from each of four semantic categories, is read to the patient, who then attempts to recall as many words as possible in any order. Performance on this single first trial gives a measure of attention/working memory which is relatively free of the influence of fatigue (immediate free recall Trial 1). This task is repeated four more times, making a total of five learning trials. Learning efficiency is approximated by the sum of the scores of all five learning trials (immediate free recall T score). Ability to access newly learned information is assessed by the number of words retained by the patient after a 20 minute interval (long delay free recall) and the percentage of words retained from those previously learnt (retention) (Delis et al., 2000).
\textbf{Digit span}: in this subtest from the WAIS-III patients are first required to listen to and verbally repeat number sequences of increasing length (digits forwards), a test of auditory attention span. Secondly patients are asked to listen to and verbally reverse number sequences of increasing length presented (digits backwards), a test of manipulation in working memory (executive function) (Wechsler, 1997).

\textbf{Verbal fluency}: the verbal fluency subtest from the Delis-Kaplan Executive Function System measures the ability to generate words meeting phonemic or semantic criteria, thereby testing executive retrieval of verbal information (Delis et al., 2001).

\textbf{Posner’s covert orienting of attention test}: measures reaction speed to a lateralised visual target, preceded by a cue prompting to either the correct or incorrect side of upcoming target presentation. This measures speed of orienting of attention (Posner, 1980). We used a customised computerised version of this test, described in detail in Chapter 4. The proportion of correct responses is also measured as a percentage of total targets presented.

\textbf{Simple and Choice Reaction Times}: these tests measure the speed of psychomotor responses to visual targets where the stimulus is either predictable (simple reaction time) or unpredictable (choice reaction time), providing surrogate measures of alertness. We used the computerised version of this test included in the Cambridge Neuropsychological Test Assessment Battery (CANTAB).

Higher scores on all these tests indicate better performance, aside from measures of reaction speed (Posner’s covert orienting of attention test and Simple and Choice Reaction Times).

\section*{2.1.10 Secondary outcome measures}

Secondary outcomes included changes in the following neuropsychological tests (which together with the abbreviated battery above comprised the detailed neuropsychological battery). Higher scores are better on all these neuropsychological tests.
• **MMSE**: a brief general screening instrument for cognitive impairment (Folstein et al., 1975). Although it is the most commonly used screening instrument for dementia worldwide, its dependence on age and educational level hinders the use of a rigid cut-off score (Crum et al., 1993).

• **Mattis Dementia Rating Scale 2**: a test of global cognitive function (Jurica et al., 2001), shown to sensitively measure the degree of cognitive deficits in patients with PD/PDD (Kulisevsky and Pagonabarraga, 2009).

• **Short recognition memory test for faces**: in this test of recognition memory for faces patients are presented with a series of 25 photographs of faces at the rate of one face every 3 s, and for each face the subject is required to judge the presented stimulus as ‘pleasant’ or ‘unpleasant’ to ensure that they are attending to the stimulus items. The patient is then presented with a series of 25 pairs of faces and the task is to identify which of the two faces came from the target list (Warrington, 1996).

• **WAIS-III arithmetic and letter-number sequencing subtests**: in the former patients are asked mental arithmetic problems of increasing difficulty, a test of manipulation in working memory (executive function). In the latter patients listen to a series of numbers and letters in random order and must recall them in numerical followed by alphabetical order, a test of auditory attention span and manipulation in working memory (executive function). The age-adjusted scaled scores for both tests are combined with that for digit span to give an overall working memory index (Wechsler, 1997).

• **Trail making test**: this subtest from the Delis-Kaplan Executive Function System is an expanded version of the original trail making test and includes five conditions: visual search, number sequencing, letter sequencing, number-letter switching and motor speed. In each condition patients must draw to connect numbers and/or letters on a page in a specific order as fast as possible. Number sequencing and letter sequencing scores are measures of psychomotor processing speed, while number-letter sequencing score is a measure of behavioural regulation/set shifting (executive function). The visual search and
motor speed scores provide indexes of deficits in visual attention and motor control respectively, and can be used to control for the confounding effects of such deficits on the other task conditions. (Delis et al., 2001).

- **WAIS-III symbol search and digit-symbol coding subtests**: in the former patients are presented with a series of target symbols and must identify whether these are present in corresponding rows of symbols under a strict time limit. In the latter a key containing nine digit-symbol pairs is presented, followed by a table of digits. Under each digit in the table the patient must write down the corresponding symbol (according to the key) as fast as possible, within a strict time limit. Both are tests of executive control of attention/processing speed, and from their combined age-adjusted scaled scores a processing speed index is derived (Wechsler, 1997).

- **Florida Apraxia Screening Test**: a test of ideational praxis wherein patients must produce 15 gestures to command. A score of nine or below on the FAST has previously been shown to demonstrate good sensitivity for a diagnosis of apraxia (Rothi and Heilman, 1984).

Additional secondary outcome measures included changes on the following validated scales. Higher scores indicate greater functional impairment on all scales except for the EuroQol VAS.

- **MDS Unified Parkinson’s Disease Rating Scale (MDS-UPDRS)**: a comprehensive assessment of the severity of all motor and non-motor symptoms in PD, comprising sixty-five items (score range 0-260) (Movement Disorder Society Task Force on Rating Scales for Parkinson’s Disease, 2003). We evaluated motor symptoms using Part III (the motor subscale), both in the absence of dopaminergic medications (OFF state) and one hour after administration of dopaminergic medications (ON state).
o **Freezing of Gait (FOG) Questionnaire**: this six-item scale (range 0–24) consists of four items that assess FOG frequency and duration, and two that assess the impact of gait difficulties in general (Giladi et al., 2000). It was found that the FOG questionnaire has high test-retest reliability, internal consistency and moderately high correlations with MDS-UPDRS motor and ADL scores (Giladi et al., 2009).

o **Parkinson’s Disease Questionnaire (PDQ-39)**: a self-reported questionnaire consisting of 39 questions assessing quality of life in PD patients (score range 0-156, summary index expressed as %).

o **Scales for Outcomes in Parkinson’s Disease (SCOPA)-Sleep**: a self-reported questionnaire consisting of 11 questions assessing problems with day and night sleeping in PD patients (score range 0-33) (Marinus et al., 2003).

o **SCOPA-Autonomic symptoms scale**: a self-reported questionnaire consisting of 23 questions assessing symptoms of autonomic dysfunction in PD patients (score range 0-69) (Visser et al., 2004).

o **Non-motor symptoms questionnaire**: a self-reported questionnaire consisting of 30 questions assessing presence of a range of non-motor symptoms in PD patients (Chaudhuri et al., 2006).

o **Starkstein Apathy Scale**: a clinician-administered 14 item questionnaire assessing apathy symptoms in PD patients (score range 0-42) (Starkstein et al., 1992).

o **EuroQol visual analogue scale (VAS)**: generic self-reported measure of overall health state on a visual analogue scale of 0-100

o **The Neuropsychiatric Inventory**: a clinician-administered caregiver-reported questionnaire assessing 12 psychiatric symptoms/behavioural disturbances which may be present in dementia patients, capturing frequency and severity of each and distress caused to the caregiver (Cummings et al., 1994).
- **Blessed dementia scale**: a clinician-administered caregiver-reported questionnaire assessing presence or absence of 22 functional impairments and behavioural disturbances due to dementia (score range 0-28) (Blessed et al., 1968).

- **Hamilton Depression Scale**: a clinician-assessed 21 item inventory measuring severity of depressive symptoms (score range 0-68) (Hamilton, 1960).

- **Hamilton Anxiety Scale**: a clinician-assessed 14 item inventory measuring severity of anxiety symptoms (score range 0-56) (Hamilton, 1959).

Throughout the blinded trial period and subsequent open-label follow up period adverse events were systematically recorded.

### 2.1.11 Statistical analysis

The sample size of this study was based on practical considerations. We planned to recruit six patients on the basis of (1) an estimate of the number of eligible patients under active follow up in our clinics, and (2) the fact that the safety of NBM DBS implantation in this vulnerable patient group is currently unknown, necessitating a cautious approach to recruitment. This is therefore a pilot trial with a small sample size and even the primary outcomes are principally exploratory in nature. To maximise transparency of the results, all individual outcome data are presented. Statistical comparisons are performed simply to highlight the most consistent differences at group level according to on- vs off-stimulation, and are not corrected for multiple comparisons. Two-tailed Wilcoxon signed ranks tests were used in paired observations when the distribution of differences was symmetrical, and two-tailed related-samples Sign tests otherwise. For comparisons according to randomisation sequence two-tailed Mann-Whitney U tests were performed. All data were analysed using Statistical Package for the Social Sciences version 22.0 software.
2.2 Results

Between 26th October, 2012, and 15th July, 2015, we assessed 25 patients and enrolled eight into the study. Two patients passed inclusion/exclusion criteria but were subsequently excluded prior to surgery: one decided he wanted GPi DBS for motor symptoms only. The other was determined unsafe for anaesthesia due to severe postural hypotension. Six patients (all male, mean age 65.17 years (SD 10.74)) therefore proceeded to NBM DBS implantation. Table 1 summarises their clinical characteristics and also details the stereotactic coordinates of their active NBM contacts and the stimulation parameters used for the blinded period. Fig. 10 shows Schaltenbrand atlas locations of active NBM contacts in all patients (Schaltenbrand and Wahren, 1977), demonstrating that the most ventral active contact was successfully placed in the Ch4i subsector of NBM in each patient. In all patients with a second active contact per hemisphere (Patients A, B, C and F), this was located on the NBM/GPi border. Surgery was well tolerated and all patients were ambulatory within 24 hours and fully oriented within 48 hours. All six patients completed the blinded crossover phase and were included in analysis.

Four patients had medication changes during the blinded trial period: Patient A had his levodopa increased by 300 mg at the start of the second blinded condition (off-stimulation) due to worsening limb rigidity - an increase in daily levodopa equivalent dose (LED, (Tomlinson et al., 2010)) to 800 mg. His fludrocortisone dose was also increased by 50 micrograms halfway through the second condition due to worsening postural hypotension. Patient C commenced oxybutynin 5 mg after surgery for urinary frequency, and continued on this throughout both blinded conditions. Patient E commenced fludrocortisone 100 micrograms after surgery for postural hypotension, and continued on this throughout both blinded conditions. Patient F switched from Duodopa® to levodopa 400 mg during the first blinded condition (on-stimulation) due to jejunal tube blockage (a reduction in LED to 700 mg).
### Table 1: Baseline clinical characteristics of the PDD study sample, and parameters used during the blinded stimulation period

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at surgery (yrs)</th>
<th>Disease duration (yrs)</th>
<th>H&amp;Y stage</th>
<th>Dementia duration (yrs)</th>
<th>Visual hallucinations?</th>
<th>NART estimated premorbid IQ (mean ± SD)</th>
<th>WASI measured current IQ (95% CI)</th>
<th>MMSE (Mattis Dementia Rating Scale 2 (Raw, Scaled))</th>
<th>Hoehn &amp; Yahr stage</th>
<th>Disease duration (yrs)</th>
<th>Dementia duration (yrs)</th>
<th>Co-morbidities</th>
<th>Concomitant medications at enrolment (total daily doses)</th>
<th>Daily levodopa equivalent dose (LED, mg/day)†</th>
<th>Daily total cholinesterase inhibitor dose</th>
<th>Active contacts (monopolar)</th>
<th>Stereotactic coordinates (x,y,z)◊</th>
<th>Stimulation parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M</td>
<td>61</td>
<td>14</td>
<td>3</td>
<td>4</td>
<td>yes</td>
<td>122 (91-103) (average)</td>
<td>25</td>
<td>124, scaled 5 (moderately impaired)</td>
<td>Psoriasis, renal calculi, prolapsed intervertebral disc</td>
<td>L-dopa 500 mg, rivastigmine 3 mg, fludrocortisone 100 mg</td>
<td>500</td>
<td>3</td>
<td>0.1, 0.9</td>
<td>-17.6, 8.5, -6.3</td>
<td>3.0 V, 60 μs, 20Hz</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>M</td>
<td>65</td>
<td>11</td>
<td>2</td>
<td>3</td>
<td>no</td>
<td>95* (average)</td>
<td>24</td>
<td>116, scaled 3 (severely impaired)</td>
<td>None</td>
<td>L-dopa 525 mg, CR L-dopa 300 mg, entacapone 600 mg, rivastigmine 4.6 mg, simvastatin 40 mg</td>
<td>923.25</td>
<td>4.6</td>
<td>0.1, 0.9</td>
<td>-19.0, 5.0, -3.8</td>
<td>18.5, 5.6, 3.8</td>
<td>3.0 V, 60 μs, 20Hz</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>M</td>
<td>75</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>yes</td>
<td>101 (76-87) (low average)</td>
<td>25</td>
<td>126, scaled 6 (mildly impaired)</td>
<td>Cholelithiasis, renal calculi</td>
<td>L-dopa 500 mg, pramipexole 0.7 mg, rasagiline 1mg, venlafaxine 75 mg, rivastigmine 6 mg, lansoprazole 30 mg</td>
<td>670</td>
<td>6</td>
<td>0.1, 0.9</td>
<td>-19.0, 5.0, -3.8</td>
<td>17.9, 5.0, 3.9</td>
<td>3.0 V, 60 μs, 20Hz</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>M</td>
<td>73</td>
<td>15</td>
<td>3</td>
<td>1</td>
<td>yes</td>
<td>120 (92-104) (average)</td>
<td>25</td>
<td>110, scaled 3 (severely impaired)</td>
<td>Benign prostatic hypertrophy</td>
<td>CR L-dopa 400 mg, ropinirole 4mg, pramipexole 160 mg, rivastigmine 6mg, pantoprazole 20 mg, simvastatin 50 mg, afluoxin 10 mg, finasteride 5 mg</td>
<td>380</td>
<td>6</td>
<td>1.9</td>
<td>-21.4, 5.8, -5.8</td>
<td>23.0, 5.3, 3.8</td>
<td>3.0 V, 60 μs, 20Hz</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>M</td>
<td>46</td>
<td>10</td>
<td>2</td>
<td>5</td>
<td>no</td>
<td>113 (high average)</td>
<td>22</td>
<td>108, scaled 2 (severely impaired)</td>
<td>None</td>
<td>L-dopa 500 mg, CR L-dopa 100 mg, rivastigmine 12 mg, fevosterol, atorazol 10 mg</td>
<td>575</td>
<td>12</td>
<td>0.8</td>
<td>-22.2, 5.2, -5.2</td>
<td>20.2, 5.4, -6.2</td>
<td>3.0 V, 60 μs, 20Hz</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>M</td>
<td>71</td>
<td>15</td>
<td>3</td>
<td>3</td>
<td>no</td>
<td>114 (high average)</td>
<td>21</td>
<td>101, scaled 2 (severely impaired)</td>
<td>None</td>
<td>CR L-dopa 400 mg, Duodopa 24 mls, quetiapine 12.5 mg, rivastigmine 9mg</td>
<td>833</td>
<td>9</td>
<td>0.1, 0.9</td>
<td>-20.8, 4.9, -6.4</td>
<td>15.7, 7.0, -5.5</td>
<td>3.0 V, 60 μs, 20Hz</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Group Mean Group SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
|---------------------|------|----|------|----|------|----|------|----|------|----|------|----|------|----|------|----|------|----|------|----|------|----|------|----|------|----|------|----|------|----|------|----|------|----|------|----|------|----|
|                     | 65.17| 12.67| 2.50 | 3.00 | 110.83| 79.17| 23.67| 114.17| 646.88| 6.77 | 204.71| 3.24 | 20.6, -5 | 20.6, -5 | 95 |

Dementia duration was estimated by examining the patient's medical notes and collateral history from the caregiver to determine the time at which cognitive decline began to interfere with normal occupational or social function. The Mattis Dementia Rating Scale 2 Scaled score is corrected for age but not education. CR = controlled release preparation. SD = standard deviation.

* English was not the first language for Patient B, therefore premorbid and actual IQs may be underestimated.

† LED calculation as per protocol in Tomlinson et al., 2010. ◊ Mean stereotactic co-ordinates of the active contacts in left and right hemispheres respectively, with reference to the mid-commissural point of the AC-PC plane.
Figure 10a: Coronal sections from Schaltenbrand atlas taken 7.5mm and 5.0mm anterior to the midcommissural point to indicate location of active DBS contacts during the blinded phase (Patients A through F). The optic tract (II) and the lateral extension of the anterior commissure (Cm.a) are coloured light blue. The Nucleus Basalis of Meynert (B) lies between these two structures (yellow) and inferior to the globus pallidus (Pars medialis interna, medialis externa and lateralis: P.mi, P.me and P.l). Figure adapted from Schaltenbrand and colleagues (plates 25–26) by permission of Thieme.
Figure 10b: Location of active DBS contacts for each patient on their individual MRI images (Patients A through F). The locations of the most ventral active contacts (in NBM) are shown on representative coronal and axial post-operative MRI images for each patient, and correspond to those shown in Figure 10a above.
Trial stimulation through the externalised electrodes in the immediate post-operative period did not produce any subjective or objective side effects in the majority of the patients (B, C, D, E, F). In Patient A however, bipolar stimulation of the right NBM (0-, 1+, 60µs, 20Hz) at 0.4V induced subjective paraesthesias in the left toes. As stimulation voltage was increased the subjective paraesthesias increased and spread up the left leg to include the thigh. These were described by the patient as a “feeling of quivering” in the left leg, though no actual movement of the left leg was observed. Increasing the frequency of stimulation to the right NBM to 130Hz induced visual illusions between 1.5-3.0V: across these voltages the patient perceived his bedclothes to be “billowing”, when in reality they were motionless on his bed. We then switched over to bipolar stimulation of the left NBM (0-, 1+, 60µs, 20Hz). At 3.0V the patient reported seeing a figure dressed as Aladdin at the end of his bed. This was followed after a couple of minutes by the perception of a watering can on his bed, and thereafter by the perception of a blue fish swimming near the end of his bed. On cessation of NBM stimulation these visions abruptly disappeared. Increasing the frequency of stimulation to the left NBM to 130Hz produced a feeling of inner unease in the patient up to 3.0V, but no visual misperceptions. Bipolar stimulation of right and then left GPis (2-, 3+, 60µs), first at 20Hz (up to 3.0V) and then at 130Hz (up to 3.0V) produced no perceptible side effects on either side. Therefore, stimulation of the left NBM in this patient appeared to reversibly induce complex formed visual hallucinations, which were specific to bipolar stimulation at low frequency. However, trial stimulation was conducted only one day post-operative in Patient A, and it seems likely that anaesthetic side effects would still have been present and might have contributed to the observations described above.

When he returned for open-label parameter screening one month later, Patient A did not experience any perceptible side effects from low frequency NBM stimulation at all, and no objective side effects were observed by the trial team. None of the other patients experienced any perceptible side effects from low frequency NBM DBS at parameter screening either, and
again no objective side effects were observed by the trial team. In light of this it seems likely that patient and carer blinding was adequately maintained throughout the blinded trial period.

Table 2 summarises the pre-specified primary outcome data at group level, while individual results for all patients are presented in Tables 3 and 10. None of the blinded on- vs off-primary outcome comparisons achieved conventional threshold for statistical significance.

The most consistent finding was that three of six patients (Patients D, E and F) showed an improvement in CVLT-II retention in memory Z scores on-stimulation compared to both off-stimulation and baseline, with no between-group differences according to randomisation order (p= 0.7 and p=0.4 for scores off- and on-stimulation respectively). Comparison at group level of retention in memory Z scores between baseline and on-stimulation conditions showed a significant median improvement of 1.5 (p=0.042). A similar pattern was seen with acute NBM stimulation, with four of six patients showing improvement in retention in memory Z scores after 24 hours on-stimulation (Table 9). In contrast however, percentage accuracy on Posner’s covert attention test was worse on-stimulation in five of the six patients (Table 3).
Table 2:  Group level primary and selected secondary outcome measures

<table>
<thead>
<tr>
<th></th>
<th>Group mean (standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td><strong>Primary Outcome Measures</strong></td>
<td></td>
</tr>
<tr>
<td>California Verbal Learning Test II</td>
<td></td>
</tr>
<tr>
<td>Immediate free recall (Trial 1) (Z score)</td>
<td>-2.67 (0.98)</td>
</tr>
<tr>
<td>Immediate free recall (T score)</td>
<td>37.83 (8.33)</td>
</tr>
<tr>
<td>Long delay free recall (Z score)</td>
<td>-1.50 (0.95)</td>
</tr>
<tr>
<td>Retention (Z score)</td>
<td>-1.00 (0.89)</td>
</tr>
<tr>
<td>WAIS-III digit span (raw scores)</td>
<td></td>
</tr>
<tr>
<td>Digits forwards (range 0-16)</td>
<td>9.00 (2.00)</td>
</tr>
<tr>
<td>Digits backwards (range 0-14)</td>
<td>4.00 (1.67)</td>
</tr>
<tr>
<td>D-KEFS Verbal Fluency Test (scaled scores)</td>
<td></td>
</tr>
<tr>
<td>Letter Fluency</td>
<td>4.00 (2.00)</td>
</tr>
<tr>
<td>Category Fluency</td>
<td>2.67 (2.07)</td>
</tr>
<tr>
<td>Category Switching Total Correct</td>
<td>2.00 (1.67)</td>
</tr>
<tr>
<td>Posner’s covert attention test</td>
<td></td>
</tr>
<tr>
<td>Total accuracy (0-100%)</td>
<td>62.03 (22.44)</td>
</tr>
<tr>
<td>Posner effect - reorienting time (ms)◊</td>
<td>4.52 (57.81)</td>
</tr>
<tr>
<td>CANTAB Reaction Time Test</td>
<td></td>
</tr>
<tr>
<td>Simple Reaction Time (ms)</td>
<td>552.80 (209.44)</td>
</tr>
<tr>
<td>Choice Reaction Time (ms)</td>
<td>490.50 (4.95)</td>
</tr>
<tr>
<td><strong>Selected Secondary Outcome Measures</strong></td>
<td></td>
</tr>
<tr>
<td>Mini-Mental State Examination (MMSE)</td>
<td>23.67 (1.75)</td>
</tr>
<tr>
<td>Mattis Dementia Rating Scale 2 (raw score)</td>
<td>114.17 (9.68)</td>
</tr>
<tr>
<td>Neuropsychiatric Inventory</td>
<td></td>
</tr>
<tr>
<td>Total score (0-144)</td>
<td>14.00 (6.54)</td>
</tr>
<tr>
<td>Caregiver distress score (0-60)</td>
<td>8.17 (6.15)</td>
</tr>
<tr>
<td>Hallucinations subscale (0-12)</td>
<td>2.33 (3.39)</td>
</tr>
<tr>
<td>MDS-UPDRS Part IV score (range 0-24)</td>
<td>7.17 (3.87)</td>
</tr>
</tbody>
</table>

All scaled scores/Z scores/T scores are age-adjusted. Posner task total accuracy is % of presented targets correctly responded to. Higher scores are better on all tests, except for measures of reaction time, Neuropsychiatric Inventory subscales and MDS-UPDRS (Movement Disorders Society Unified Parkinson’s Disease Rating Scale) Part IV scores, in which lower scores are better. ◊ = scores closer to zero are better for Posner reorienting time.
Table 2: Primary outcome measures at baseline, end of the blinded off-stimulation period and end of the blinded on-stimulation period

<table>
<thead>
<tr>
<th>Patient</th>
<th>California Verbal Learning Test II</th>
<th>D-KEFS Verbal Fluency Test (scaled scores)</th>
<th>Posner's covert attention test</th>
<th>CANTAB Reaction Time Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immediate free recall</td>
<td>Digits forwards (range 0-14)</td>
<td>Digit Span (raw scores)</td>
<td>Immediate free recall</td>
</tr>
<tr>
<td></td>
<td>Trial 1 (2 score)</td>
<td>Long delay free recall (2 score)</td>
<td>Letter Fluency</td>
<td>Target accuracy (0-100%)</td>
</tr>
<tr>
<td>A</td>
<td>-1.50 -2.00 -2.00</td>
<td>-5.50 -2.00 -2.00</td>
<td>6.00 1.94</td>
<td>1.67 2.00</td>
</tr>
<tr>
<td>B</td>
<td>-3.00 -2.00 -2.00</td>
<td>3.00 2.00 2.00</td>
<td>3.00 1.94</td>
<td>1.67 2.00</td>
</tr>
<tr>
<td>C</td>
<td>-3.00 2.00 -2.00</td>
<td>3.00 2.00 2.00</td>
<td>3.00 1.94</td>
<td>1.67 2.00</td>
</tr>
<tr>
<td>D</td>
<td>-1.50 -1.50 -1.50</td>
<td>-5.50 -2.00 -2.00</td>
<td>6.00 1.94</td>
<td>1.67 2.00</td>
</tr>
<tr>
<td>E</td>
<td>-1.00 -2.50 -0.50</td>
<td>3.00 2.00 2.00</td>
<td>3.00 1.94</td>
<td>1.67 2.00</td>
</tr>
<tr>
<td>F</td>
<td>-3.00 -1.00 -2.50</td>
<td>3.00 2.00 2.00</td>
<td>3.00 1.94</td>
<td>1.67 2.00</td>
</tr>
<tr>
<td>Group</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>OFF</td>
<td>ON</td>
<td>OFF</td>
</tr>
<tr>
<td></td>
<td>37.83 38.17 31.17</td>
<td>4.95 1.11</td>
<td>8.09 0.95</td>
<td>6.03 0.63</td>
</tr>
<tr>
<td></td>
<td>37.83 38.17 31.17</td>
<td>4.95 1.11</td>
<td>8.09 0.95</td>
<td>6.03 0.63</td>
</tr>
</tbody>
</table>

SD = standard deviation. WAIS-III = Wechsler Adult Intelligence Scale-III.
Figure 11: Effects of nucleus basalis of Meynert DBS on selected primary and secondary outcome measures. All graphs show individual results for all patients (dotted lines) and superimposed group mean results (solid lines) as per key. Please note that in both Graphs A and B (left side of figure) higher scores are better, whereas in Graphs C and D (right side of figure) lower scores are better. In Graph A all Z-scores are age-adjusted. CVLT-II = California Verbal Learning Test II, MDS-UPDRS = Movement Disorders Society Unified Parkinson's Disease Rating Scale, MMSE = mini-mental state examination.
Tables 2, 4 and 10 present secondary outcome cognitive measures. There was no consistent advantage on-stimulation vs off-stimulation on global cognitive measures, or on focal measures of working memory, psychomotor speed, facial recognition memory, task-switching or praxis.

Tables 2, 5 and 10 present secondary outcome psychiatric measures. Four of six patients (Patients A, C, D and E) showed improvement in Neuropsychiatric Inventory total scores (NPI total, Tables 2 and 5) on-stimulation compared to both off-stimulation and baseline (see also Fig. 11). Comparison at group level between on- and off-stimulation conditions showed a significant median improvement of -5 points with NBM DBS on (p=0.027). There were no between-group differences in NPI total scores according to randomisation sequence (p=1.0 and p=0.7 for scores off- and on-stimulation respectively). The improvement in NPI total score with NBM DBS on was primarily driven by a reduction in hallucinations subscale scores in Patients A and D (Table 5). At baseline Patients A, C and D reported daily complex visual hallucinations while Patient B reported regular extracampine hallucinations only. Patients A and D both experienced near complete cessation of visual hallucinations after surgery when NBM stimulation was turned on, followed by a resurgence of hallucinations when stimulation was subsequently turned off (Fig. 12). There was no effect of either surgery or stimulation on the more minor visual or extracampine hallucinations of Patients C and B respectively. There was no consistent advantage on-stimulation vs off-stimulation on measures of anxiety, depression or apathy (Tables 5 and 10).
## Table 4: Secondary outcome measures (cognitive) at baseline, end of the blinded off-stimulation period and end of the blinded on-stimulation period

<table>
<thead>
<tr>
<th>Patient</th>
<th>MMSE (range 0-30)</th>
<th>Mattis Dementia Rating Scale 2 (total) (range 0-144)</th>
<th>WAIS-III (indices of scaled scores)</th>
<th>Short Recognition Memory for Faces (range 0-25)</th>
<th>D-KEFS Trail Making Test (scaled scores)</th>
<th>Florida Apraxia Screening Test (range 0-15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B OFF ON</td>
<td>B OFF ON</td>
<td>B OFF ON</td>
<td>B OFF ON</td>
<td>B OFF ON</td>
<td>B OFF ON</td>
</tr>
<tr>
<td>A</td>
<td>25 24 24</td>
<td>124 122 114</td>
<td>75 73 75</td>
<td>71 66 60</td>
<td>24 24 23</td>
<td>8 1 1</td>
</tr>
<tr>
<td>B</td>
<td>24 25 24</td>
<td>116 130 125</td>
<td>82 73 75</td>
<td>63 63 63</td>
<td>20 19 21</td>
<td>1 1 3</td>
</tr>
<tr>
<td>C</td>
<td>25 22 22</td>
<td>126 112 114</td>
<td>73 80 78</td>
<td>63 60 60</td>
<td>21 21 17</td>
<td>4 1 1</td>
</tr>
<tr>
<td>D</td>
<td>25 20 28</td>
<td>110 112 122</td>
<td>80 67 94</td>
<td>69 71 66</td>
<td>21 20 19</td>
<td>NC NC NC</td>
</tr>
<tr>
<td>E</td>
<td>22 20 19</td>
<td>108 122 118</td>
<td>63 65 61</td>
<td>57 57 57</td>
<td>21 21 20</td>
<td>1 1 1</td>
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<tr>
<td>F</td>
<td>21 20 20</td>
<td>101 103 104</td>
<td>63 71 67</td>
<td>63 63 NC</td>
<td>17 19 20</td>
<td>1 1 1</td>
</tr>
<tr>
<td>Group</td>
<td>Mean</td>
<td>23.67 21.83 22.83</td>
<td>114.17 116.83 116.17</td>
<td>72.67 71.50 75.00</td>
<td>64.33 63.33 61.20</td>
<td>20.67 20.67 20.00</td>
</tr>
<tr>
<td>Group</td>
<td>SD</td>
<td>1.75 2.23 3.25</td>
<td>9.68 9.64 7.39</td>
<td>8.16 5.28 11.22</td>
<td>5.01 4.84 3.42</td>
<td>2.25 1.86 2.00</td>
</tr>
</tbody>
</table>

B = Baseline, OFF = end of blinded off-stimulation period. ON = end of blinded on-stimulation period. All scaled scores are age-adjusted. Higher scores are better on all tests. Results highlighted in green indicate those where ON stimulation score was better than both OFF stimulation and baseline scores, by at least one scaled score/10% raw score increase compared to both. Results highlighted in red indicate those where ON stimulation score was worse than both OFF stimulation and baseline scores, by at least one scaled score/10% raw score decrease compared to both. NC = not completed. SD = standard deviation.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Hamilton Depression Scale score</th>
<th>Hamilton Anxiety Scale score</th>
<th>Starkstein Apathy Scale score</th>
<th>Neuropsychiatric Inventory (12 item version)</th>
<th>Blessed Dementia Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(range 0-68)</td>
<td>(range 0-56)</td>
<td>(range 0-42)</td>
<td>total score (0-144)</td>
<td>(range 0-28)</td>
</tr>
<tr>
<td>B OFF ON</td>
<td>B OFF ON</td>
<td>B OFF ON</td>
<td>B OFF ON</td>
<td>caregiver distress (0-60)</td>
<td>hallucinations subscale (0-12)</td>
</tr>
<tr>
<td>A</td>
<td>3 6 3</td>
<td>4 6 2</td>
<td>13 19 17</td>
<td>5 8 4</td>
<td>1 4 0</td>
</tr>
<tr>
<td>B</td>
<td>2 2 2</td>
<td>2 2 2</td>
<td>5 5 5</td>
<td>14 29 24</td>
<td>10 15 15</td>
</tr>
<tr>
<td>C</td>
<td>2 2 2</td>
<td>2 2 2</td>
<td>13 4 5</td>
<td>12 10 4</td>
<td>0 0 1</td>
</tr>
<tr>
<td>D</td>
<td>5 7 7</td>
<td>4 4 4</td>
<td>17 19 13</td>
<td>16 14 13</td>
<td>10 7 5</td>
</tr>
<tr>
<td>E</td>
<td>7 6 6</td>
<td>3 2 2</td>
<td>7 10 14</td>
<td>12 9 4</td>
<td>0 0 0</td>
</tr>
<tr>
<td>F</td>
<td>8 9 8</td>
<td>4 8 4</td>
<td>24 13 16</td>
<td>25 38 26</td>
<td>18 14 14</td>
</tr>
<tr>
<td></td>
<td>Group Mean</td>
<td></td>
<td></td>
<td>Group SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.50 5.33 4.67</td>
<td></td>
<td></td>
<td>2.59 2.80 2.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.17 4.00 2.67</td>
<td></td>
<td></td>
<td>0.98 2.53 1.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13.17 11.67 11.67</td>
<td></td>
<td></td>
<td>6.88 6.56 5.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.00 18.00 12.50</td>
<td></td>
<td></td>
<td>6.54 12.51 10.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.17 7.33 6.83</td>
<td></td>
<td></td>
<td>6.15 5.99 6.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.33 1.83 1.00</td>
<td></td>
<td></td>
<td>3.39 1.60 1.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.67 10.67 10.33</td>
<td></td>
<td></td>
<td>2.34 2.42 1.51</td>
<td></td>
</tr>
</tbody>
</table>

B = Baseline, OFF = end of blinded off-stimulation period. ON = end of blinded on-stimulation period.
Results highlighted in green indicate those where ON stimulation score was 10% better than both OFF stimulation and baseline scores. Results highlighted in red indicate those where ON stimulation score was 10% worse than both OFF stimulation and baseline scores. SD = standard deviation.
Figure 1: Effect of nucleus basalis of Meynert DBS on hallucinations subscale scores on the Neuropsychiatric Inventory (NPI). For clarity of viewing, separate graphs of NPI hallucination subscale scores are presented above one another for those patients in the stimulation on-first group (Patients A and D, above) and those in the stimulation off-first group (Patients B and C, below). Lower scores indicate lower frequency and severity of hallucinations. Please note the re-starting of numbering on the y-axis between graphs. Patients E and F are not shown as they did not suffer from hallucinations at baseline or at any point during the trial.

Table 2 and Table 6 present secondary outcome measures relating to motor symptoms and quality of life. Three of the six patients (Patients A, C and D) showed improvement in UPDRS Part IV scores on-stimulation compared to both off-stimulation and baseline (see also Fig. 11). These patients suffered from levodopa-induced dyskinesias and their Part IV improvements were due to a reduction in the frequency and severity of these. Patients A, D and E reported subjective improvement in their health-related quality of life (EQ-VAS health
state) on-stimulation compared to both off-stimulation and baseline. Patients B and F reported no change, and Patient C reported a decline. This pattern was mirrored by the subjective reporting of distress levels by their carers on the NPI (Table 5).

Other secondary outcome measures relating to non-motor symptoms (SCOPA-sleep, SCOPA-Aut, Non-motor symptoms questionnaire) are reported in Table 10. There were no consistent differences seen across the group with regard to these measures with NBM DBS off vs on.
### Table 6: Secondary outcome measures (motor symptoms and quality of life) at baseline and ends of the blinded off- and on-stimulation periods

<table>
<thead>
<tr>
<th>Patient</th>
<th>Movement Disorders Society Unified Parkinson's disease Rating Scale</th>
<th>Freezing of Gait Questionnaire</th>
<th>PDQ - 39 summary index</th>
<th>EQ-VAS Health state</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Part I score (range 0-52)</td>
<td>Part II score (range 0-52)</td>
<td>Part III score (range 0-132)</td>
<td>Part IV score (range 0-24)</td>
</tr>
<tr>
<td>A</td>
<td>B OFF ON</td>
<td>B OFF ON</td>
<td>B OFF ON</td>
<td>B OFF ON</td>
</tr>
<tr>
<td>B</td>
<td>20 18 18</td>
<td>30 40 31</td>
<td>56 68 71</td>
<td>27 24 37</td>
</tr>
<tr>
<td>C</td>
<td>24 24 20</td>
<td>23 23 21</td>
<td>39 49 42</td>
<td>26 28 37</td>
</tr>
<tr>
<td>D</td>
<td>21 16 17</td>
<td>30 28 26</td>
<td>62 68 69</td>
<td>42 54 66</td>
</tr>
<tr>
<td>E</td>
<td>12 16 19</td>
<td>31 31 36</td>
<td>49 51 51</td>
<td>37 42 39</td>
</tr>
<tr>
<td>F</td>
<td>31 26 24</td>
<td>36 42 40</td>
<td>24 47 39</td>
<td>16 22 22</td>
</tr>
</tbody>
</table>

| Group Mean | B OFF ON | 21.33 19.33 19.50 | 27.50 29.50 28.17 | 46.67 54.17 52.00 | 30.17 33.83 38.17 | 7.17 5.50 4.00 | 86.17 88.17 89.83 | 10.50 11.00 12.17 | 49.33 53.33 50.67 | 63.17 48.33 61.83 |

B = Baseline, OFF = end of blinded off-stimulation period. ON = end of blinded on-stimulation period. Lower scores are better in all cases, except EQ-VAS health state where a higher score is better.

Results highlighted in green indicate those where ON stimulation score was 10% better than both OFF stimulation and baseline scores. Results highlighted in red indicate those where ON stimulation score was 10% worse than both OFF stimulation and baseline scores. EQ-VAS = EuroQol five dimensions questionnaire visual analogue scale. PDQ-39 = Parkinson’s Disease Questionnaire, 39 item. SD = standard deviation.
No serious adverse events occurred during the trial period, however in Patient C erosion of the right electrode cap through the scalp occurred 15 months after trial start date (during open label follow up), necessitating surgical removal. This event caused inconvenience to the patient as he had to undergo a further daycase surgical procedure, but the event caused no extra morbidity and there were no harmful sequelae. Table 7 lists all adverse events. Scores on the abbreviated cognitive battery one week after NBM DBS implant did not show any significant worsening on primary outcome measures as a result of surgery (Table 8). In fact there were median improvements at group level in both CVLT-II immediate free recall and CVLT-II retention in memory scores after surgery (+1.25 Z scores, p=0.041, and +1.00 Z scores, P=0.026, respectively).
### Table 7: Adverse events in the six patients during and beyond the study period

<table>
<thead>
<tr>
<th>Category</th>
<th>All adverse events</th>
<th>Resolved adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serious adverse events</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Related to surgery or device</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erosion of right electrode cap through scalp</td>
<td>1◊</td>
<td>1◊</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1 (17%)*</td>
<td>1 (100%)**</td>
</tr>
<tr>
<td><strong>Non-serious adverse events</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Related to surgery or device</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial scalp wound infection</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Urethral tear from traumatic catheterisation</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Post-operative transient confusion/paranoia</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Burr hole cap discomfort</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><strong>Related to stimulation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual hallucinations</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased limb rigidity</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Worsened postural hypotension</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Worsened urinary frequency</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Jejunal tube blockage</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>13 (100%)*</td>
<td>11 (85%)**</td>
</tr>
</tbody>
</table>

Data are n or n (%). ◊ Erosion of the right electrode cap through the scalp occurred in patient 3 fifteen months after trial start date, during open label follow up. This necessitated surgical removal of the right electrode for safety. *Proportion of patients. ** Proportion of events.
### Table B: Effect of the surgical procedure on primary outcome measures at one week post-operative

<table>
<thead>
<tr>
<th>Patient</th>
<th>California Verbal Learning Test II (Z scores)</th>
<th>WASI-II digit span (raw scores)</th>
<th>D-KEFS Verbal Fluency Test (scaled scores)</th>
<th>Posner’s covert attention test</th>
<th>CANTAB Reaction Time Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immediate free recall (Trial 1)</td>
<td>Long delay free recall</td>
<td>Retention</td>
<td>Digits forwards (range 0-16)</td>
<td>Digits backwards (range 0-14)</td>
</tr>
<tr>
<td>A</td>
<td>-1.50</td>
<td>-1.50</td>
<td>0.00</td>
<td>-2.00</td>
<td>-0.50</td>
</tr>
<tr>
<td>B</td>
<td>-3.00</td>
<td>-2.00</td>
<td>-2.50</td>
<td>-2.00</td>
<td>0.00</td>
</tr>
<tr>
<td>C</td>
<td>-3.00</td>
<td>0.00</td>
<td>0.00</td>
<td>-5.00</td>
<td>0.00</td>
</tr>
<tr>
<td>D</td>
<td>-1.50</td>
<td>0.00</td>
<td>0.00</td>
<td>-5.00</td>
<td>0.00</td>
</tr>
<tr>
<td>E</td>
<td>-4.00</td>
<td>-3.00</td>
<td>-1.50</td>
<td>-1.50</td>
<td>0.00</td>
</tr>
<tr>
<td>F</td>
<td>-3.00</td>
<td>-1.50</td>
<td>-1.50</td>
<td>-2.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>-2.67</td>
<td>-1.33</td>
<td>-1.50</td>
<td>-1.50</td>
<td>0.00</td>
</tr>
<tr>
<td>Group Mean</td>
<td>0.98</td>
<td>1.17</td>
<td>0.95</td>
<td>0.83</td>
<td>0.89</td>
</tr>
</tbody>
</table>

**B** = Baseline assessments. **P** = Assessments at one week post-operative (off-stimulation). All scaled scores/Z scores are age-adjusted. Higher scores are better on all tests, except for measures of reaction time and reorienting time. Results highlighted in green indicate those where one week post-operative score was better than baseline score, by at least one Z score/scaled score/10% raw score/50 ms increase. Results highlighted in red indicate those where one week post-operative score was worse than baseline score, by at least one Z score/scaled score/10% raw score/50 ms decrease. **"** = lower scores better. 0 = scores closer to zero are better. NA = performance too poor, no normative data available to standardise score. NC = not completed.
Table 9 presents data showing the effects of acute NBM stimulation over 24 hours on the primary outcome measures. The data for each patient is taken from the visits when they performed the abbreviated cognitive battery immediately before and 24 hours after simulation was switched on during the blinded period, whether this was at the start of Condition 1 or Condition 2. None of these blinded off- vs 24 hours on- primary outcome comparisons achieved conventional threshold for statistical significance. Three of the six patients (A, C and E) demonstrated improvement on measures of verbal fluency after 24 hours on NBM stimulation, particularly on the category switching condition (a test of set shifting - a component of executive function). However, this improvement was not sustained with chronic stimulation (Table 3). There was also a trend towards worsened simple movement time scores after 24 hours of NBM DBS, with five of six patients demonstrating worsening on this measure. However, this worsening in simple movement times was not accompanied by a deterioration in corresponding simple reaction times, suggesting that acute low-frequency NBM stimulation might have a selective deleterious effect on motor performance, but not on psychological processing speed. This divergent pattern in simple movement and reaction times was not sustained with chronic NBM stimulation (Tables 3 and 10) and therefore might only have been a transient effect.
Table 5: Effects of acute NBM stimulation over 24 hours on primary outcome measures.

<table>
<thead>
<tr>
<th>Patient</th>
<th>California Verbal Learning Test II</th>
<th>WMS-III Digit Span (raw scores)</th>
<th>D-KEFS Verbal Fluency Test (scaled scores)</th>
<th>Posner’s covert attention test</th>
<th>CANTAB Reaction Time Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immediate free recall (Z score)</td>
<td>Immediate free recall (Z score)</td>
<td>Long delay free recall (Z score)</td>
<td>Digits forward (range 0-16)</td>
<td>Digits backward (range 0-14)</td>
</tr>
<tr>
<td>A</td>
<td>OFF</td>
<td>-3.00</td>
<td>-3.00</td>
<td>28.00</td>
<td>31.00</td>
</tr>
<tr>
<td>B</td>
<td>ON</td>
<td>-2.00</td>
<td>-3.50</td>
<td>25.00</td>
<td>30.00</td>
</tr>
<tr>
<td>C</td>
<td>OFF</td>
<td>-1.00</td>
<td>-1.50</td>
<td>47.00</td>
<td>44.00</td>
</tr>
<tr>
<td>D</td>
<td>ON</td>
<td>-1.50</td>
<td>-1.50</td>
<td>35.00</td>
<td>30.00</td>
</tr>
<tr>
<td>E</td>
<td>OFF</td>
<td>-1.50</td>
<td>-1.00</td>
<td>26.00</td>
<td>29.00</td>
</tr>
<tr>
<td>F</td>
<td>ON</td>
<td>-2.50</td>
<td>-1.50</td>
<td>29.00</td>
<td>22.00</td>
</tr>
<tr>
<td></td>
<td>Group SD</td>
<td>0.74</td>
<td>0.88</td>
<td>8.29</td>
<td>7.41</td>
</tr>
</tbody>
</table>

Off = immediately prior to patient being set to blinded on-stimulation condition. ON = 24 hours after patient set to blinded on-stimulation condition. All scaled scores/Z scores are age-adjusted. Higher scores are better on all tests, except for measures of reaction time and reorienting time. Results highlighted in green indicate those where ON stimulation score was better than OFF stimulation score by at least one Z score/one T score/one scaled score/one raw score/50 ms increase. Results highlighted in red indicate those where ON stimulation score was worse than OFF stimulation score by at least one Z score/one T score/one scaled score/one raw score/50 ms decrease. * = lower scores better. 0 = scores closer to zero are better. NA = performance too poor; no normative data available to standardise score. NC = not completed.
### Table 10

Other secondary outcome measures at baseline, end of the blinded off-stimulation period and end of the blinded on-stimulation period

<table>
<thead>
<tr>
<th>Patient</th>
<th>WASI-II (scaled scores)</th>
<th>CANTAB Reaction Time Test</th>
<th>Neuropsychiatric Inventory (12 item version)</th>
<th>SCOPA-sleep</th>
<th>SCOPA-AUT</th>
<th>Non Motor Symptoms Questionnaire</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symbol Search</td>
<td>Digit Symbol Coding</td>
<td>Simple Movement Time (ms)</td>
<td>Choice Movement Time (ms)</td>
<td>Attention/ agression subscale (0-12)</td>
<td>Depression/ dysphoria subscale (0-12)</td>
</tr>
<tr>
<td>A</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>10</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>F</td>
<td>2</td>
<td>3</td>
<td>NC</td>
<td>NA</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Group Mean</td>
<td>1.37</td>
<td>1.05</td>
<td>0.84</td>
<td>1.12</td>
<td>1.37</td>
<td>0.84</td>
</tr>
</tbody>
</table>

B = Baseline, OFF = end of blind off-stimulation period; ON = end of blinded on-stimulation period. Lower scores are better for all measures except WASI-II symbol search and digit symbol coding where higher scores are better. All scaled scores are age-adjusted. Results highlighted in green indicate those where ON stimulation score was worse than both OFF stimulation and baseline score, by at least one scaled score/50 raw score/50 increase compared to both. Results highlighted in red indicate those where ON stimulation score was worse than both OFF stimulation and baseline scores, by at least one scaled score/50 raw score/50 decrease compared to both. Scores for the Non Motor Symptoms Questionnaire are expressed as number of patients in the group with active symptom during each condition. SCOPA-AUT = Scales for outcomes in Parkinson's disease - autonomic symptoms assessment scale. SCOPA-sleep = Scales for outcomes in Parkinson's disease - sleep assessment scale. NC = not completed. RDN = rapid eye movement. SD = standard deviation.
2.3 Discussion

In this double-blind, crossover trial of six patients with Parkinson’s disease dementia, low frequency (20Hz) stimulation of the nucleus basalis of Meynert appears to be safe and well tolerated. Our objective data provides preliminary indication that this intervention might be associated with subtle improvements in memory and in neuropsychiatric symptoms, particularly visual hallucinations. However, given the small sample size, the results should be interpreted cautiously and viewed as being predominantly hypothesis generating to guide further studies.

The improvements across the group in CVLT-II retention in memory scores were observed initially one week after surgery compared to baseline (+1.0 Z scores, p=0.026, Table 8), and improved further on-stimulation compared to baseline (+1.5 Z scores, p=0.042, Table 2, Table 3 and Fig. 11). The acute effects of NBM stimulation were also consistent with this, with four of six patients showing improved retention in memory scores after 24 hours on-stimulation (Table 9).

Interestingly, there was also a significant improvement across the group in CVLT-II immediate free recall (Trial 1) one week after surgery compared to baseline (p=0.041, Table 8), and although this was not obtained with chronic stimulation, a similar pattern was present following acute NBM stimulation with three of six patients showing improved scores after 24 hours (Table 9). Improvements in free recall were also observed with NBM DBS in both the single PDD patient reported by Freund and colleagues and in the six Alzheimer’s disease patients reported by Kuhn and colleagues (Freund et al., 2009; J Kuhn et al., 2015).

The NBM plays a key role in memory encoding (see Section 1.6.6 above and (Gratwicke et al., 2013)), and so the improvements in immediate recall and retention in memory scores suggest that modulation of NBM activity might have the potential to improve this. However, immediate recall and retention of information are also dependent upon alertness/attention mechanisms, which the NBM is also proposed to play a role in, so the results could equally
reflect improvements in these cognitive processes. However, performance on many of the
cognitive tests assessing attention remained unchanged and in some instances worsened with
stimulation, including response accuracy on Posner’s covert orienting of attention test (Table
3). Thus although the NBM and its cholinergic projections are strongly implicated in
attentional processes, the impact of 20Hz stimulation in the Ch4i subregion in which the
electrodes were located did not consistently improve the range of attentional deficits seen in
these patients. The clinical impact of the objective improvements on memory subtests with
NBM DBS also remains to be determined as we did not assess specific functional capacities
to determine if and how these might translate to daily activities for patients.

Outside our pre-specified primary outcome measures, the improvement across the group in
NPI total scores on-stimulation compared to off-stimulation (-5 points, p=0.027), driven by
marked improvements in visual hallucinations in Patients A and D on-stimulation compared
to both off-stimulation and baseline, is interesting. It seems unlikely that these improvements
were due to changes in medication, since Patient A had his levodopa increased by 300mg
only after the start of the second blinded condition (after his visual hallucinations had already
been supressed on-stimulation compared to baseline, Fig. 12), while Patient D (who
demonstrated the most powerful suppression of hallucinations with stimulation, Fig. 12) had
no changes made to his medications throughout the blinded trial period. The observation that
low frequency trial stimulation in Patient A in the immediate post-operative period appeared
to induce complex formed visual hallucinations in a time-locked manner also points towards
an effect of electrical stimulation of the NBM on visual hallucinations. The reasons why acute
low frequency NBM stimulation in that situation appeared to induce visual hallucinations,
whereas chronic low frequency NBM stimulation later on appeared to suppress those same
symptoms are currently unclear, but could reflect a superimposed post-operative stun effect
on NBM functioning (as is commonly observed in the immediate aftermath of STN DBS
implantation) which later resolves.
As discussed in subsections 1.3.1.10 and 1.3.1.11 above, the generation of visual hallucinations in PDD is poorly understood, but is strongly linked to impairments in alertness and attentional control (Bronnick et al., 2011; Gratwicke et al., 2015b; Meppelink et al., 2008). AChEIs significantly improve attentional deficits in PDD and consequently improve visual hallucinations (Emre et al., 2004; Wesnes et al., 2005). The possibility that hallucinations may be reduced by NBM DBS further supports the hypothesis that stimulation might modulate cholinergic transmission in a similar way to AChEIs. Visual hallucinations have a particularly negative impact on quality of life for both PDD patients and caregivers (Aarsland et al., 2000; Goetz and Stebbins, 1993; Schrag et al., 2006), and the reduction in these symptoms may explain why Patients A and D and their carers all independently reported subjective improvements in their quality of life during the on-stimulation period compared to both off-stimulation and baseline (EQ-VAS scores, Table 6, and caregiver distress scores, Table 5, respectively).

An unexpected finding was the improvement in UPDRS Part IV scores on-stimulation compared to both off-stimulation and baseline in Patients A, C and D, driven by reduction in their dyskinesias (Fig. 11). These results are likely explicable by current spread from NBM to the overlying GPi in addition to any possible microlesion effect of the surgery. However, conventional GPi DBS for dyskinesia control in Parkinson’s disease is generally delivered at high frequency (130Hz), and so the finding that low frequency (20Hz) stimulation directed towards the NBM also attenuates dyskinesias warrants further study. The possibility that improvement in dyskinesias could have compromised blinding of patients or assessing clinicians cannot be completely excluded.

We did not observe the dramatic changes across multiple cognitive domains as reported in the only other case report of low frequency NBM DBS in PDD (Freund et al., 2009). Reasons for this could include the fact that the previous patient suffered more severe dementia than the patients in our trial, leading to a more apparent step-change in cognitive ability with stimulation, or possibly a synergistic effect from the combined subthalamic nucleus and NBM
stimulation that he received. Nevertheless one similarity between our experience and that of Freund and colleagues is that in both studies the patient’s carers reported a (blinded) general subjective improvement in cognition when NBM stimulation was on, reflected by the fact that all our patients opted to continue with NBM DBS beyond trial end rather than switching over to pallidal stimulation for motor impairments.

It is difficult to compare our study directly to the two previously published double-blind trials of DBS in dementia since both were conducted in Alzheimer’s disease, and only one of them targeted the NBM (J Kuhn et al., 2015; Lozano et al., 2016). Considering the latter study by Kuhn and colleagues, differences in the effects seen in our study might reflect the fact that there is a larger cortical cholinergic deficit in PDD than in Alzheimer’s disease (Gratwicke et al., 2013), meaning that stimulation of the cholinergic NBM may produce a greater effect in the former group. However, differences in NBM active contact locations between the two trials (the spatial range of targets was greater in the Kuhn study, extending to Ch4p in some patients) might equally account for differences in observed effects.

This pilot trial has a number of key strengths. We used a prolonged blinded period, allowing us to report more accurately on the sustained effects of NBM DBS than other studies have been able to. We also included a washout period of two weeks between experimental conditions, such that any prolonged effects of NBM DBS which might have persisted after cessation of stimulation would have been less likely to confound the second condition. Additionally, our primary outcome measures included detailed assessment of individual cognitive subdomains, rather than purely measures of global cognitive function. Due to the overlapping effects of impairments in individual cognitive domains upon performance in others (see Section 1.3.3 above and also (Gratwicke et al., 2015b)), use of global measures makes it difficult to determine what the specific cognitive effects of NBM DBS might be. We also performed detailed assessments of neuropsychiatric symptoms, motor symptoms and patient and carer quality of life, which allowed us to assess a broad range of potential beneficial effects.
The major limitation to our study is the small sample size, which although appropriate for exploratory data collection, is not designed to detect significant differences between the blinded on- and off-stimulation periods. Given the multiple comparisons we have performed, our data must not be interpreted as evidence for efficacy, but can be used in the planning and design of formal trials to test the hypotheses generated by the current dataset. Another relevant issue is that patients continued AChEI therapy during the trial, and although there were no dose alterations, it still means that the potential physiological effects of NBM DBS on the cholinergic system, and any consequent clinical effects on symptoms, may be partially masked. However, given the relative safety of AChEI medications in comparison to DBS, we did not feel it was appropriate to expose patients to surgical risks who might gain sufficient cognitive benefits from the use of medications alone. In addition, we did not include a randomised non-operated control group of PDD patients, and so cannot objectively determine whether NBM DBS made a difference to the natural progression of cognitive deficits during the trial period. Likewise this also makes it difficult to determine whether the deterioration across the group in some outcome measures (Tables 3 and 4) was due to effects of surgery, stimulation or disease progression. Although we tried to minimise potential confounding influences of task familiarity and practice effects by using parallel test versions, this was not possible for all outcome measures. Inclusion of a non-operated group would also have been useful to control for this. Finally, we only investigated the effects of low frequency NBM stimulation at 20Hz, however the scientific rationale for this is limited (as discussed in Section 1.7.2. above), and stimulation at a different frequency might produce different results. Use of a titration schedule investigating cognitive responses to different frequencies would have shed light on this issue. However, to implement this would have required a much longer study with an even greater frequency of assessment visits, and it seems unlikely that patients would have been easily able to comply with this.

Looking forwards, one of the aims of this pilot study was to identify parameters which will inform sample size calculations for future trials of NBM DBS in PDD. In accordance with our
results, detailed measurements of the frequency and severity of visual hallucinations, as well as immediate free recall and retention in memory should comprise future primary outcome measures. Given the possible negative effect of NBM DBS on a measure of attentional shifting (Posner’s covert orienting of attention test) it would also be important to include more detailed assessment of different components of attention (as discussed in Section 1.2.2. above and in (Gratwicke et al., 2015b). As discussed earlier, secondary outcomes should include assessments of functional capacity to further determine the clinical impact that changes in neuropsychological test scores have on patient’s daily activities.

In conclusion, data from this pilot clinical trial have shown that NBM DBS is both technically feasible and safe in carefully selected PDD patients, and provide preliminary evidence that this therapy should be further evaluated regarding its effects on memory and neuropsychiatric symptoms, particularly visual hallucinations. The outcomes from the study serve to justify further exploration of NBM DBS as a therapy for PDD patients whose cognitive and behavioural symptoms are refractory to medical therapy. Future studies should explore the effects of stimulation amongst subregions within the NBM and formally test whether stimulation can reproducibly impact on cognitive and neuropsychiatric symptoms in PDD patients.
Chapter 3: Deep brain stimulation of the nucleus basalis of Meynert for dementia with Lewy bodies

3.1. Patients and methods

3.1.1. Experimental design

The experimental design for this trial was almost identical to our trial of NBM DBS for PDD (see Chapter 2): We conducted a randomised, double-blind, crossover trial of bilateral NBM DBS to compare deficits on a short battery of cognitive tests after six weeks of active stimulation and six weeks of sham stimulation. One major difference in terms of design was that in this trial NBM DBS was achieved without any requirement for the dorsal electrode contacts to be positioned in the overlying GPi. The reason for this is that GPi DBS is not used as a conventional treatment for motor symptoms in DLB. Therefore, the constraints imposed upon optimal targeting of the NBM in the PDD trial by the requirement to have dorsal contacts in the anteroposterior GPi did not apply here, allowing direct targeting of NBM only in the DLB patients.

A second major difference was that this trial was conducted across two sites, the primary site being UCL’s Institute of Neurology, and the secondary site being Newcastle University’s Clinical Ageing Research Unit. This collaboration with colleagues in Newcastle was established as the research team there have a large cohort of DLB patients under their care, which facilitated more rapid recruitment of participants. The study was sponsored by University College London, and all neurosurgery, LFP and MEG recordings were performed at the National Hospital for Neurology and Neurosurgery, London, UK. All other trial assessments were performed at each individual participants’ original recruiting site.
3.1.2. Patients

Participants were recruited from the populations of DLB patients referred to clinics at both sites. Patients were eligible for inclusion if they: met consensus criteria for the diagnosis of DLB (McKeith et al., 2005); were appropriate surgical candidates aside from the existence of dementia; were aged 50-80 years (reflective of the older average age of onset of DLB compared to PD/PDD (Aarsland et al., 2008)); were able to give informed consent; had a score between 2-12 on the Clinician Assessment of Fluctuations Scale (CAFS, see below). DLB patients have more prominent cognitive fluctuations than PDD patients of matched dementia severity (Bonanni et al., 2008), and given the scientific rationale for a potential role of the NBM in modulating arousal (see Section 1.6.8 above) we restricted recruitment of DLB subjects to those with significant cognitive fluctuations in order to evaluate the clinical effects of NBM DBS on this specific symptom; had an MMSE score between 21-27 (in order to select those with moderate dementia severity with retained capacity to give informed consent). Having found from the PDD trial that the MMSE score tends to underestimate cognitive impairment in LBDs (Hoops et al., 2009) we increased the MMSE score inclusion criteria to 21-27 in this trial to facilitate more rapid recruitment of eligible patients; had minimal atrophy on MRI brain scans (to ensure technical feasibility of electrode implantation); were living at home with a carer-informant; were willing to comply with the trial protocol (i.e. attend the National Hospital for Neurology and Neurosurgery in London for two weeks for DBS surgery and electrophysiological testing, and also necessary clinic visits in London/Newcastle); if taking either AChEIs or glutamate-antagonist medication (such as memantine) the patient had to have been taking a stable dosage for the last three months (with either an established suboptimal response to this medication or intolerance of higher dosage) and this was continued throughout the trial. Changes in AChEI/glutamate antagonist doses were not permitted during the trial period to avoid confounding any cognitive effects of NBM DBS.
Exclusion criteria were: diagnosis or suspicion of other cause for dementia; known abnormality on CT or MRI brain imaging considered likely to compromise compliance with the trial protocol; prior intra-cerebral surgical intervention.

3.1.3.Ethics and consent
The trial conformed to the Seoul revision of the Declaration of Helsinki (2008) and Good Clinical Practice guidelines, and was approved by the East of England Research Ethics Committee. All potential participants were assessed for their capacity to provide written informed consent by an experienced neuropsychologist independent from the trial team. This trial is registered with ClinicalTrials.gov, Identifier: NCT02263937

3.1.4.Randomisation and blinding
We randomly assigned participants to either the stimulation off-first group (sham stimulation for six weeks, followed by active stimulation for six weeks) or the stimulation on-first group (vice versa). We used computer-generated pairwise randomisation according to order of enrolment, so that equal numbers of patients were recruited to each group and the order of those receiving on- followed by off- stimulation and vice-versa was counterbalanced. The randomisation sequence was held by an unblinded clinician who was also responsible for programming the stimulation. Participants and assessing clinicians were blinded to the stimulation condition. The unblinded clinician spent the same time adjusting each patient’s stimulator at the start of both active and sham stimulation periods. The stimulation parameters were selected to avoid any immediate or long-lasting side effect that could be perceived by the patient or blinded clinicians, and thus have the potential to unblind them.

3.1.5.Baseline procedures
All enrolled participants underwent a baseline assessment (Fig. 13) which included a detailed neuropsychological battery, and motor, non-motor and psychiatric symptom scales (discussed in detail below). During baseline assessment the participants also completed two separate
measures of IQ; the two subtest form of the Wechsler Abbreviated Scale of Intelligence gives a brief estimate of current IQ by combining scores on a measure of crystallised verbal intelligence, the vocabulary subtest for measuring word knowledge, and a measure of fluid performance intelligence, the matrix reasoning subtest for measurement of visual information processing and abstract reasoning skills (Wechsler, 1999). In comparison, the Test of Premorbid Function (TOPF) estimates pre-morbid intelligence in English speaking patients with dementia (Wechsler, 2011). It is an untimed measure consisting of 70 words with atypical phonemic pronunciation, thereby testing the patient’s vocabulary rather than their ability to apply regular pronunciation rules. The pronunciation of previously learnt words is thought to be spared in cognitive decline, allowing it to be used as a proxy for estimating pre-morbid crystallised verbal intelligence. We switched from using the National Adult Reading Test (used to measure premorbid intelligence in the PDD trial) to the Test of Premorbid Function in the DLB trial because (1) the latter is a more recently developed reading test with updated population reference ranges, and (2) The TOPF is standardised with the Wechsler Adult Intelligence Scale Fourth Edition (WAIS-IV; Wechsler, 2008), which we used in this trial instead of the older WAIS-III (see below). Comparing the scores on these two IQ tests at baseline allowed us to estimate the degree of cognitive decline in each individual participant relative to their pre-morbid state.
3.1.6. **Neurosurgical procedure**

Within one month of completing baseline assessments participants underwent stereotactic implantation of bilateral DBS electrodes. All patients were operated under general anaesthesia (average time anaesthetised was 3-4 hours) using a Leksell stereotactic frame (Elekta Instrument AB, Stockholm), without microelectrode recording. Following attachment of the frame the NBM was visualised in each patient using pre-operative stereotactic axial and coronal proton-density MRI scans on which the pallidum, optic tract, anterior commissure and the adjacent NBM were visible (1.5T Siemens Espree, PDw Turbo Spin-echo; 1.0 x 1.0 x 2.0 mm; TR 4000ms TE 13ms). Target selection for placement of the deepest contact/s was at the level of maximal NBM diameter, the Ch4i subsector (around 5mm below the AC-PC plane). As in the PDD trial, Ch4i was chosen as it is both the largest subsector of NBM, giving highest probability of successful electrode placement, and has the most widespread cortical projections, thus potentially influencing more cortical areas (see Section 1.6.4. above.
and (Gratwicke et al., 2013)). Planning of the surgical trajectory in each individual patient was undertaken using commercially available software (Framelink, Medtronic, Minneapolis, USA). The NBM was visualised as the hyperintense signal lateral to the hypointense optic tract and medial to the hypointense anterior commissure (lateral extension) on proton density MRI. The entry point was chosen to ensure a trajectory which avoided both sulci and the ventricular system, while maximising the length of the trajectory within the visible NBM hyperintensity.

Further surgical details are identical to those presented in Section 2.1.6 above. All six DLB patients received model 3389 quadripolar DBS electrodes (Medtronic, Minneapolis, MN, USA). The accuracy of DBS lead contact location was confirmed immediately with postoperative stereotactic MRI (see Fig. 9 above): The distance between the intended MRI target and the actual position of the implanted electrode was calculated and surgery was not considered complete until acceptable placement of the electrodes had been image-confirmed. In all six cases (twelve electrode implants) actual position of the most ventral electrode contact was within 1mm of intended MRI target location.

Disposable extension connectors were attached to the end terminals of both subcutaneous leads, and these extensions were externalised though the scalp. Patient’s leads were externalised in this manner for a five-seven day period in order to allow trials of external stimulation to take place, as well as direct recording of LFPs from NBM and GPi (even though surgical trajectory in the DLB patients was planned without consideration of contact location in GPi, dorsal electrode contacts were still located in GPi in all patients due to its anatomical proximity to NBM). Lead externalisation also facilitated LFP and MEG recordings during this time (see Chapters 4 and 5). Prophylactic systemic antibiotics (Cefuroxime 1.5 g) were administered intra-operatively, and three more doses administered within the following 24 hours. Patients were reviewed in the recovery suite both by the operating neurosurgeon and a neurologist (myself) to confirm that there was no new
neurological deficit following surgery. Once awake the patients were transferred back to the main ward to recover.

Following recovery on the ward over the interval five-seven day period each patient underwent a second shorter operation (average time under general anaesthesia two hours). Again, details of the surgical procedure were identical to those presented in Section 2.1.6 above. Once awake the patients were transferred back to the main ward to recover over the following few days. Patients were only discharged home once they had fully recovered to their baseline pre-operative level of functioning (on average 3-4 days post-operative). DBS systems remained switched off during this period, and on discharge.

All adverse events were recorded immediately post-operatively, throughout the rest of the trial period, and beyond into the open label follow up period.

3.1.7. Externalised assessments

As mentioned above, following electrode implantation all patients had their electrodes externalised on the ward for five to seven days before IPG implantation. During this time three types of assessment were performed on each patient: trial stimulation, LFP recordings and combined MEG-LFP recordings. Details of these assessments are identical to those presented in Section 2.1.7 above.

3.1.8. Post-operative procedures

All participants underwent their first post-operative assessment one week after pulse generator implantation (Fig. 13). An abbreviated cognitive battery was performed, consisting of: Hopkins Verbal Learning Test - Revised (HVLT-R), the Wechsler Adult Intelligence Scale-IV (WAIS-IV) digit span, verbal fluency, Posner’s covert orienting of attention test and Simple and Choice Reaction Times (these tests are discussed in more detail both in Section 2.1.9 above and Section 3.1.9 below). These selected tests from the detailed
neuropsychological battery are amenable to repeated administration due to tests being either less susceptible to practice effects or parallel versions being available.

Three weeks later patients attended for 24 hours and were screened for the effects of stimulation in an open label manner, using the WAIS-IV digit span (a test of attention and executive function) as an objective measure. Only low frequency stimulation at 20Hz was used (for the reasons discussed above in Chapter 1). Only monopolar stimulation was used at a pulsewidth of 60 µs. Optimum stimulation voltages were determined as those producing highest digit spans with the lowest energy, without side-effects, and these were adopted for the blinded phase (Table 11). DBS was subsequently turned off for two weeks, and then patients were randomised into the simulation off-first or on-first group for the subsequent six weeks. Following this there was a two-week washout period (DBS off), then patients were switched over to the opposite condition for a further six weeks. All assessments performed at baseline were repeated at the end of each six-week period, except for measures of IQ (Fig. 13). The abbreviated cognitive battery was performed immediately prior to each change in DBS condition, as well as 24 hours afterwards. All trial assessments were performed by a clinician blinded to stimulation status (myself). Minor adjustments to concomitant licenced medications were permitted throughout the blinded trial period, aside from changes to the doses of any AChEIs or glutamate antagonists being taken.

After the blinded crossover phase patients were invited to routine follow-up with neuropsychological assessments and open-label NBM DBS adjustments at least every six months.

3.1.9. Primary outcome measures

The pre-specified primary outcomes were the differences in scores on each item of the abbreviated cognitive battery between the two blinded stimulation conditions (after six weeks on-stimulation vs after six weeks off-stimulation). These included:
• **HVLT-R:** A list of 12 nouns, with four items drawn from each of three semantic categories, is read to the patient, who then attempts to recall as many words as possible in any order. This task is repeated two more times, for a total of three learning trials. Learning efficiency is approximated by the sum of the scores of all three learning trials (total recall). Ability to access newly learned information is assessed by the number of words retained by the patient after a 20-minute interval (delayed recall) and the percentage of words retained from those previously learnt (retention). Finally, a list of 24 randomly ordered words is read, containing both the original 12 target words and 12 non-target words, and the patient must identify which were the target words – this provides a measure of retention in memory that is relatively free from the influence of effortful memory search and retrieval (recognition – discrimination index) (Brandt and Benedict, 2001). We switched from using the CVLT-II (used to measure verbal learning and memory in the PDD trial) to the HVLT-R in this trial primarily because the latter has six equivalent alternate forms (as opposed to only two alternate forms of the CVLT-II), making it less susceptible to practice effects due to item familiarity (Kuslansky et al., 2004). In addition the CVLT-II was developed with relatively healthy populations in mind, and therefore the length and complexity of its administration makes it challenging for moderately cognitively impaired patients. In contrast the HLVT-R was developed primarily for use with brain disordered populations and is brief and easier to administer, and is therefore well-tolerated in moderately demented patients (Brandt and Benedict, 2001).

• **Digit span:** in this subtest from the WAIS-IV patients are first required to listen to and verbally repeat number sequences of increasing length (digits forwards), a test of auditory attention span. Secondly patients are asked to listen to and verbally reverse number sequences of increasing length presented (digits backwards), a test of manipulation in working memory (executive function). In the WAIS-IV version of the digit span test there is also a third section wherein patients listen to random sequences of numbers and letters
of increasing length and are asked to repeat them back verbally in numerical followed by alphabetical order (digit sequencing), a more demanding test of manipulation in working memory (executive function) (Wechsler, 2008). We switched from using the WAIS III (used in the PDD trial) to using the subtests of the WAIS IV in this trial as this more recent version uses up to date population reference ranges for standardisation of scores.

- **Verbal fluency**: the verbal fluency subtest from the Delis-Kaplan Executive Function System measures the ability to generate words meeting phonemic or semantic criteria, thereby testing executive retrieval of verbal information (Delis et al., 2001).

- **Posner’s covert orienting of attention test**: measures reaction speed to a lateralised visual target, preceded by a cue prompting to either the correct or incorrect side of upcoming target presentation. This measures speed of orienting of attention (Posner, 1980). We used a customised computerised version of this test, described in detail in Chapter 4. The proportion of correct responses is also measured as a percentage of total targets presented.

- **Simple and Choice Reaction Times**: these tests measure the speed of psychomotor responses to visual targets where the stimulus is either predictable (simple reaction time) or unpredictable (choice reaction time), providing surrogate measures of alertness. We used the computerised version of this test included in the Cambridge Neuropsychological Test Assessment Battery (CANTAB).

- **Clinician Assessment of Fluctuations Scale**: this scale consists of a series of screening questions, put to an informant, to assess for subjective episodes of fluctuating confusion and impaired consciousness occurring in the patient during the preceding month (Walker, 2000). If present, the frequency and duration of episodes are both rated on a scale of 0-4, and these are multiplied together to give an overall severity score from 0-12 (0 = no fluctuating confusion, 12 = severe fluctuating confusion, 16 would indicate a state of continuous confusion which, be definition, would denote no fluctuation).
Higher scores on all these tests indicate better performance, aside from measures of reaction speed (Posner’s covert orienting of attention test and Simple and Choice Reaction Times) and the Clinician Assessment of Fluctuations Scale, where lower scores are better.

3.1.10. Secondary outcome measures

Secondary outcomes included changes in the following neuropsychological tests (which together with the abbreviated battery above comprised the detailed neuropsychological battery). Higher scores are better on all these neuropsychological tests.

- **MMSE**: a brief general screening instrument for cognitive impairment (Folstein et al., 1975). Although it is the most commonly used screening instrument for dementia worldwide, its dependence on age and educational level hinders the use of a rigid cut-off score (Crum et al., 1993).

- **Mattis Dementia Rating Scale 2**: a test of global cognitive function (Jurica et al., 2001), shown to sensitively measure the degree of cognitive deficits in patients with PD/PDD (Kulisevsky and Pagonabarraga, 2009).

- **Short recognition memory test for faces**: in this test of recognition memory for faces patients are presented with a series of 25 photographs of faces at the rate of one face every 3 s, and for each face the subject is required to judge the presented stimulus as ‘pleasant’ or ‘unpleasant’ to ensure that they are attending to the stimulus items. The patient is then presented with a series of 25 pairs of faces and the task is to identify which of the two faces came from the target list (Warrington, 1996).

- **WAIS-IV arithmetic and letter-number sequencing subtests**: in the former patients are asked mental arithmetic problems of increasing difficulty, a test of manipulation in working memory (executive function). In the latter patients listen to a series of numbers and letters in random order and must recall them in numerical followed by alphabetical order, a test of auditory attention span and manipulation in working memory (executive function).
function). The age-adjusted scaled scores for both tests are combined with that for digit span to give an overall working memory index (Wechsler, 2008).

- **Trail making test**: this subtest from the Delis-Kaplan Executive Function System is an expanded version of the original trail making test and includes five conditions: visual search, number sequencing, letter sequencing, number-letter switching and motor speed. In each condition patients must draw to connect numbers and/or letters on a page in a specific order as fast as possible. Number sequencing and letter sequencing scores are measures of psychomotor processing speed, while number-letter sequencing score is a measure of behavioural regulation/set shifting (executive function). The visual search and motor speed scores provide indexes of deficits in visual attention and motor control respectively, and can be used to control for the confounding effects of such deficits on the other task conditions. (Delis et al., 2001).

- **Colour-word interference test**: this subtest from the Delis-Kaplan Executive Function System is an expanded version of the original Stroop test and includes four conditions: Colour naming and word reading act as screening tests which measure basic naming of colours and reading of words respectively (both key component skills necessary to attempt the higher tasks). The Inhibition condition is identical to the original Stroop test wherein the patient must inhibit reading words in order to name the dissonant ink colours in which those words are printed. This tests the patient’s ability to inhibit an overlearned prepotent response. Finally, in the inhibition/switching condition the patient is asked to switch back and forth between naming the dissonant ink colours and reading the words, a test of both response inhibition and cognitive flexibility (rule switching) (Delis et al., 2001).

- **WAIS-IV symbol search and digit-symbol coding subtests**: in the former patients are presented with a series of target symbols and must identify whether these are present in corresponding rows of symbols under a strict time limit. In the latter a key containing nine digit-symbol pairs is presented, followed by a table of digits. Under each digit in the
table the patient must write down the corresponding symbol (according to the key) as fast as possible, within a strict time limit. Both are tests of executive control of attention/processing speed, and from their combined age-adjusted scaled scores a processing speed index is derived (Wechsler, 2008).

- **Florida Apraxia Screening Test**: a test of ideational praxis wherein patients must produce 15 gestures to command. A score of nine or below on the FAST has previously been shown to demonstrate good sensitivity for a diagnosis of apraxia (Rothi and Heilman, 1984).

- **Sustained Attention to Response Test (SART)**: In this computerised test the digits “1” through “9” are presented randomly in white colour against a black screen, in the lower left visual field. Patients are instructed to respond with their dominant hand (press a button) to all digits (Go trials) except “5”, at which they should inhibit their response (NoGo trial). On each trial the digits vary randomly in size and font to ensure that patients process the identity of the digits rather than focussing on specific perceptual features. Response speed and errors are recorded. This is a long task with repetitive trials in a non-arousing visual environment, and thus can be used to measure the patient’s level of sustained attention/vigilance over time (Manly et al., 1999; O’Connell et al., 2009). Attentional lapses are manifest in both increased response time variability (Weissman et al., 2006) and errors (O’Connell et al., 2009). We used a customised computerised version of this test, described in detail in Chapter 4.

- **The Benton Judgement of Line Orientation Test**: In this test patients are asked to match two angled lines on a page to a set of 11 lines which are arranged in a semicircle and separated 18 degrees from one another. The test measures accuracy of angular judgement, which is a proxy for visuospatial ability. Feedback is provided during five practice trials prior to initiating the test items. Compared to the practice trials, lines in the test items have part of the line erased to increase task difficulty. There are 30 trials, each with two
lines to identify, and responses are scored as being correct only when both lines are identified correctly (Benton et al., 1978).

Additional secondary outcome measures included changes on the following validated scales. Higher scores indicate greater functional impairment on all scales except for the Quality of Life–AD scale.

- **MDS Unified Parkinson’s Disease Rating Scale (MDS-UPDRS):** a comprehensive assessment of the severity of all motor and non-motor symptoms in PD, comprising sixty-five items (score range 0-260) (Movement Disorder Society Task Force on Rating Scales for Parkinson’s Disease, 2003). We evaluated motor symptoms using Part III (the motor subscale), both in the absence of dopaminergic medications (OFF state) and one hour after administration of dopaminergic medications (ON state).

- **Freezing of Gait (FOG) Questionnaire:** this six-item scale (range 0–24) consists of four items that assess FOG frequency and duration, and two that assess the impact of gait difficulties in general (Giladi et al., 2000). It was found that the FOG questionnaire has high test-retest reliability, internal consistency and moderately high correlations with MDS-UPDRS motor and ADL scores (Giladi et al., 2009).

- **SCOPA-Autonomic symptoms scale:** a self-reported questionnaire consisting of 23 questions assessing symptoms of autonomic dysfunction in PD patients (score range 0-69) (Visser et al., 2004).

- **Starkstein Apathy Scale:** a clinician-administered 14 item questionnaire assessing apathy symptoms in PD patients (score range 0-42) (Starkstein et al., 1992).

- **The Neuropsychiatric Inventory:** a clinician-administered caregiver-reported questionnaire assessing 12 psychiatric symptoms/behavioural disturbances which may be present in dementia patients, capturing frequency and severity of each and distress caused to the caregiver (Cummings et al., 1994).
- **Blessed dementia scale**: a clinician-administered caregiver-reported questionnaire assessing presence or absence of 22 functional impairments and behavioural disturbances due to dementia (score range 0-28) (Blessed et al., 1968).

- **Hamilton Depression Scale**: a clinician-assessed 21 item inventory measuring severity of depressive symptoms (score range 0-68) (Hamilton, 1960).

- **Hamilton Anxiety Scale**: a clinician-assessed 14 item inventory measuring severity of anxiety symptoms (score range 0-56) (Hamilton, 1959).

- **The North East Visual Hallucinations Interview**: a clinician-administered semi-structured interview for use with the patient, which helps to identify the presence of visual hallucinations. The questionnaire also assesses the frequency of any hallucinations, and quantifies emotions and behaviours associated with them (Mosimann et al., 2008).

- **Clinical Global Impression of Change Scale**: a brief, stand-alone assessment of the clinician's view of the patient's global functioning prior to and after an intervention. The patient's history, psychosocial circumstances, symptoms, behaviour, and the impact of the symptoms on the patient's ability to function are taken into account when rating. The scale comprises two companion one-item measures evaluating (a) severity of psychopathology from 1-7 and (b) change/improvement from the initiation of treatment from 1-7 (Busner and Targum, 2007).

- **Quality of Life – Alzheimer’s Disease Scale**: This measure comprises two clinician-administered scales, one for use with the patient and the other for use with the caregiver. Both scales independently rate the perceived quality of life of the patient across a number of domains (including physical, emotional, social, financial and occupational status) to give an overall index measure of their quality of life at that point in time (Logsdon et al., 2002).

- **Mayo Fluctuations Composite Scale**: a clinician-administered measure for use with the caregiver which assesses the presence of four composite features of cognitive fluctuation in DLB patients (daytime drowsiness and lethargy, daytime sleep of 2 or more hours,
staring into space for long periods, and episodes when the patient’s flow of ideas seem disorganised) (Ferman et al., 2004).

- **Carer Strain Index (Zarit burden interview):** a 22-item self-reported questionnaire completed by the carer, which measures their level of burden at home due the functional and behavioural impairments of the patient with dementia. The items are worded subjectively, focusing on the affective response of the caregiver. Each question is scored on a 5 point Likert scale ranging from ‘never’ to ‘nearly always’ present. Total scores range from 0 (low burden) to 88 (high burden) (Bédard et al., 2001).

Throughout the blinded trial period and subsequent open-label follow up period adverse events were systematically recorded.

### 3.1.11. Statistical analysis

The sample size of this study was based on practical considerations. We planned to recruit six patients on the basis of (1) an estimate of the number of eligible patients under active follow up in our clinics, and (2) the fact that the safety of NBM DBS implantation in this vulnerable patient group is currently unknown, necessitating a cautious approach to recruitment. This is therefore a pilot trial with a small sample size and even the primary outcomes are principally exploratory in nature. To maximise transparency of the results, all individual outcome data are presented. Statistical comparisons are performed simply to highlight the most consistent differences at group level according to on- vs off-stimulation, and are not corrected for multiple comparisons. Two-tailed Wilcoxon signed ranks tests were used in paired observations when the distribution of differences was symmetrical, and two-tailed related-samples Sign tests otherwise. For comparisons according to randomisation sequence two-tailed Mann-Whitney U tests were performed. All data were analysed using Statistical Package for the Social Sciences version 22.0 software.
3.2. Results

Between 20th May, 2014, and 8th February, 2016, we assessed 15 patients and enrolled six into the study (five males, mean age 71.33 years (SD 3.67)). Table 11 summarises their clinical characteristics and also details the stereotactic coordinates of their active NBM contacts and the stimulation parameters used for the blinded period. Fig. 14 shows Schaltenbrand atlas locations of active NBM contacts in all patients (Schaltenbrand and Wahren, 1977), demonstrating that the most ventral active contact was successfully placed in the Ch4i subsector of NBM in each patient. In all patients with a second active contact per hemisphere (Patients A, B, C and F), this was located on the NBM/GPi border. Surgery was well tolerated and all patients were ambulatory within 24 hours and fully oriented within 48 hours. All six patients completed the blinded crossover phase and were included in analysis.

Only two patients had medication changes during the blinded trial period: Patient D had his dose of immediate-release levodopa increased from 125 mg three times daily at baseline to 187.5 mg four times daily in the initial post-operative period due to worsening limb rigidity (an increase in total LED to 843.75 mg). During condition 1 his dose of immediate-release levodopa was subsequently reduced to 187.5 mg three times daily (a reduction in total LED to 656.25 mg). Following this he had no further changes made to his medications throughout the rest of conditions 1 and 2. Patient E had his immediate-release levodopa increased from 62.5 mg once daily at baseline to 62.5 mg twice daily (an increase in total LED to 125 mg) three weeks after surgery due to worsening bradykinesia and rigidity. He remained on this dose throughout both blinded conditions.
<table>
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<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at surgery (yrs)</th>
<th>Disease duration (yrs)</th>
<th>Hoehn &amp; Yahr stage</th>
<th>Visual hallucinations?</th>
<th>TASP estimated premorbid IQ (IQ 95%)</th>
<th>MMSE</th>
<th>Mattis Dementia Rating Scale 2 (Base, Scaled)</th>
<th>Concomitant medications at enrolment (total daily doses)</th>
<th>Daily levodopa equivalent dose (LED, mg/day)</th>
<th>Daily total cholinesterase inhibitor dose (mg/day)</th>
<th>Active contacts (monopolar)</th>
<th>Stereotactic coordinates (x,y,z)</th>
<th>Stimulation parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M</td>
<td>65</td>
<td>5</td>
<td>3</td>
<td>No</td>
<td>12</td>
<td>52</td>
<td>83 (78-89)</td>
<td>Hypertension, appendicectomy, osteoarthritis, Nasus hemis</td>
<td>187.5</td>
<td>10</td>
<td>1, 9</td>
<td>-20.4, 5.1, -5.3</td>
<td>18.5, 8.8, -5.4</td>
</tr>
<tr>
<td>B</td>
<td>M</td>
<td>73</td>
<td>4</td>
<td>2</td>
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<td>9</td>
<td>102</td>
<td>90 (85-96)</td>
<td>Coronary artery disease, cataracts</td>
<td>375</td>
<td>9</td>
<td>0, 1, 8, 9</td>
<td>-21.4, 9.2, -4.5</td>
<td>20.3, 8.8, -4.2</td>
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<td>C</td>
<td>F</td>
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<td>2</td>
<td>101</td>
<td>90 (85-96)</td>
<td>Anaest, vulvodynia</td>
<td>1000</td>
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<td>69</td>
<td>5</td>
<td>3</td>
<td>Yes</td>
<td>12</td>
<td>91</td>
<td>69 (85-76)</td>
<td>Sciatica</td>
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<td>21.4, 11.5, -7.3</td>
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<td>Yes</td>
<td>12</td>
<td>NC</td>
<td>65 (61-72)</td>
<td>Age-related macular degeneration, coeliac disease, chronic obstructive pulmonary disease</td>
<td>62.5</td>
<td>10</td>
<td>0, 1, 8, 9</td>
<td>-18.8, 7.4, -4.6</td>
<td>18.0, 8.8, -4.9</td>
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<tr>
<td>F</td>
<td>M</td>
<td>73</td>
<td>4</td>
<td>2</td>
<td>No</td>
<td>12</td>
<td>96</td>
<td>90 (85-96)</td>
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<td>0</td>
<td>10</td>
<td>0.8</td>
<td>-18.9, 4.2, -8.0</td>
<td>19.7, 5.7, -7.3</td>
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<tr>
<td>Group Mean</td>
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<td>2.50</td>
<td>9.83</td>
<td>96.40</td>
<td>81.17</td>
<td>22.83</td>
<td>113.33</td>
<td>148.96</td>
<td>10.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group SD</td>
<td></td>
<td>3.67</td>
<td>2.48</td>
<td>0.55</td>
<td>4.02</td>
<td>5.08</td>
<td>11.37</td>
<td>1.17</td>
<td>17.53</td>
<td>365.73</td>
<td>1.02</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Disease duration was estimated by examining the patient’s medical notes and collateral history from the caregiver to determine the time at which cognitive decline began to interfere with normal occupational or social function. The Mattis Dementia Rating Scale 2 Scaled score is corrected for age but not education. Patient F had very poor vision due to severe macular degeneration which impaired his completion of all visuoperceptual tasks, therefore WASI, MMSE and Mattis DRS-2 scores likely underestimated his cognitive ability. He is also alexic and so could not complete the TOPF. CR = controlled release preparation. SD = standard deviation. † LED calculation as per protocol in Tomlinson et al., 2010. ⊙ Mean stereotactic co-ordinates of the active contacts in left and right hemispheres respectively, with reference to the mid-commissural point of the AC-PC plane.
Figure 14a: Coronal sections from Schaltenbrand atlas taken 11.5mm, 7.5mm and 5.5mm anterior to the midcommissural point to indicate location of active DBS contacts during the blinded phase (DLB patients A-F). The optic tract (II) and the lateral extension of the anterior commissure (Cm.a) are coloured light blue. The Nucleus Basalis of Meynert (B) lies between these two structures (yellow) and inferior to the globus pallidus (Pars medialis interna, medialis externa and lateralis: P.mi, P.me and P.l). Figure adapted from Schaltenbrand and colleagues (plates 25–26) by permission of Thieme.
Figure 14b: Location of active DBS contacts for each patient on their individual MRI images (Patients A through F). The locations of the most ventral active contacts (in NBM) are shown on representative coronal and axial post-operative MRI images for each patient, and correspond to those shown in Figure 14a above.
Trial stimulation through the externalised electrodes in the immediate post-operative period did not produce any subjective or objective side effects in any of the patients. Additionally, none of the patients experienced any perceptible side effects from low frequency NBM DBS at parameter screening, and again no objective side effects were observed by the trial team. In light of this it seems likely that patient and carer blinding was adequately maintained throughout the blinded trial period.

Table 12 summarises the pre-specified primary outcome data at group level, while individual results for all patients are presented in Tables 13 and 20, and Figure 11. None of the blinded on- vs off- primary outcome comparisons achieved conventional threshold for statistical significance. The most consistent finding here was that four of six patients (Patients A, B, C and D) showed an improvement in simple reaction time on-stimulation compared to both off-stimulation and baseline (Table 13), with no between group differences according to randomisation order (p= 0.7 and p=0.7 for scores off- and on-stimulation respectively).

However, although comparisons of simple reaction time scores at group level showed median improvements on-stimulation compared to both off-stimulation and baseline these failed to reach significance (64.5 s, p=0.600, and 10.5 s, p=0.753, respectively). Interestingly, however, three of these four patients (Patients A, B and C) demonstrated worsening of their corresponding simple movement times on-stimulation compared to both off-stimulation and baseline (Table 20), again with no group differences according to randomisation sequence (p=1.0 for scores both off- and on-stimulation). Comparisons at group level of simple movement times showed a significant median worsening of 745.5 s (p=0.028) on-stimulation compared to baseline, and a non-significant median worsening of 629.5 s (p=0.249) on-stimulation compared to off-stimulation. This dissociation between simple reaction and simple movement times on-stimulation suggests that the improvements seen in simple reaction speeds with chronic NBM stimulation were not due to improvements in motor symptoms, and thus may represent improvements in central (cognitive) processing speed,
which is a surrogate measure of alertness. However, the absence of improvements on-stimulation on other measures of attention and recall makes this less likely.

The group level results for both simple reaction time and simple movement time are skewed against the on-stimulation condition due to the fact that Patient D was so somnolent both at baseline and during the off-stimulation condition (due to his marked cognitive fluctuation) that his performance was too poor to record a result. The only time when he was alert enough to record meaningful results was during the on-stimulation condition, and those results, though representing a significant qualitative improvement in performance for him, were markedly impaired compared to the rest of the group. However, even when his results are removed completely from the analysis the overall pattern remains unchanged, with group level results showing median improvements in simple reaction time on-stimulation compared to both off-stimulation and baseline (128.5 s, p=0.138, and 66 s, p=0.686 respectively), and median worsening in simple movement times on-stimulation compared to both off-stimulation and baseline (387 s, p=0.5, and 503 s, p=0.043 respectively).
Table 12: Group level primary and selected secondary outcome measures

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group mean</th>
<th>(standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Outcome Measures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hopkins Verbal Learning Test Revised (T scores)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Recall</td>
<td>29.50</td>
<td>(7.61)</td>
</tr>
<tr>
<td>Delayed recall</td>
<td>30.83</td>
<td>(7.08)</td>
</tr>
<tr>
<td>Retention</td>
<td>38.17</td>
<td>(11.51)</td>
</tr>
<tr>
<td>Recognition discrimination Index</td>
<td>36.00</td>
<td>(12.93)</td>
</tr>
<tr>
<td>WAIS-IV digit span (raw scores)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digits forwards (range 0-16)</td>
<td>8.40</td>
<td>(1.67)</td>
</tr>
<tr>
<td>Digits backwards (range 0-14)</td>
<td>6.40</td>
<td>(1.52)</td>
</tr>
<tr>
<td>Digits Sequencing (range 0-16)</td>
<td>5.60</td>
<td>(1.52)</td>
</tr>
<tr>
<td>D-KEFS Verbal Fluency Test (scaled scores)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Letter Fluency</td>
<td>8.00</td>
<td>(2.19)</td>
</tr>
<tr>
<td>Category Fluency</td>
<td>4.17</td>
<td>(2.64)</td>
</tr>
<tr>
<td>Category Switching Total Correct</td>
<td>3.00</td>
<td>(2.28)</td>
</tr>
<tr>
<td>Category Switching Total Accuracy</td>
<td>3.00</td>
<td>(3.16)</td>
</tr>
<tr>
<td>Posner's covert attention test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total accuracy (0-100%)</td>
<td>80.00</td>
<td>(22.43)</td>
</tr>
<tr>
<td>Posner effect - reorienting time (ms)Δ</td>
<td>5.76</td>
<td>(48.76)</td>
</tr>
<tr>
<td>CANTAB Reaction Time Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple Reaction Time (ms)</td>
<td>511.14</td>
<td>(225.03)</td>
</tr>
<tr>
<td>Choice Reaction Time (ms)</td>
<td>439.55</td>
<td>(79.10)</td>
</tr>
<tr>
<td>Clinician Assessment of Fluctuation Score</td>
<td>9.50</td>
<td>(4.81)</td>
</tr>
<tr>
<td><strong>Selected Secondary Outcome Measures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mini-Mental State Examination (MMSE)</td>
<td>22.83</td>
<td>(1.17)</td>
</tr>
<tr>
<td>Mattis Dementia Rating Scale 2 (raw score)</td>
<td>113.33</td>
<td>(17.53)</td>
</tr>
<tr>
<td>Neuropsychiatric Inventory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total score (0-144)</td>
<td>18.67</td>
<td>(12.08)</td>
</tr>
<tr>
<td>Caregiver distress score (0-60)</td>
<td>10.00</td>
<td>(5.97)</td>
</tr>
<tr>
<td>Hallucinations subscale (0-12)</td>
<td>2.83</td>
<td>(4.75)</td>
</tr>
</tbody>
</table>

All scaled scores/T scores are age-adjusted. Posner task total accuracy is % of presented targets correctly responded to. Higher scores are better on all tests, except for measures of reaction time, the Clinicians Assessment of Fluctuations Scale scores and Neuropsychiatric Inventory subscales, in which lower scores are better. Δ = scores closer to zero are better for Posner reorienting time.
Table 13: Primary outcome measures at baseline, end of the blinded off-stimulation period and end of the blinded on-stimulation period

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hopkins Verbal Learning Test - Revised (T scores)</th>
<th>WAIS-IV digit span (raw scores)</th>
<th>D-KEFS Verbal Fluency Test (scaled scores)</th>
<th>Power's covert attention test</th>
<th>CANTAB Reaction Time Test</th>
<th>CAFS (range 0-14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total recall</td>
<td>Delayed recall</td>
<td>Retention</td>
<td>Recognition</td>
<td>Category</td>
<td>Acceptance</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>discrimination index</td>
<td>discrimination index</td>
<td>discrimination index</td>
<td>discrimination index</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>OFF</td>
<td>B</td>
<td>OFF</td>
<td>B</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>33</td>
<td>28</td>
<td>40</td>
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<td>36</td>
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<td>18</td>
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<td>34</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>21</td>
<td>25</td>
<td>30</td>
<td>31</td>
<td>34</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>20</td>
<td>20</td>
<td>25</td>
<td>25</td>
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<tr>
<td>E</td>
<td></td>
<td>38</td>
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<td>33</td>
<td>27</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Group</td>
<td>Mean</td>
<td>38.00</td>
<td>31.50</td>
<td>5.06</td>
<td>9.83</td>
<td>9.83</td>
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<tr>
<td></td>
<td>Mean SD</td>
<td>33.33</td>
<td>32.50</td>
<td>2.83</td>
<td>4.17</td>
<td>4.17</td>
</tr>
<tr>
<td></td>
<td>CAFS</td>
<td>9.83</td>
<td>9.83</td>
<td>2.83</td>
<td>4.17</td>
<td>4.17</td>
</tr>
</tbody>
</table>

*Higher scores are better on all tests, except for measures of reaction time and reorienting time. Results highlighted in green indicate those where ON stimulation score was better than both OFF stimulation and baseline scores, by at least one T score/one raw scaled score/10% increase in raw scores compared to both. Results highlighted in red indicate those where (ON stimulation score was worse than both OFF stimulation and baseline scores, by at least one T score/one raw scaled score/10% decrease in raw scores compared to both. *"= lower scores better = scores closer to zero are better. NA = performance too poor, no normative data available to standardize score. SD = standard deviation. CAFS = Clinician Assessment of Fluctuations Scale score. WAIS-IV = Wechsler Adult Intelligence Scale-IV.
Figure 15: Effects of nucleus basalis of Meynert DBS on selected primary and secondary outcome measures. All graphs show individual results for all patients (dotted lines) and superimposed group mean results (solid lines) as per key. Please note that in both Graphs A and B (left side of figure) higher scores are better, whereas in Graphs C and D (right side of figure) lower scores are better. In Graph A all T-scores are age-adjusted. CAFS = Clinician Assessment of Fluctuations Scale. HVLT-R = Hopkins Verbal Learning Test – Revised. MMSE = mini-mental state examination.
Tables 12, 14 and 20 present secondary outcome cognitive measures (and MMSE scores also shown in Figure 11). There was no consistent advantage on-stimulation vs off-stimulation on global cognitive measures, or on focal measures of working memory, psychomotor speed, facial recognition memory, task-switching or praxis. Four of six patients (Patients B, C, D and E) showed an improvement in commission error rate on the SART task on-stimulation compared to both off-stimulation and baseline (Table 14), with no between group differences according to randomisation sequence (p= 1.0 for scores both off- and on-stimulation respectively). Comparison at group level of commission error rates showed median improvements on-stimulation compared to both off-stimulation and baseline (-4 %, p=0.104, and -20 %, p=0.08 respectively).

Tables 12 and 15 present secondary outcome psychiatric measures. Although none of the group level comparisons reached statistical significance there was a trend for improvement in Neuropsychiatric Inventory total scores on-stimulation compared to both off-stimulation and baseline (median improvements of -12.5 points, p=0.066, and -8.5 points, p=0.080 respectively, Figure 11), not due to between group differences due to randomisation sequence (p= 1.0 for scores both off- and on-stimulation respectively). This was mirrored by a similar trend for improvement in Neuropsychiatric Inventory caregiver distress scores on-stimulation compared to both off-stimulation and baseline (median improvements of -6.5 points, p=0.068, and -6.5, p=0.144, respectively), again with no between group differences according to randomisation sequence (p= 0.7 for scores both off- and on-stimulation respectively).

In a similar manner to that seen in the PDD trial, the trend for improvement in Neuropsychiatric Inventory total scores with NBM DBS on was partially driven by a reduction in hallucinations subscale scores in two patients, in this case Patients D and E (Table 15). At baseline Patient D reported daily illusions and complex formed visual hallucinations several times a week on the North-East Visual Hallucinations Interview (NEVHI). Following surgery and six weeks of blinded off-stimulation he did not report a major change in his hallucination frequency, but reported fewer illusions on the NEVHI.
Following a subsequent six weeks on-stimulation he reported no visual hallucinations in the last month on the NEVHI. At baseline Patient E reported multiple daily complex formed visual hallucinations of people in his house, which were a major cause of distress. In the immediate three weeks post-operative (NBM DBS off) both he and his partner reported a major reduction in the frequency of his visual hallucinations to only once per week. However, thereafter his visual hallucinations gradually increased in frequency back to occurring daily, with no qualitative difference reported on the NEVHI between the blinded on- and off-stimulation conditions.

There was no consistent advantage on-stimulation vs off-stimulation on measures of anxiety, depression or apathy (Table 15).
### Table 14: Secondary outcome measures (cognitive) at baseline, end of the blinded off-stimulation period and end of the blinded on-stimulation period

<table>
<thead>
<tr>
<th>Patient</th>
<th>MMSE (range 0-30)</th>
<th>Mattis Dementia Rating Scale 2 (total raw score)</th>
<th>WAIS-IV (composite scores)</th>
<th>Short Recognition Memory for Faces (range 0-25)</th>
<th>D-KEFS Trail Making Test (scaled scores)</th>
<th>Sustained Attention to Response Task</th>
<th>Florida Apraxia Screening Test (range 0-15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Working memory index</td>
<td>Processing speed index</td>
<td>Visual scanning</td>
<td>number sequencing</td>
<td>letter sequencing</td>
<td>number-letter switching</td>
</tr>
<tr>
<td>A</td>
<td>23 26 28 25 126 130 123</td>
<td>77 77 77</td>
<td>65 56 50</td>
<td>17 22 20</td>
<td>1 1 6</td>
<td>4 1 2</td>
<td>1 2 1</td>
</tr>
<tr>
<td>B</td>
<td>23 27 24 121 138 133</td>
<td>86 83 77</td>
<td>56 50 53</td>
<td>18 23 21</td>
<td>1 1 1</td>
<td>1 1 1</td>
<td>1 1 1 NA</td>
</tr>
<tr>
<td>C</td>
<td>21 25 24 123 126 121</td>
<td>83 83 60</td>
<td>56 50 NC</td>
<td>22 21 19</td>
<td>1 1 1</td>
<td>1 1 1</td>
<td>1 1 1</td>
</tr>
<tr>
<td>D</td>
<td>22 18 19 103 84 106</td>
<td>69 NA NA</td>
<td>50 NA NA</td>
<td>11 16 22</td>
<td>1</td>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td>E</td>
<td>24 25 23 82 76 85</td>
<td>95 89 92</td>
<td>NC</td>
<td>NC</td>
<td>11 15 10</td>
<td>NC</td>
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<tr>
<td>F</td>
<td>24 23 23 125 126 131</td>
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<td>NC 79 84</td>
<td>14 19 20</td>
<td>NC 9 5</td>
<td>NC 15 12</td>
<td>NC 12 12</td>
</tr>
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</table>

<table>
<thead>
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<th>Group SD</th>
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<th></th>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>22.83</td>
<td>24.13</td>
<td>23.00</td>
<td>113.33</td>
<td>113.33</td>
<td>116.50</td>
<td>82.00</td>
<td>88.80</td>
<td>79.60</td>
<td>56.75</td>
<td>58.75</td>
<td>62.13</td>
<td>15.50</td>
<td>19.33</td>
<td>18.67</td>
<td>1.00</td>
<td>3.00</td>
<td>2.80</td>
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</table>

B = Baseline, OFF = end of blinded off-stimulation period. ON = end of blinded on-stimulation period. All scaled scores are age-adjusted. Higher scores are better on all tests. Results highlighted in green indicate those where ON stimulation score was better than both OFF stimulation and baseline scores, by at least one scaled score/10% raw score increase compared to both. Results highlighted in red indicate those where ON stimulation score was worse than both OFF stimulation and baseline scores, by at least one scaled score/10% raw score decrease compared to both. Patient E was not able to complete the processing speed index tasks, Trail Making Test, SART or Stroop Test at any point due to his poor vision rendering him alesic. NA = performance too poor to attempt test properly. NC = not completed. SD = standard deviation.
Table 15: Secondary outcome measures (psychiatric) at baseline, end of the blinded off-stimulation period and end of the blinded on-stimulation period

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hamilton Depression Scale score (range 0-68)</th>
<th>Hamilton Anxiety Scale score (range 0-56)</th>
<th>Starkstein Apathy Scale score (range 0-42)</th>
<th>Neuropsychiatric Inventory (12 item version) total score (0-144)</th>
<th>caregiver distress (0-60)</th>
<th>hallucinations subscale (0-12)</th>
<th>Depression/dysphoria subscale (0-12)</th>
<th>Apathy subscale (0-12)</th>
<th>Blessed Dementia Scale (range 0-28)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>OFF</td>
<td>ON</td>
<td>B</td>
<td>OFF</td>
<td>ON</td>
<td>B</td>
<td>OFF</td>
<td>ON</td>
</tr>
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<td>4</td>
<td>3</td>
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<td>17</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>20</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>3</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>7</td>
<td>14</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>27</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>E</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>12</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>3</td>
<td>11</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>12</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Group Mean</td>
<td>4.83</td>
<td>5.33</td>
<td>7.33</td>
<td>4.50</td>
<td>4.50</td>
<td>4.67</td>
<td>16.33</td>
<td>15.50</td>
<td>16.33</td>
</tr>
<tr>
<td>Group SD</td>
<td>3.25</td>
<td>2.73</td>
<td>3.20</td>
<td>2.17</td>
<td>2.07</td>
<td>2.34</td>
<td>6.02</td>
<td>4.89</td>
<td>5.85</td>
</tr>
</tbody>
</table>

B = Baseline, OFF = end of blinded off-stimulation period. ON = end of blinded on-stimulation period. Lower scores are better on all above measures. Results highlighted in green indicate those where ON stimulation score was 10% better than both OFF stimulation and baseline scores. Results highlighted in red indicate those where ON stimulation score was 10% worse than both OFF stimulation and baseline scores. SD = standard deviation.
Table 16 presents secondary outcome measures relating to motor symptoms and quality of life. There was no consistent advantage on-stimulation vs off-stimulation on measures of motor function, freezing of gait, patient-reported quality of life, carer reported quality of life or blinded clinician-rated change in clinical state.

Other secondary outcome measures relating to non-motor symptoms (SCOPA-AUT, Mayo Fluctuations Composite Scale) are reported in Table 20. There were no consistent differences seen across the group with regard to these measures with NBM DBS on vs off.
Table 16: Secondary outcome measures (motor symptoms and quality of life) at baseline, end of the blinded off-stimulation period and end of the blinded on-stimulation period

<table>
<thead>
<tr>
<th>Patient</th>
<th>Movement Disorders Society Unified Parkinson’s disease Rating Scale</th>
<th>Freezing of Gait Questionnaire</th>
<th>Carer strain index</th>
<th>Quality of Life - AD score</th>
<th>Clinical Global Impressions Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Part I score (range 0-52)</td>
<td>Part II score (range 0-52)</td>
<td>Part III score ON medication (range 0-132)</td>
<td>Part IV score (range 0-24)</td>
<td>Total score ON medication (range 0-260)</td>
</tr>
<tr>
<td>B</td>
<td>16</td>
<td>20</td>
<td>18</td>
<td>B OFF</td>
<td>ON</td>
</tr>
<tr>
<td>A</td>
<td>13</td>
<td>12</td>
<td>15</td>
<td>C OFF</td>
<td>ON</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>23</td>
<td>21</td>
<td>D OFF</td>
<td>ON</td>
</tr>
<tr>
<td>E</td>
<td>20</td>
<td>18</td>
<td>20</td>
<td>E OFF</td>
<td>ON</td>
</tr>
<tr>
<td>F</td>
<td>10</td>
<td>5</td>
<td>7</td>
<td>E OFF</td>
<td>ON</td>
</tr>
<tr>
<td>Group</td>
<td>Mean</td>
<td>16.50</td>
<td>15.60</td>
<td>16.83</td>
<td>18.00</td>
</tr>
<tr>
<td>Group</td>
<td>SD</td>
<td>4.28</td>
<td>7.16</td>
<td>5.27</td>
<td>10.18</td>
</tr>
</tbody>
</table>

B = Baseline, OFF = end of blind stimulation period. ON = end of blinded on-stimulation period. Lower scores are better in all cases, except Quality of Life AD subcores, where a higher score is better. Results highlighted in green indicate those where ON stimulation score was 10% better than both OFF stimulation and baseline scores. Results highlighted in red indicate those where ON stimulation score was 10% worse than both OFF stimulation and baseline scores. EQ-VAS = EuroQol five dimensions questionnaire visual analogue scale. PDQ-39 = Parkinson’s Disease Questionnaire, 39 item. SD = standard deviation.
Only one serious adverse event occurred during the trial period: Patient D developed antibiotic-associated \textit{Clostridium difficile} colitis post-operatively (as a result of the prophylactic cefuroxime administered intra-operatively), which necessitated a prolongation of his hospital stay by two weeks while he completed an extended course of antibiotic therapy and recovered. Once he had made a full recovery there were no ongoing sequelae and no long-term extra morbidity. Table 17 lists all adverse events. The patients were generally fatigued in the immediate post-operative period and consequently most of them failed to completed the one-week post-operative assessment battery. Consequently a judgement cannot be made regarding whether surgery itself had any effects on cognitive performance (Table 18).

\textbf{Table 17: Adverse events in the six patients during and beyond the study period}

<table>
<thead>
<tr>
<th>All adverse events</th>
<th>Resolved adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Serious adverse events}</td>
<td></td>
</tr>
<tr>
<td>Related to surgery or device</td>
<td></td>
</tr>
<tr>
<td>Antibiotic-associated C. difficile diarrhoea prolonging hospitalisation</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>1 (17%)*</td>
</tr>
<tr>
<td>\textit{Non-serious adverse events}</td>
<td></td>
</tr>
<tr>
<td>Related to surgery or device</td>
<td></td>
</tr>
<tr>
<td>Post-operative transient confusion/paranoia</td>
<td>2</td>
</tr>
<tr>
<td>Post-operative transient increased daytime somnolence</td>
<td>1</td>
</tr>
<tr>
<td>Burr hole cap discomfort</td>
<td>2</td>
</tr>
<tr>
<td>Other</td>
<td></td>
</tr>
<tr>
<td>Increased limb rigidity</td>
<td>2</td>
</tr>
<tr>
<td>Fall</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>8 (83%)*</td>
</tr>
</tbody>
</table>

Data are \( n \) or \( n \% \). *Proportion of patients. ** Proportion of events. C.difficile = \textit{Clostridium difficile}
<table>
<thead>
<tr>
<th>Patient</th>
<th>Hopkins Verbal Learning Test - Revised (T scores)</th>
<th>WAIS-IV digit span (raw scores)</th>
<th>D-KEFS Verbal Fluency Test (scaled scores)</th>
<th>Posner's covert attention test</th>
<th>CANTAB Reaction Time Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Recall</td>
<td>Delayed recall</td>
<td>Retention</td>
<td>Recognition discrimination index</td>
<td>Digits forwards (range 0–16)</td>
</tr>
<tr>
<td>B</td>
<td>33 NC</td>
<td>37 NC</td>
<td>41 NC</td>
<td>52 NC</td>
<td>10 7 9</td>
</tr>
<tr>
<td>C</td>
<td>21 10</td>
<td>23 11</td>
<td>37 39</td>
<td>37 26</td>
<td>10 7 5</td>
</tr>
<tr>
<td>D</td>
<td>20 NC</td>
<td>25 NC</td>
<td>56 NC</td>
<td>20 NC</td>
<td>10 NC</td>
</tr>
<tr>
<td>E</td>
<td>38 NC</td>
<td>38 NC</td>
<td>30 NC</td>
<td>43 NC</td>
<td>10 NC</td>
</tr>
<tr>
<td>F</td>
<td>36 6</td>
<td>20 20</td>
<td>20 20</td>
<td>43 32</td>
<td>NC 7</td>
</tr>
<tr>
<td>Group A</td>
<td>29 29.50</td>
<td>29.00</td>
<td>30.83</td>
<td>26.50</td>
<td>26.00</td>
</tr>
<tr>
<td>Group B</td>
<td>38 7.61</td>
<td>11.31</td>
<td>7.08 2.32</td>
<td>12.51 9.19</td>
<td>12.63</td>
</tr>
</tbody>
</table>

*B = Baseline assessments. P = Assessments at one-week post-operative (off-stimulation). All scaled scores/T scores are age-adjusted. Higher scores are better on all tests, except for measures of reaction time and reorienting time. Results highlighted in green indicate those where one-week post-operative score was better than baseline score, by at least one T score/one scaled score/10% raw score/50 ms decrease. Results highlighted in red indicate those where one-week post-operative score was worse than baseline score, by at least one T score/one scaled score/10% raw score/50 ms decrease. ◊ = lower scores better. B = scores closer to zero are better. NA = performance too poor, no normative data available to standardize score. NC = not completed.
Table 19 presents data showing the effects of acute NBM stimulation over 24 hours on the primary outcome measures. The data for each patient is taken from the visits when they performed the abbreviated cognitive battery immediately before and 24 hours after simulation was switched on during the blinded period, whether this was at the start of Condition 1 or Condition 2. None of these blinded off- vs 24 hours on- primary outcome comparisons achieved conventional threshold for statistical significance. Four of the six patients (A, B, D and E) demonstrated improvement on measures of total recall after 24 hours on NBM stimulation, indicating improved learning efficiency. These improvements were sustained with chronic stimulation for three of these patients (A, B and D, Table 13). Five of the six patients (the same four plus patient C) also demonstrated improvements on recognition-discrimination index scores after 24 hours on NBM stimulation, indicating improved retention of information in memory. However, these improvements were only sustained in two of the patients with chronic stimulation (B and D, Table 13).
Table 10: Effects of acute NBM stimulation over 24 hours on primary outcome measures.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hopkins Verbal Learning Test - Revised (T scores)</th>
<th>WAIS-IV digit span (raw scores)</th>
<th>D-KEFS Verbal Fluency Test (scaled scores)</th>
<th>Posner's covert attention test</th>
<th>CANTAB Reaction Time Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total recall</td>
<td>Delayed recall</td>
<td>Retention</td>
<td>Recognition discrimination index</td>
<td>Digits forwards (range O-16)</td>
</tr>
<tr>
<td>A</td>
<td>20</td>
<td>35</td>
<td>21</td>
<td>27</td>
<td>43</td>
</tr>
<tr>
<td>B</td>
<td>21</td>
<td>34</td>
<td>27</td>
<td>42</td>
<td>33</td>
</tr>
<tr>
<td>C</td>
<td>29</td>
<td>20</td>
<td>31</td>
<td>23</td>
<td>33</td>
</tr>
<tr>
<td>D</td>
<td>T5</td>
<td>T5</td>
<td>T5</td>
<td>T5</td>
<td>T5</td>
</tr>
<tr>
<td>E</td>
<td>21</td>
<td>29</td>
<td>27</td>
<td>27</td>
<td>41</td>
</tr>
<tr>
<td>F</td>
<td>34</td>
<td>23</td>
<td>27</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Group</td>
<td>21.00</td>
<td>26.83</td>
<td>26.60</td>
<td>27.33</td>
<td>34.80</td>
</tr>
<tr>
<td>Group</td>
<td>6.20</td>
<td>6.79</td>
<td>7.56</td>
<td>7.66</td>
<td>6.94</td>
</tr>
</tbody>
</table>

OFF = immediately prior to patient being set to blinded on-stimulation condition. ON = 24 hours after patient set to blinded on-stimulation condition. All scaled score/T scores are age-adjusted. Higher scores are better on all tests, except for measures of reaction time and reorienting time.

Results highlighted in green indicate those where ON stimulation score was better than OFF stimulation score by at least one T score/one scaled score/10% raw score/50 ms increase. Results highlighted in red indicate those where ON stimulation score was worse than OFF stimulation score by at least one T score/one scaled score/10% raw score/50 ms decrease. * = lower scores better. ◊ = scores closer to zero are better. NA = performance too poor, no normative data available to standardise score. NC = not completed. TS = too somnolent to complete task.
### Table 20: Other secondary outcome measures at baseline, end of the blinded off-stimulation period and end of the blinded on-stimulation period

| Patient | WASI-IV (scaled scores) | WAIS-IV (scaled scores) | WAIS-IV (scaled scores) | WAIS-IV (scaled scores) | WAIS-IV (scaled scores) | WAIS-IV (scaled scores) | WAIS-IV (scaled scores) | WAIS-IV (scaled scores) | WAIS-IV (scaled scores) | WAIS-IV (scaled scores) | WAIS-IV (scaled scores) | WAIS-IV (scaled scores) | WAIS-IV (scaled scores) | WAIS-IV (scaled scores) |
|---------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| A       | 5 3 7 5 4 4 4 2 1      | 3 2 1 1331 991 1553    | 1 1 1 1 1 1 3 1 1      | 1 1 1 45 40 27        | -1 -13 -16             | 3 1 4 9 20 33          | 3 4                      |
| B       | 5 3 5 8 7 7 3 1 2      | 2 1 3 526 186 718      | 1 1 1 1 1 1 5 2 6      | NA 3 1 35 31 33        | 43 -16 -28             | 6 13 7 19 NC 2 1       |
| C       | 2 3 2 1 NA 1 1 NA 1    | 1 1 NA 1668 1800 1839  | 3 4 1 5 1 1 1 1 1      | NA NA 33 42 40         | -44 -87 24             | 13 5 29 NC 13 1 1      |
| D       | 3 4 5 6 7 7 3 1 1 NA 1 | 1 1 NA 1263 NA NA 1271 | 1 1 1 1 1 1 1 1 1      | NA 8 NA 9              | 32 -9 -164             | NA 72 29 19 NC 4 1     |
| E       | 6 4 6 7 7 7 6 NC NC NC | NC NC NC 779 2489 2079  | 4 4 1 NA NA NC NC NC NC | NC NC NA 10 NA NA -56 | 91 15 NC NC 13 18 1 1 |
| F       | NC 6 1 NC 3 4 6 6 8    | 1 1 1 1 2 1 4 1        | 1 3 1 58 56 57         | 64 50 NA 4 2 1 4 3 6 1 |

**Group Mean Groups**

- 6.00 6.00 6.00 6.00 6.00 6.00 6.00 6.00 6.00 6.00 6.00 6.00 6.00 6.00 6.00
- 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00
- 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00

**Notes:**
- B = Baseline, OFF = end of blinded off-stimulation period, ON = end of blinded on-stimulation period.
- Lower scores are better for all measures except WASI-IV symbol search and Digit Symbol Coding where higher scores are better. All scaled scores are age-adjusted.
- Results highlighted in green indicate that the difference from baseline at the end of the blinded stimulation period was greater in the treatment group compared to the sham group.
- Values in parentheses indicate that the difference is statistically significant at the 0.05 level.
- Group Mean Groups: Mean values for each group.

**Abbreviations:**
- CANTAB: Cambridge Neuropsychological Test Automated Battery.
- SD: Standard Deviation.
3.3. Discussion

In this double-blind, crossover trial of six patients with dementia with Lewy bodies, low frequency (20Hz) stimulation of the nucleus basalis of Meynert appears to be safe and well tolerated. Given the small sample size, the lack of contemporaneous controls and the absence of any significant findings, it is entirely possible that any variation in scores relates to chance alone. However, given that this is a hypothesis generating exercise, we shall consider whether any positive encouraging signals emerged.

None of the blinded on- vs off-stimulation comparisons reached conventional threshold for statistical significance at group level. Nevertheless, the most consistent finding amongst our primary outcomes was that four of the six patients (A, B, D and E) demonstrated improvements in HVLT-R total recall scores following 24 hours of blinded acute NBM stimulation (median group level improvement of +5.0 T scores, p=0.249, Table 19), and that this improvement was sustained in three of them (A, B and D) with chronic NBM stimulation compared to both off-stimulation and baseline (Table 13 and Figure 11). Total recall is a proxy measure of learning efficiency across trials, which could potentially be influenced by an improvement in underlying attention parameters, storage of information in memory or executive retrieval of learnt information. In order to determine which of these processes might be responsible this finding needs to be interpreted in the context of the other results. This trend for improved learning efficiency on-stimulation in the DLB group was not seen on-stimulation in the PDD group (CVLT-II immediate free recall T scores, Tables 3 and 9), and in fact worsened in the PDD group. However, these two verbal learning subtests are not equivalent, with the CVLT-II version using a longer word list (16 rather than 12 words) and more learning trials (5 rather than 3) than its HVLT-R equivalent, thus making it more likely to be confounded by fatigue in dementia patient populations (as discussed above in Section 3.1.9). The lack of a similar improvement in learning efficiency in the PDD group may therefore reflect this fact. Interestingly however, an improvement in total recall, and thus learning efficiency, was also observed with acute NBM stimulation in the single PDD patient
reported by Freund and colleagues (an improvement in Rey Auditory Verbal Learning Test (AVLT) summary score from 11 to 20 words after 24 hours of NBM stimulation) (Freund et al., 2009).

Of note the significant improvement in immediate free recall (Trial 1) seen in the PDD group after 24 hours of acute NBM stimulation does not equate to the improvement in total recall seen here in the DLB patients with acute stimulation, as the former is a measure of recall from one single trial only (reflecting working memory/attention, but not learning), whereas the latter is a measure of learning efficiency across three separate trials.

Five of the six patients (the same ones that showed improvement in HVLT-R total recall, plus patient C) also demonstrated improvements in HVLT-R recognition-discrimination index scores with acute NBM stimulation (median group level improvement of +5.5 T scores, p=0.141, Table 19), and this improvement was sustained in two of them (B and D) with chronic NBM stimulation compared to both off-stimulation and baseline (Table 13). Recognition-discrimination index is a measure of retention of information in memory, and therefore these results are in line with the improvements in CVLT-II retention in memory scores seen in the PDD patients with both acute and chronic NBM stimulation. Therefore, the combined results from both trials appear to support the hypothesis that modulation of NBM activity might have the potential to improve learning and memory processes in LBD patients.

Nevertheless, as discussed in Section 2.3 above, apparent improvements in learning and retention of information in memory could equally be due to improvements in underlying attention/alertness mechanisms, and some of the present trial results lend support to this alternate hypothesis; for example, four of the six patients (A, B, C and D) demonstrated improved simple reaction times with chronic NBM stimulation compared to both off-stimulation and baseline (Table 13), while their corresponding simple movement times simultaneously worsened with stimulation (Table 20). This divergent trend in the results
suggests that the improvements seen in simple reaction speeds with chronic NBM stimulation were not due to improvements in motor symptoms, and thus represent true improvements in central (cognitive) processing speeds. A similar dichotomous trend is seen in the corresponding choice reaction time and choice movement time results (Tables 13 and 20), however the results are harder to interpret given that two patients (D and E) performed too poorly on this part of the task for their results to be standardised. Improvements in central processing speed are often viewed as surrogate measures of improved attention/alertness (Wesnes et al., 2005), therefore this trend in reaction time results could suggest that chronic NBM DBS improved these cognitive processes in the DLB patients. Interestingly a similar dichotomous pattern in simple reaction times and simple movement times was also seen in the PDD trial with both acute and chronic NBM stimulation (Tables 3 and 10, and 9 respectively).

The fact that four of the five DLB patients tested on the SART task (Patients B, C, D and E) showed improvements in commission error rate with chronic NBM stimulation compared to both off-stimulation and baseline (Table 14) also supports a possible effect on attention/alertness mechanisms. A lower commission error rate can only be considered a true improvement as long as the corresponding omission error rate also remains stable or improves (otherwise patients could have a low commission error rate simply because they are not performing the task correctly). In one of these four patients (Patient C) her omission error rate worsened markedly during on-stimulation testing, therefore her apparent improvement in commission error rate on-stimulation was simply due to poor task performance at that time point (Table 20). However, the omission error rates of the other three patients remained stable or improved on-stimulation (Patients B, D and E, Table 20) indicating that their corresponding improvements in commission error rates were true improvements in task performance. Commission errors on the SART task are reflective of lapses in sustained attention (O’Connell et al., 2009), therefore the relative reduction in error rate in these three patients on-stimulation further suggests that NBM DBS may have an effect on
attention/alertness mechanisms. An alternative explanation for the improvement in SART commission error rates in these patients on-stimulation could be an improvement in response inhibition, however this seems less likely given that they failed to show improvements on-stimulation on more traditional measures of response inhibition such as the Trail Making Test and Colour-Word Interference Test (Tables 14 and 20 respectively).

Qualitative evidence supporting a role of NBM DBS in improving attention/alertness comes from Patient D, who displayed marked cognitive fluctuation and frank daytime somnolence both at baseline and during the blinded off-stimulation condition, precluding his ability to perform many of the tasks at these time points (see Tables 13, 14, 19 and 20). However, during the blinded on-stimulation condition he was much more alert and attentive, and consequently was able to engage with and complete all tasks at this time point. This is reflected in the improvements in his blinded scores on the Clinician Assessment of Fluctuations Scale and Mayo Fluctuations Composite Scale on-stimulation compared to both off-stimulation and baseline (Table 13 and Figure 11, and Table 20 respectively).

However, as in the PDD trial, performance on many of the cognitive tests remained unchanged on-stimulation. This argues against a general improvement in global attention functions with NBM DBS as in that situation one would have expected to see a general improvement in performance across all measures. Instead, our results currently suggest that low frequency NBM DBS may have a more specific effect on one particular mode of attention, particularly sustained attention/vigilance or level of arousal. Such an improvement in sustained attention or arousal could account for the improvements in learning efficiency and retention of information in memory with NBM DBS described above, however improvements in these two cognitive domains are not necessarily mutually exclusive.

Outside our cognitive outcome measures, the improvement across the group in NPI total scores on-stimulation compared to both off-stimulation and baseline (median improvements of -12.5 points, p=0.066, and -8.5 points, p=0.080 respectively, Table 15 and Figure 11)
mirrors the improvement in blinded NPI total scores seen with NBM DBS in the PDD trial. However, whereas the improvement in the PDD patients was primarily driven by a reduction in complex formed visual hallucinations, in the DLB patients it appears to be due to a general reduction in neuropsychiatric symptoms across the board, with modest reductions in caregiver-rated subscale scores for hallucinations, depressive symptoms and apathy while on-stimulation all contributing equally (Table 15). This is paralleled by more marked reductions in NPI caregiver distress scores on-stimulation in the DLB group (median improvements of -6.5 points, p=0.068, and -6.5, p=0.144, compared to off-stimulation and baseline respectively, in comparison to -1.5 points, p=0.257, and -4.5 points, p=0.459 in the PDD group), although the median NPI caregiver distress score at baseline was higher to start with in the DLB group (10.5 points as opposed to 8.5 points in the PDD group). Results from both trials therefore support the hypothesis that low frequency NBM DBS improves neuropsychiatric symptoms in LBD patients, and suggest that greater symptom burden at baseline may predict a greater response to treatment. The exact mechanisms by which low frequency NBM DBS may influence these symptoms remains unclear. A recent DTI study has shown strong associations between degradation of corticopetal cholinergic projections from NBM and the occurrence of particular neuropsychiatric symptoms in dementia patients, including hallucinations, apathy, delusions and anxiety (van Dalen et al., 2016). In light of this we can hypothesise that low frequency NBM DBS may relieve such symptoms in LBD patients by increasing cortical cholinergic transmission in the degenerative NBM network. However, exactly how increased cortical cholinergic tone may influence diverse neuropsychiatric symptoms remains to be determined. As discussed in Sections 1.3.1.10, 1.3.1.11 and 2.3 above there are strong links between cortical cholinergic deficiency, impairments in alertness/attentional control, and generation of visual hallucinations, and the same may hold true for other neuropsychiatric symptoms in LBDs such as apathy and depression.

We did not observe any improvements in UPDRS motor subscores or freezing with NBM DBS in the DLB group. In particular, we did not observe the same improvement in UPDRS
Part IV scores that was seen on-stimulation in the PDD group, likely because none of the DLB patients suffered from levodopa-induced dyskinesias, therefore negating any effect that current spread to the neighbouring GPi might have had on such symptoms.

In common with the PDD trial, this pilot trial of NBM DBS in DLB has a number of key strengths. We again used a prolonged blinded period, allowing us to report more accurately on the sustained effects of NBM DBS than other studies have been able to so far. We also included a washout period of two weeks between experimental conditions, such that any prolonged effects of NBM DBS which might have persisted after cessation of stimulation would have been less likely to confound the second condition. Additionally, our primary outcome measures included detailed assessment of individual cognitive subdomains, rather than purely measures of global cognitive function. Due to the overlapping effects of impairments in individual cognitive domains upon performance in others (see Section 1.3.3 above and also (Gratwicke et al., 2015b)), use of global measures makes it difficult to determine what the specific cognitive effects of NBM DBS might be. We also performed detailed assessments of neuropsychiatric symptoms, motor symptoms and patient and carer quality of life, which allowed us to assess a broad range of potential beneficial effects. A key strength of this particular trial over the PDD trial is that there was no requirement for the dorsal electrode contacts to be positioned in the overlying GPi, which allowed more direct targeting of the Ch4i subsector of NBM and thus evaluation of the effects of its stimulation.

Once again the major limitation to this study was the small sample size, which although appropriate for exploratory data collection, is not designed to detect significant differences between the blinded on- and off-stimulation periods. Given the multiple comparisons performed here, our data must not be interpreted as evidence for efficacy, but can be used in the planning and design of formal trials to test the hypotheses generated by the current dataset. Another relevant issue is that patients continued AChEI therapy during the trial, and although there were no dose alterations, it still means that the potential physiological effects of NBM DBS on the cholinergic system, and any consequent clinical effects on symptoms,
may be partially masked. However, given the relative safety of AChEI medications in comparison to DBS, we did not feel it was appropriate to expose patients to surgical risks who might gain sufficient cognitive benefits from the use of medications alone. In addition, we did not include a randomised non-operated control group of DLB patients, and so cannot objectively determine whether NBM DBS made a difference to the natural progression of cognitive deficits during the trial period. Likewise, this also makes it difficult to determine whether deterioration across the group in some outcome measures (Tables 13 and 14) was due to effects of surgery, stimulation or disease progression. Although we tried to minimise potential confounding influences of task familiarity and practice effects by using parallel test versions, this was not possible for all outcome measures. Inclusion of a non-operated group would also have been useful to control for this. Finally, as in the PDD trial, we only investigated the effects of low frequency NBM stimulation at 20Hz, however the scientific rationale for this is limited (as discussed in Section 1.7.2. above), and stimulation at a different frequency might produce different results. Use of a titration schedule investigating cognitive responses to different frequencies would have shed light on this issue. However, to implement this would have required a much longer study with an even greater frequency of assessment visits, and it seems unlikely that patients would have been easily able to comply with this, especially given the difficulty that some of the DLB patients had in completing all tests under the current trial design. Finally, the clinical impact of the individual objective improvements on memory subtests with NBM DBS also remains to be determined as we did not assess specific functional capacities to determine if and how these might translate to daily activities for patients.

Looking forwards, one of the aims of this pilot study was to identify parameters which will inform sample size calculations for future potential trials of NBM DBS in LBDs. In accordance with the results of the PDD trial our results here suggest that detailed assessments of verbal memory performance, including learning efficiency and retention in memory, as well as detailed measurements of the frequency and severity of neuropsychiatric symptoms
should comprise future primary outcome measures. Given the possible beneficial effects of NBM DBS on a measure of vigilance (Sustained Attention to Response Task) it would also be important to include more detailed assessment of different components of attention (as discussed in Section 1.2.2. above and in (Gratwicke et al., 2015b). As previously mentioned, secondary outcomes should include assessments of functional capacity to further determine the clinical impact that changes in neuropsychological test scores and/or neuropsychiatric symptoms have on patient’s daily activities.

In conclusion, data from this pilot clinical trial have shown that NBM DBS is both technically feasible and safe in carefully selected DLB patients, and provide preliminary evidence that this therapy should be further evaluated regarding its effects on memory, attention and neuropsychiatric symptoms. The outcomes from the study serve to justify further exploration of NBM DBS as a therapy for DLB patients whose cognitive and behavioural symptoms are refractory to medical therapy. Future studies should explore the effects of stimulation amongst subregions within the NBM and formally test whether stimulation can reproducibly impact on cognitive and neuropsychiatric symptoms in DLB patients. In addition, given that the preliminary evidence from these trials suggests that NBM DBS may have similar beneficial effects in both PDD and DLB, and the fact that the boundary between these two dementia syndromes is largely artificial, it may prove useful to recruit patients with both dementias into future trials to increase numbers and thereby better determine the effects of NBM DBS in Lewy body dementias as a whole.
Chapter 4: Local field potential recordings from the nucleus basalis of Meynert in patients with Lewy body dementias

4.1. Introduction

Local field potentials (LFPs) recorded from a macro- or microelectrode reflect the temporal and spatial summation of synaptic neuronal activity in a certain volume of tissue (Goldberg et al., 2004), with the major influence likely to be slow sub-threshold post-synaptic potentials (Eccles, 1951). Consequently the LFP amplitude or power indexes the strength of synchronization, density and spatial extent of the involved neural pool (Little et al., 2012).

Although it is not entirely clear how LFP activity relates to neural spike firing, the locking of neurons to LFP activity over a wide range of frequencies provides support for its use as a measure of the pattern of underlying neuronal synchronization (Moran et al., 2008). Extensive evidence from LFP recordings from the human STN and GPi in PD patients undergoing DBS for motor symptoms, and animal models of the disease, have demonstrated synchronised neural oscillations in the beta band (13-35 Hz) in the motor network (Brown et al., 2001; Hammond et al., 2007; Kühn et al., 2004; Williams et al., 2002). Their suppression by dopamine, DBS and salient cues has been shown to correlate with changes in motor performance (Brown, 2003; Brown and Williams, 2005; A. a Kühn et al., 2006; Kühn et al., 2004; Oswal et al., 2012). Thus such recordings from patients undergoing DBS have offered unique insights into the physiological properties of distributed brain networks and consequently shed light on their function.

As discussed in Sections 1.6.6 – 1.6.8. above, the exact physiological function of the human NBM remains open to debate as thus far its function has only ever been investigated by indirect methods such as functional imaging. However, our trials of NBM DBS afforded us the unique opportunity to record bilateral LFPs directly from NBM in our awake patients during the period of electrode externalisation prior to IPG implantation. Therefore, we were
able to directly investigate the physiological properties of the human NBM in vivo for the first time, and thereby gain novel insight into its possible functions.

4.2. Materials and methods

4.2.1 Patients

As mentioned in both preceding chapters all PDD and DLB patients who participated in the clinical trials agreed and provided informed consent to undergo LFP recordings in the period of electrode externalisation on the ward, between electrode implantation and IPG implantation (a period of 4-7 days on average). Clinical characteristics of the patients and stereotactic co-ordinates of the most ventral electrode contacts in each hemisphere are detailed in Tables 1 and 11 above. Figures 10 and 14 above show anatomical locations of most ventral DBS contacts in the PDD and DLB patients respectively. Ethical approval and consent for externalised LFP recordings were included in the main ethics applications and consent forms respectively for the clinical trials (as described above in Sections 2.1.3 and 3.1.3).

4.2.2 Rest recordings and paradigms

Patients attended our research laboratory during the daytime having taken their usual medications (including their usual doses of both levodopa and AChEI medications). Patients were seated comfortably in a chair. We first conducted a resting recording with no task paradigm to complete. We recorded resting bilateral LFPs from NBM and Gpi simultaneously in each patient. Our hypothesis was that the NBM would show significantly different resting state activity to neighbouring Gpi, reflective of the anatomical differentiation and proposed functional differentiation of the two structures. We subsequently conducted two separate recordings while patients completed two different attention task paradigms, Posner’s covert attention task (PCAT) and the sustained attention to response task (SART). We chose
these two tasks since the NBM has previously been proposed to play a role in both orienting of attention and sustained attention/vigilance (Buzsaki et al., 1988; Fuller et al., 2011; Sarter et al., 2005, 2006; Voytko et al., 1994). Therefore, we hypothesized that by directly comparing NBM LFP activity during these two tasks we would be able to determine if activity in the nucleus preferentially reflected one or other mode of attention. By recording simultaneous GPi LFPs during the tasks we would be able to determine whether any evoked activity seen in NBM during the attention tasks was specific to that nucleus, or a reflection of more widespread neural activation. The rest recording and both task recordings were conducted in one sitting, with breaks in between to allow patients to rest and to provide instructions. Initial set-up and wiring took some time, and so it was preferable from the patient’s perspective to only go through this procedure once, hence why all three recordings were conducted in one sitting rather than on separate days.

4.2.2.1 Resting state recordings

LFPs were simultaneously recorded from both NBM and GPi while patients sat still in a quiet room for three minutes with eyes open. No task was administered. These recordings were conducted in order to characterise baseline resting activity in the human NBM in the awake and alert state, and compare it to resting GPi activity (which has previously been well characterised through LFP recordings (Brown et al., 2001), and therefore acted as a control). Our prediction was that there would be a different resting LFP pattern in NBM compared to GPi reflecting their different proposed physiological roles.

4.2.2.2 Posner’s Covert Attention Test (PCAT)

LFPs were simultaneously recorded from both NBM and GPi while patients performed a customised version of this classical test of orienting of visual attention, displayed on a laptop computer. The recordings were made in order to investigate the proposed role of NBM in orienting of visual attention (see Section 1.6.7). Our hypothesis was that NBM activity would reflect orienting of visual attention to a novel stimulus, and that activity would be stronger
when required to reorient visual attention to an unexpected target location compared to an expected one, reflective of the greater attentional demand in the former. Meanwhile GPi activity would only reflect the motor aspect (response) in the task.

Patients were seated comfortably in a chair facing the laptop screen 50 cm in front of them. They held an analogue trigger button in each hand and registered responses by pressing either the right or left button. Each trial began with a white fixation cross presented in the centre of a black screen, followed 2500 ms later by a warning cue (presented for 500 ms) which indicated either right or left (Figure 16). This was followed 1000-1500 ms later by a target on either the right or left of the screen (presented for 140 ms). After target appearance patients had to respond as fast as possible within a 1750 ms window by pressing the right or left hand button to indicate on which side of the screen the target had been presented. However, the warning cue only accurately predicted correct target side 70% of the time, so two types of trial were possible; ‘valid’ trials, where cue and target side were congruent, and ‘invalid’ trials, where cue and target side were incongruent. Thus valid trials only required orientation of visual attention to the expected target location, whereas invalid trials required a re-orientation (shift) of visual attention to the location of the unexpected target (Posner, 1980). By keeping the balance of probability in favour of the occurrence of a valid trial patients were encouraged to respond rapidly contingent upon the cue rather than waiting to plan their response based on ultimate target location. In this way the potential requirement for a rapid re-orientation of visual attention was maintained throughout the task. Feedback was provided to patients at the end of each trial in the form of either a green ‘correct’ or red ‘error’ word being displayed for 500 ms, following which the fixation cross would reappear and the next trial would begin.

The task requirements were verbally explained to the patient prior to completing an initial training block of 30 trials to ensure that instructions had been fully understood. After training, patients performed ten blocks of 30 trials each, with breaks between. The maximum total
number of trials that could be completed per patient was therefore 300, of which a maximum of 210 were valid and 90 invalid.

Of note, this version of the PCAT was identical to that administered as a primary outcome measure to the patients in both clinical trials.

**Figure 16**: Schematic illustration of a valid and an invalid trial in Posner's Covert Attention Test (PCAT). In either trial type a fixation cross was initially displayed for 2500 ms. A warning cue was then briefly displayed (for 500 ms), indicating right or left. After this a blank screen was shown for 1000-1500ms, followed by brief presentation of a target on either the right or left side of the screen (for 140 ms). After target appearance patients had to respond as fast as possible within a 1750 ms window by pressing the right or left hand button to indicate on which side of the screen the target had been presented. However, the warning cue only accurately predicted correct target side 70% of the time, so two trial types were possible; ‘valid’ trials, where cue and target side were congruent (left hand trial schematic), and ‘invalid’ trials, where cue and target side were incongruent (right hand trial schematic). Brief feedback was provided at the end of each trial in the form of either a green ‘correct’ or red ‘error’ word (displayed for 500 ms), following which the fixation cross would reappear and the next trial would begin. This customised version was used for both LFP recordings and as a primary outcome measure in both clinical trials.

4.2.2.3 Sustained Attention to Response Test (SART)

LFPs were simultaneously recorded from both NBM and GPi while patients performed a customised version of this test of sustained attention/vigilance, displayed on a laptop computer. The recordings were made in order to investigate the proposed role of NBM in sustained attention/alertness (see Section 1.6.8). Our prediction was that NBM activity would
reflect lapses in sustained attention leading to errors, while GPi activity would only reflect the motor aspect (response) in the task.

Patients were seated comfortably in a chair facing the laptop screen 50 cm in front of them. They held an analogue trigger button in their dominant hand and registered responses with button presses. Each trial began with a white fixation cross (size 0.25 dva) presented in the centre of a black screen. This was constantly present, and subjects were instructed to fixate on this throughout the paradigm (although gaze fixation was not controlled for with an eye-tracker). After 1000 ms a digit (varying randomly between “1” and “9” on each trial) was presented in the lower left corner of the screen for 70 ms. After presentation of the digit stimulus patients had to respond as fast as possible within a 1750 ms window by pressing the button. They were instructed to respond to all digits in this way (Go trials) except the digit “5”, to which they should inhibit their response (NoGo trial). NoGo trials accounted for 11% of the total trials administered in each block, and Go trials accounted for 89%. On each trial the digit presented varied randomly in size (0.84–1.17 dva) and font. This was to ensure that subjects processed the identity of the digits rather than focusing on specific perceptual features. Following the response window feedback was only given in three situations: (1) if the patient had responded too fast (within 100 ms of stimulus presentation, which is too fast to be a true physiological response and must therefore be a random error), in this case “too fast” was presented for 75 ms; (2) if the patient failed to make any response on a Go trial (an omission error) then “too slow” was presented for 75 ms; (3) if the patient made a response on a NoGo trial (a commission error), in which case “error” was presented for 75 ms. However, if the patient had made a correct response on a Go trial then no feedback was given. After any necessary feedback had been briefly displayed the next trial began.

The task requirements were verbally explained to the patient prior to completing an initial training block of 36 trials, to ensure that the instructions had been fully understood. After training, patients performed ten blocks of 36 trials each, with optional breaks between. The maximum total number of trials that could be completed per patient was therefore 360.
The SART task was developed by Robertson et al. as an alternative to traditional Continuous Performance Tasks (CPTs) for measuring sustained attention/vigilance (Robertson et al., 1997). In traditional CPTs participants are asked to monitor a stream of repetitive stimuli in a non-arousing visual environment over an extended period of time for the occurrence of a rare target stimulus to which they must make a response. Such tasks are designed to be monotonous and boring by virtue of their low signal probability, and rely on time-on-task effects to place the participant at increasing risk of a critical lapse of attention. However, in real life we are susceptible to fluctuations in attention over much shorter periods (Manly et al., 1999; O'Connell et al., 2009). Imaging studies support this observation by showing that the frontoparietal attention network is engaged over periods of less than a minute (Paus et al., 1997), and that brief lapses of attention are preceded by momentary reductions of activity in frontal control regions (Weissman et al., 2006). Hence, the large temporal gaps between target presentations in traditional CPTs make them insensitive to these moment-to-moment lapses in sustained attention. This problem is addressed by the SART, which is in effect the inverse of the traditional CPT; it still presents a long task with repetitive trials in a non-arousing visual environment, but the much more frequent occurrence of target stimuli mean that this task is more sensitive to the relatively brief lapses of sustained attention that occur in the absence of time-on-task effects. Additionally, in the context of the present experiment, since fluctuations in attentional control have been shown to occur on a second-by-second basis in LBD patients (Walker et al., 2000) then use of the SART paradigm appears likely to be a more sensitive measure of sustained attention in this particular patient group.

Of note, this version of the SART was identical to that administered as a secondary outcome measure to the patients in the DLB trial.
Figure 17: Schematic illustration of the Sustained Attention to Response Task. Each trial began with a white fixation cross presented in the centre of a black screen. This was constantly present, and subjects were instructed to fixate on this throughout the paradigm (although gaze fixation was not controlled for with an eye-tracker). After 1000 ms a digit (varying randomly between “1” and “9” on each trial) was presented in the lower left corner of the screen for 70 ms. After presentation of the digit stimulus patients had to respond as fast as possible within a 1750 ms window by pressing the button. They were instructed to respond to all digits in this way (Go trials) except the digit “5”, to which they should inhibit their response (NoGo trial). This customised version was used for both LFP recordings and as a secondary outcome measure in the DLB trial.

4.2.3 Electrophysiological data acquisition

For all three paradigms data acquisition procedures were identical. NBM and GPi LFPs and analogue signals related to the cues, targets and button presses were recorded using a D360 amplifier (Digitimer Ltd, Hertfordshire, UK), in combination with an analog-to-digital converter, and sampled onto a laptop computer using Spike 2 V6 software (Cambridge Electronic Design, UK). Electrophysiological signals were collected in a monopolar fashion (recorded across eight separate DBS channels: R0, R1, R2, R3, L0, L1, L2, L3) referenced to the left clavicle. Signals were amplified (X 50,000), filtered (1.0-1000 Hz) and sampled at a common rate of 2480 Hz. Raw data files in Spike 2 were converted to MATLAB file format for subsequent off-line pre-processing and spectral analysis.
4.2.4 Data pre-processing

The data were imported into MATLAB (The Mathworks, Inc, Natick, MA) and analysed using custom scripts in conjunction with the SPM (http://www.fil.ion.ucl.ac.uk/spm/) and FieldTrip (http://www.ru.nl/neuroimaging/fieldtrip/) toolboxes (Litvak, Mattout, et al., 2011; Oostenveld et al., 2011). For task files, an events array was generated for each specific file by decoding the digitised trigger signals generated by the cues, targets and button presses (responses) during the task and thereby defining the events which occurred and their corresponding timings. LFP signals were then converted to a bipolar montage; this involved computing the difference between the adjacent but dorsal electrode contact from each contact to give three bipolar contacts per electrode representing the potential difference between contacts 0-1, 1-2, and 2-3. A bipolar montage was preferred to limit the effects of volume conduction from both nearby and distant sources; given that we were trying to resolve differences in LFP signal between two distinct yet closely anatomically-related neighbouring structures (NBM and GPi), then taking all steps to limit the possible confounding influences of any current spread between these structures, or current spread from common distant sources, was important. Bipolar montages focus on the net difference in current between adjacent contacts (within the same structure), thereby reducing the influence of external current sources, whereas monopolar montages summate all current detected by each individual contact, and are therefore theoretically more vulnerable to their signal being confounded by distant current spread. In light of this, we felt that a bipolar montage offered a higher likelihood of being able to resolve differences in the LFP signals between the two structures. Given that in most patients contact 0 on each electrode was located in the body of the NBM, contact 1 was located around the NBM-GPi boarder and more dorsal contacts were located in GPi (see Figures 10 and 14), then the 0-1 bipole corresponded to the NBM, the 2-3 bipole corresponded to the GPi, and the 1-2 bipole was taken as a mixed signal intermediate between the other two. This therefore meant that the LFP file for each paradigm for each patient contained six different channels of data: Right NBM, Right NBM/GPi and Right GPi,
Left NBM, Left NBM/GPi, Left GPi. Each individual file containing these six channels then underwent the same pre-processing steps as follows.

Data were acquired at a high sampling rate (2480 Hz), which increases data storage and computational requirements, but allows for higher frequencies to be examined (the Nyquist frequency limit equals half the sampling rate\(^4\)). In this experiment we focussed on oscillatory activity at frequencies less than 100 Hz, and therefore we felt it reasonable to downsample the data to 300 Hz in order to reduce computational requirements while maintaining the potential for sensitive analysis of our frequency range of interest.

Although the acquired data had been hardware-filtered during acquisition by the LFP recording system, further filtering was necessary at the pre-processing stage in order to remove unwanted frequency components. A high-pass fifth-order Butterworth filter (> 1 Hz) was used to remove low frequency fluctuations in the signal due to gradual build-up of electrical charge, which can saturate the recording equipment. Fifth-order notch filters at 50 Hz and its harmonics were then applied to the data to remove noise from mains interference.

In our data there were numerous sources of artefacts which required removal; these included artefacts generated by the experimental procedures, recording equipment and physiological artefacts generated from eye movements, muscle activity or the cardiac cycle. In most cases these minor artefacts would be handled later by our spectral analysis methods. However, we prospectively applied artefact detection methods to the data, including thresholding of the data itself and thresholding of the difference between adjacent samples, in order to detect large amplitude jumps and mark them. This enabled us to check later on that all artefacts had been sufficiently removed by spectral analysis, and apply further strategies for removal if not.

\(^4\) In order to recover all Fourier components of a periodic waveform, it is necessary to use a sampling rate at least twice the highest waveform frequency you are interested in. The Nyquist frequency limit is defined as this highest frequency that can be coded at a given sampling rate in order to be able to fully reconstruct the original signal (Oppenheim et al., 1999).
4.2.5 Spectral Analysis

The aim of these experiments was to investigate spectral activity in the NBM, in both the resting state condition and under manipulations of orienting and sustained attention. In order to do this we used a standard spectral timeseries analysis method, the Fourier Transform. This is a powerful technique for decomposing complex neural signals into simpler, biologically salient signals (Gabbiani and Cox, 2010), and is based on the principle of the Fourier Series: that any periodic function \( f(t) \) with period \( T \) can be decomposed into a sum of sine and cosine waves:

\[
f(t) = \frac{a_0}{2} + \sum_{m=1}^{\infty} \left( a_m \cos \frac{m2\pi t}{T} + b_m \sin \frac{m2\pi t}{T} \right)
\]

The Fourier Transform can be viewed as an extension of the Fourier series to non-periodic functions (i.e. when \( T \) trends towards infinity), and therefore can be applied to a signal to determine what frequencies are present and in what proportions. The standard equation for the Fourier Transform:

\[
f(\omega) = \int_{-\infty}^{\infty} f(t) e^{-i\omega t} dt
\]

In practical use we usually perform the transform on a time series of discrete length (as in these experiments where the time series is epoch), and therefore we use an approximation known as the Discrete Fourier Transform. This can be evaluated over multiple frequencies and the magnitude square of the Fourier transform for a particular frequency \( |f(\omega)|^2 \) tells us how much weight, or power, the original signal contains at that frequency in that discrete time series. The distribution of power over different frequencies is a standard method to visualise the spectral properties of a signal and is termed the power spectrum.
A difficulty arises with performing Discrete Fourier Transforms on complex real world signals; a non-integer number of cycles of an underlying component frequency is likely to be sampled in a discrete time series. When a Fourier transform is applied to this signal then the time discontinuity will mean that other apparent frequencies close to the ‘true’ frequency of that component signal will also be erroneously detected (‘aliasing’). This is termed spectral leakage and can interfere with the ability to accurately resolve different component frequencies within a complex signal. The effects of spectral leakage can be reduced by pre-multiplying the raw data with a window function (‘taper’) which suppresses time discontinuities. A common taper used is the Hanning taper, which has the advantage of very low aliasing at the expense of slightly decreased frequency resolution (Harris 1978). We applied a multitaper approach, which involves obtaining multiple independent spectral estimates by the use of multiple Hanning tapers, then averaging them together to yield a robust estimation of the true underlying spectrum (Thomson 1982).

### 4.2.6 Analysis Pipelines

Slightly different analysis pipelines were employed for resting and task recordings:

LFP files from the resting experiments consisted of one long recording without discrete events. For each file we epoched this continuous data into discrete 1000 ms sections, before performing repeated spectral analyses using the discrete Fourier transform on each section (with frequency resolution of 1:100 Hz). We then averaged across these transforms to obtain a closer approximation to the true spectral decomposition of the recorded resting signal in each channel. For each patient the data in corresponding channels in each hemisphere were then averaged together to generate three individual spectral datasets: NBM, NBM/GPi and GPi. These spectra were then normalised to the mean power across the 55-95 Hz frequency range. Finally, these individual spectra were averaged across all patients to generate grand average resting power spectra.
For LFP files from the Posner and SART experiments spectral analysis using Fourier
transformation was first applied to the whole recording. Following this data was rescaled
using a log function and a high-pass Butterworth filter (> 1 Hz) was again applied to remove
low frequency artefacts. Epoching was then implemented according to the timings in the
corresponding events array decoded for each recording. Robust averaging was then applied to
the whole spectral dataset (the benefits of this method for increasing signal-to-noise ratio in
data with low trial numbers has been previously demonstrated (Litvak et al., 2012)). For each
patient the data from corresponding channels in each hemisphere were then averaged together
to generate three individual spectral datasets: NBM, NBM/GPi and GPi. Finally these
individual spectra were averaged across all patients to generate grand-average time frequency
spectra for each task.

For the Posner task I separately analysed the spectra locked to the warning cue, the targets
and the responses in overlapping windows of -1000 ms to +1000 ms. For the SART task I
separately analysed the spectra locked to the cues and responses, again in overlapping
windows of -1000 ms to +1000 ms.

4.2.7 Statistical Analysis

4.2.7.1. Resting State Recordings

For resting state recordings statistical analysis was performed using the Fieldtrip software
package. To look for a significant difference between the resting state power spectra in NBM
and GPi we first calculated sequential Monte-Carlo estimates (conditional probabilities, or
‘best estimates’) of their respective true original signals from our observed data. We then
performed a two-tailed paired samples T test on these estimated signals, using a cluster-based
method for multiple comparison correction.
The reason for employing Monte Carlo methodology is that, statistically-speaking, our data is vulnerable to the filtering problem – i.e. due to (1) the fact that in our original recordings it was inherent that only partial observations of the true biological signal were made, (2) the introduction of random perturbations in the recorded signal from our sensors, and (3) potential artefacts introduced by our use of non-linear transforms (such as re-scaling our data using logarithmic functions), we cannot be sure that our data accurately represent the true original signal. We therefore need to establish a ‘best estimate’, termed the conditional probability, of the true original signal from our incomplete, potentially noisy observations. Sequential Monte Carlo methods are a form of mathematical particle filter which addresses this problem. The algorithm uses a genetic type mutation-selection sampling approach, taking a set of particles (data points) to represent the probable true signal and assigning them likelihood weights representing the probability of that particle being sampled from the true signal according to the probability density function. Once this is calculated the data is then resampled, and the particles with negligible weights are removed and replaced by new particles in the proximity of the particles with higher weights. Thus the particles with higher relative weights gradually become multiplied, and consequently the sequential models of the data trend towards the ‘best estimate’ (conditional probability) of the true original signal (Del Moral, 1996; Crisan et al., 1999).

4.2.7.2 Task recordings

For the tasks statistical analysis was performed by using a general linear model (GLM) based approach, implemented in the SPM12 (Statistical Parametric Mapping) software package. SPM is a MATLAB toolbox that was designed to make inferences about regionally specific effects in the brain, but whose statistical principles can be used to make inferences about regionally specific effects in three dimensional space generally, and is thus applicable to analysis of subcortical LFP data (Kilner and Friston, 2010).
SPM employs a mass univariate approach to the statistical analysis of images, meaning that the observed data at each voxel, pixel or time point (in the case of 3D, 2D or 1D images respectively) is modelled as a linear combination of one or more explanatory variables with additive Gaussian noise. For example, in an experiment with ten conditions and ten observations per condition then the recorded activity in a single image voxel would take a different value for each observation, and is represented in SPM as a vector $Y$ containing 100 x 1 values). $X$ is a design matrix with each explanatory variable as a column vector, here a 100 x 10 block diagonal matrix. $\beta$ represents a vector of parameters which optimally fit the model to the data whilst $e$ is a vector of errors containing the mismatch between the observations and the model.

$$Y = X\beta + e$$

It is relatively straightforward to estimate the $\beta$ parameter when assuming that the errors are independent and identically distributed, however this assumption is rarely true with repeated measures designs. SPM therefore uses additional methods to handle such errors (Worsley and Friston, 1995).

Statistical analysis was performed at the group level using a summary statistic based approach, whereby a single image for each experimental condition of interest for each subject was analysed at the between subject level. Once the GLM had been applied to each data point, SPM produced an image of the $\beta$ parameters for each regressor in the design matrix.

Statistical testing of hypothesis can then be performed using the $\beta$ images, after specifying appropriate contrasts to test. Once again two-tailed paired samples T tests were used. Images of the t-statistic for each contrast can be generated in SPM for visualisation and these may be thresholded to a certain p-value, such that surviving data points are those significantly activated by an experimental manipulation (Friston et al., 1995). However, this approach runs the risk of false positives (Type 1 errors) due to the multiple comparisons performed.
Correcting for this requires an estimate of the number of independent statistical tests being performed, but Bonferroni correction is not possible here as spatial or temporal correlations in the data mean that the number of independent tests is not simply equal to the number of datapoints. Instead, SPM uses a branch of mathematics called Random Field Theory to calculate a corrected p-value: having estimated the smoothness of the data, the expected distribution of topological features such as peaks or clusters is calculated according to the Euler characteristic, and this allows more accurate thresholding of the statistical parametric maps (Worsley et al., 1992).
4.3. Results

All six PDD patients and five of the DLB patients underwent LFP recording in the immediate post-operative period. DLB Patient D was too unwell in the post-operative period (due to his contraction of antibiotic-associated *C. Difficile* diarrhoea) to undergo LFP recording. Of the 11 LBD patients who underwent recordings, all 11 completed the resting state recording, eight also completed the Posner task recording, and seven also completed the SART task recording. One patient (DLB Patient C) was too fatigued to complete either task recording, two patients (PDD Patients B and E) could not complete the task recordings due to equipment failures, and one patient (DLB Patient 5) could complete the Posner task, but could not accurately complete the SART task due to alexia. All completed recordings were included in the respective analyses.

4.3.1 Resting state recordings

Individual resting NBM power spectra from each hemisphere in all 11 patients (22 hemispheres in total) are shown in both Figures 18 and 20 (latter is zoomed view of former to illustrate low frequency power more clearly). Corresponding individual resting GPi power spectra from each hemisphere in all 11 patients (22 hemispheres in total) are shown in both Figures 19 and 21 (latter is zoomed view of former to illustrate low frequency power more clearly). Both nuclei show spectral peaks in the delta band (0.1-3 Hz) range, however note the differences in Y axis scales between the NBM and GPi plots, indicating that delta power is generally much higher in resting state NBM compared to GPi in these LBD patients.

Minor spectral peaks are also seen in the theta band (4-7 Hz) and alpha band (8-13 Hz) in the NBM spectra, and in the theta band and beta band (16-30 Hz) in the GPi spectra. The relatively low activity in the beta band in the GPi spectra is consistent with the fact that all patients had taken their usual levodopa medication prior to LFP recording, which has previously been shown to suppress GPi beta activity (Brown and Williams, 2005).
Figure 18 (right): Resting NBM power spectra for all patients. Each coloured line represents the resting power spectrum from a single NBM (one hemisphere) from each patient. Most individual NBM spectra demonstrate peaks in the delta band (0.1 – 3 Hz). Most spectra show a second peak in the theta band (4-7 Hz) range. See Figure 20 for a zoomed in view of the low frequency spectra. The apparent loss of power between 48-52 Hz is due to artefact from the stop band filter applied at 48-52 Hz to remove electrical mains interference at these frequencies. These power spectra are normalised to the mean power across the 55-95 Hz frequency range.

Figure 19 (left): Resting GPi power spectra for all patients. Each coloured line represents the resting power spectrum from a single GPi (one hemisphere) from each patient, colours correspond to those in Figure 18, identifying NBM and GPi from the same individual hemisphere. Most individual GPi spectra demonstrate peaks in the delta band (0.1 – 3 Hz), however note the difference in Y axis scales between Figures 18 and 19, indicating that delta band power is generally much lower in GPi compared to NBM. Most spectra show a second peak in the theta band (4-7 Hz) range and a third in the beta band (15-30 Hz) range. See Figure 21 for a zoomed in view of the low frequency spectra. The apparent loss of power between 48-52 Hz is due to artefact from the stop band filter applied at 48-52 Hz to remove electrical mains interference at these frequencies. These power spectra are normalised to the mean power across the 55-95 Hz frequency range.
Figure 20 (right): Resting NBM power spectra for all patients, zoomed in view on low frequencies. Each coloured line represents the resting power spectrum from a single NBM (one hemisphere) from each patient. Most individual NBM spectra demonstrate peaks in the delta band (0.1 – 3 Hz). Most spectra show a second peak in the theta band (4-7 Hz) range and a few show a peak in the alpha band (8-13 Hz) range. These power spectra are normalised to the mean power across the 55-95 Hz frequency range.

Figure 21 (left): Resting GPi power spectra for all patients, zoomed in view on low frequencies. Each coloured line represents the resting power spectrum from a single GPi (one hemisphere) from each patient, colours correspond to those in Figure 20, identifying NBM and GPi from the same individual hemisphere. Most individual GPi spectra demonstrate peaks in the delta band (0.1 – 3 Hz), however note the difference in Y axis scales between Figures 20 and 21, indicating that delta band power is generally much lower in GPi compared to NBM. Most spectra show a second peak in the theta band (4-7 Hz) range and a third in the beta band (16-30 Hz) range. These power spectra are normalised to the mean power across the 55-95 Hz frequency range.
Figure 22 shows the grand average resting NBM and GPi power spectra averaged over all respective hemispheres in all patients. The resting spectral peaks in the delta band, and to a lesser extent in the theta band, in both nuclei can now be more clearly visualised. The difference in resting delta band power between NBM and GPi is also readily apparent.

**Figure 22: Grand average resting NBM and GPi normalised power spectra.** A) Group average resting NBM and GPi power spectra for all LBD patients (n=11). Spectral peaks are evident in the delta and theta bands in both nuclei, however higher power is seen in NBM relative to GPi in both frequency bands. B) and C) Group average resting NBM and GPi power spectra for PDD (n=6) and DLB (n=5) patients separately. Spectral peaks are again evident in the delta and theta bands in both nuclei in both patient groups, with higher power seen in both frequency bands in the NBM compared to the GPi (note the difference in Y axis scales). In the GPi, but not the NBM, PDD patients showed higher power in the delta and theta bands than their DLB counterparts (although these differences were not significant). All power spectra are normalised to the mean power across the 55-95 Hz frequency range. The shaded regions represent standard errors of the mean.
Figure 23 below shows a plot of the differences between the corresponding NBM and GPi power spectra (NBM - GPi power, from each individual hemisphere) at each individual frequency. The mean difference across all corresponding pairs is plotted as a red line. At group level, statistically significant differences in resting state power between NBM and GPi were found in the delta, theta, and alpha bands (p=0.004 for all). The individual frequencies at which these significant differences in resting power were found (1-16 Hz) are plotted as red stars on the figure. Figure 24 shows a zoomed-in version of the previous figure, focussing on the low frequencies with statistically significant differences in resting state power between NBM and GPi.

Figure 23: Differences in power between corresponding NBM and GPi resting power spectra. Plots of NBM-GPi power from all 22 hemispheres are included. The red line represents the mean difference in resting state power between NBM and GPi across the group. Red stars indicate the frequencies at which mean differences in resting state power between NBM and GPi were statistically significant at group level. See Figure 24 below for a zoomed-in view of these particular frequencies. The power is normalised to the mean power across the 55-95 Hz frequency range.
Figure 24: Differences in power between corresponding NBM and GPi resting power spectra, zoomed-in view of low frequencies. Plots of NBM-GPi power from all 22 hemispheres are included. The red line represents the mean difference in resting state power between NBM and GPi across the group. Red stars indicate the frequencies at which mean differences in resting state power between NBM and GPi were statistically significant at group level. The power is normalised to the mean power across the 35-95 Hz frequency range.

4.3.2 Posner’s Covert Attention Test (PCAT)

**Behavioural results:** across the eight patients (4 PDD and 4 DLB) who successfully completed the PCAT LFP recording a total of 1422 valid trials and 474 invalid trials were completed. However, average response accuracy was only 55%, which was not significantly different from chance level of 50% accuracy (one-sample T-test, p=0.617). This indicated that only half of all trials (valid or invalid) were completed correctly, and therefore that patients struggled to complete the task properly in the post-operative period. The mean response times at group level for valid and invalid trials were 731.57 ms (SD 243.96 ms) and 761.31 (SD 273.57) respectively (Fig. 25). These reaction speeds are slow compared to speeds of 300 ms and under in normal controls (Posner, 1980), but are within the expected range for this patient.
group (pre-operative reaction times in the PDD group were 769.49 ms (SD 232.61 ms) for valid trials and 774.06 ms (SD 198.49 ms) for invalid trials). Although there was a difference between the mean response times for valid and invalid trials of 29.74 ms (SD 57.46 ms), consistent with the so-called ‘Posner effect’ (wherein mean reaction time for invalid trials is prolonged compared to that for valid trials due to the requirement to re-orient (shift) attention in the former) this failed to reach significance for the group (two-tailed paired samples T-test, p=0.187).

![Average response time across all subjects](chart.png)

**Figure 25: Behavioural results from the Posner paradigm.** The red line shows the difference between the group level average reaction times to valid and invalid trial types, the so-called ‘Posner effect’ or reaction time cost caused by the requirement to re-orient visual attention to the unexpected target location in the latter. Black bars show the standard error of the mean for valid and invalid trials.
Electrophysiological results: Grand average time-frequency power spectra (averaged across all sixteen hemispheres) for both NBM and GPi during the Posner task are shown in Figure 26 below. There are no clear differences in the visualised spectra when locked to cue, target or response, or between the two nuclei when compared to one another. This is not surprising given that the behavioural results show that the majority of the patients struggled to complete the task correctly, with response accuracies of around 50% (chance level) in six of the eight patients. Consequently, any evoked activity in NBM and/or GPi during correctly completed trials is likely to have been lost when averaged with noise from the high number of incorrectly completed trials across the patient group.

Despite these limitations, analysis of the group level results in SPM did reveal some effects of trial validity on low frequency NBM activity: analysis of the contrast ‘invalid trials’ > ‘valid trials’ on the NBM LFPs confirmed a main effect of the invalid target on NBM activity with a significant cluster at 1.26 Hz, 767 ms pre-target appearance (Ke 39, p=0.003 corrected), possibly representing an anticipatory effect to the target. Analysis of the reverse contrast (valid trials > invalid trials) also showed a main effect of the valid target on NBM activity with a significant cluster at 1.98 Hz, 600 ms pre-target appearance (Ke 48, p<0.001 corrected). Again this may represent an anticipatory effect to the target. Since both these effects occurred within a similar timeframe prior to target presentation then it seems likely that they both represent an anticipatory effect to target presentation generally, but not specifically to the nature of the target (valid/invalid). Analysis of the same contrasts in GPi did not show any effect of either target type on activity in that nucleus, suggesting that the anticipatory activity seen to the target was specific to the NBM.

Further contrasts did not show any effects of trial validity on activity locked to the response in either nucleus. There were also no significant differences detected between the two nuclei in activity locked to the cue, target or response for either trial validity.
When restricting the analysis to include only the two patients who performed the task well (both achieved response accuracies of >95%, and were the only two patients with response accuracies >55%) analysis of the contrast ‘invalid trials’ > ‘valid trials’ on the NBM LFPs again confirmed a main effect of the invalid target on NBM activity with a significant cluster at 1.34 Hz, 267 ms pre-target appearance (Ke 26, p=0.014 corrected). The reverse contrast did not show any significant clusters, in this case suggesting that the anticipatory activity was specific only to invalid targets. No other contrasts found significant results.
Figure 26: Grand average time-frequency power spectra for NBM and GPi locked to cue, target and response for both valid and invalid trial types. For the NBM and GPi plots aligned to the onset of the warning cue the time window extends to 1000 ms post-cue presentation, to prevent contamination from motor responses which would occur after this time period. Contamination from anticipatory activity related to the upcoming target cannot be excluded however. Power was calculated relative to a 0.8 s period prior to the appearance of the warning cue. Warm colours indicate an increase in power at the respective frequencies, with cooler colours indicating a reduction in power (see colour bar).
4.3.3 Sustained attention to response task (SART)

**Behavioural results:** Across the seven patients who completed the SART LFP recordings a total of 2282 total trials were completed. As discussed previously (Chapter 3 Discussion, Section 3.3), correct performance on the SART task is shown by a low overall omission error rate. As shown by Figure 27 below only two of the patients (2 and 7) achieved a good rate of omission errors (<5%). Two further patients (1 and 4) displayed an omission error rate of 25-30%, but their commission error rates were still higher (55% and 43% respectively), indicating that they were making a good attempt to complete the task correctly, even if they found it difficult. In the remaining three patients (3, 5 and 6) their omission error rates were much higher than their commission error rates, which suggests that they were not performing the task correctly as they were making very few responses at all to any trial type.

This pattern in individual performance by the patients is generally reflected by their reaction times in Figure 28: those who completed the task correctly (patients 1, 2, 4 and 7) showed faster average reaction times when making commission errors (responding on NoGo trials) than when making correct responses on Go trials. This is because the fast repetitive nature of the SART task encourages patients to generate a pre-potent response to any presented digit. The pre-potent response is automatic, and is faster than a volitionally-guided response under attentional control. If there is a lapse in sustained attention then the subject will fail to exert volitional control over the pre-potent response, which leads to failure to inhibit the response during NoGo trials and consequent commission errors. Although a lapse in sustained attention (and consequent switch to pre-potent responses) will mean reaction speeds are faster for both correct Go and incorrect NoGo trials, the relative scarceness of the latter trial type compared to the former means that the average reaction time for incorrect NoGo trials (commission errors) will be lower overall (Robertson et al., 1997).

Conversely, subjects who are not performing the task correctly (not paying adequate attention) will tend to show equal reaction times on correct Go and incorrect NoGo trials, or
faster reaction times on the former. This is because the majority of all responses will be pre-potent in this situation, and the greater number of correct Go trials therefore biases the mean reaction time in the direction of this trial type. This pattern can be seen in the reaction times for patients 3 and 6 (Fig. 28), again indicating that they did not perform the task correctly.

The reaction times for Patient 5 appear to suggest that he was performing the task correctly since his average reaction time on incorrect NoGo trials was much faster than on correct Go trials (Fig. 28). However, further scrutiny of his individual results showed that he actually only made incorrect responses on two NoGo trials in the whole experiment, which consequently placed undue bias towards his reaction times on these trials. As discussed above his high omission error rate (Fig. 27) indicates that he was not performing the task correctly.

Only the four patients (1, 2, 4 and 7) who had performed the task correctly according to the analysis of their behavioural results were included in the electrophysiological analysis.

![Figure 27: Behavioural results from the SART paradigm: Error rates. Commission error rates are percentage of total NoGo trials presented on which the patient erroneously made a response. Omission error rates are percentage of total Go trials presented on which the patient failed to make the required response. Good performers are those patients where the commission error rate is higher than the omission error rate. Poor performers are those where the omission error rate is higher than the commission error rate. Average error rates for the group as a whole are shown in the final column. Error bars represent the standard error of the mean error rates across trial blocks.](image-url)
Figure 28: Behavioural results from the SART paradigm: Average reaction times. Good performers are those patients who displayed faster average reaction times on incorrect NoGo trials compared to correct Go trials. Average reaction times for the group as a whole are shown in the final column. Error bars represent the standard error of the mean for each subject.

**Electrophysiological results:** Grand average time-frequency power spectra (averaged across the eight hemispheres from the four patients) for both NBM and GPi during the SART task are shown in Figure 29 below. Cyclical high frequency artefacts are present in many of the spectra except in those for correct Go trials. This is because correct Go trials occurred in the highest number (969 completed across the four patients), and averaging across such a high number served to remove such artefacts. However, the total number of other trial types completed was much smaller due to their relative infrequency in the paradigm (totals of 189 incorrect Go, 85 correct NoGo and 64 incorrect NoGo trials), and averaging over these smaller numbers was not sufficient to remove such high frequency artefacts.

Nevertheless, at lower frequencies, several differences in the individual plots are apparent:
First, there appears to be a greater reduction of delta power in the NBM immediately following presentation of NoGo cues compared to following Go cues (comparing Plots C and D to Plots A and B). This suggests that there may be a greater desynchronisation of delta power in NBM in conditions where the pre-potent response requires inhibition. Analysis of the contrast ‘correct NoGo trials’ > ‘correct Go trials’ on the NBM LFPs in SPM confirmed a main effect of the NoGo cue on NBM activity with a significant cluster at 1.1 Hz, 567 ms post-cue (Ke 70, p<0.001 corrected). The reverse contrast ‘correct Go trials’ > ‘correct NoGo trials’ did not show any significant clusters around the same time point post-cue, confirming that the effect was specific to the NoGo cue rather than simply to the presence of a cue itself.

There also appears to be a greater reduction of delta power in the GPi immediately following presentation of NoGo cues compared to following Go cues (comparing Plots I and J to Plots G and H below). Analysis of the contrast ‘correct NoGo trials’ > ‘correct Go trials’ on the GPi LFPs in SPM also confirmed a main effect of the NoGo cue on GPi activity with a significant cluster at 0.95 Hz, 600 ms post-cue (Ke 49, p<0.001 corrected). The reverse contrast again did not show any significant clusters, confirming that the effect was specific to the NoGo cue.

Analysis of the contrasts ‘NBM’ > ‘GPi’ and ‘GPi > NBM’ on the LFPs locked to the cue on correct NoGo trials did not show any significant clusters around the 600 ms post-cue time point for either contrast. This therefore suggests that there was no significant difference in the delta band desynchronisation seen in both nuclei following NoGo cues on correct trials.

Second, in NoGo trials there appears to be a greater desynchronisation in delta band power in NBM following the cue in those trials which are correct compared to those which are incorrect (Plot C compared to Plot D). This suggests that there may be a greater desynchronisation of delta power in NBM during periods of sustained attention (correct NoGo trials) compared to during attentional lapses (incorrect NoGo trials). Analysis of the contrast ‘correct NoGo trials’ > ‘incorrect NoGo trials’ on the NBM LFPs in SPM confirmed
a main effect of correct outcome on NBM activity with a significant cluster at 1.5 Hz, 553 ms post-cue (Ke 50, p<0.001 corrected). The reverse contrast did not show any significant clusters, confirming that the effect was specific to a correct outcome.

When the same contrast (‘correct NoGo trials’ > ‘incorrect NoGo trials’) was applied to the GPi LFPs in SPM a main effect of correct outcome on GPi activity was also seen with a significant cluster at 0.71 Hz, 667 ms post-cue (Ke 44, p<0.001 corrected). However, when the reverse contrast was applied (‘incorrect NoGo trials’ > ‘correct NoGo trials’) a main effect of incorrect outcome on GPi activity was also seen with two significant clusters; one at 1.58 Hz, at 900 ms before the cue (Ke 46, p<0.001 corrected) and one at 0.71 Hz 300 ms post-cue (Ke 26, p=0.029).

Finally, looking at activity locked to the motor responses, there was no effect of cue type (Go cue vs NoGo cue) on activity in either NBM or GPi individually, which is not surprising as the motor response was identical in the two trial types where responses were made (correct Go trials and incorrect NoGo trials). Comparing activity locked to the motor responses between the two nuclei (contrasts NBM>GPi and GPi>NBM) there were no significant clusters detected for either contrast in incorrect NoGo trials. In correct Go trials however, the contrast GPi>NBM showed a significant cluster at 1.19 Hz at 33 ms post-response (Ke 44, p<0.001 corrected), while the contrast NBM>GPi showed two significant clusters, one at 1.19 Hz at 333 ms pre-response (Ke 27 p=0.03 corrected) and one at 1 Hz at 533 ms post-response (Ke 33, p=0.006 corrected).
Figure 29: Grand average time-frequency power spectra for NBM and GPi locked to cues and responses for different trial types in the SART. Time windows extent to 1000 ms pre- and post-cue/response. For the NBM and GPi plots aligned to the onset of the cues in Correct Go and Incorrect NoGo trials the plots will be contaminated by the motor responses occurring afterwards, at 750 ms and 600 ms post-cue respectively (see Figure 28 for the average response times). Likewise, the opposite is true of the NBM and GPi plots aligned to the responses for these particular trial types. In all plots contamination from anticipatory activity related to the upcoming cues cannot be excluded. Power was calculated relative to a 0.8 s period prior to the appearance of the cues. Warm colours indicate an increase in power at the respective frequencies, with cooler colours indicating a reduction in power (see colour bar).
4.4. Discussion

Overall, our LFP experiments revealed a number of interesting findings with regard to the physiological functioning of the human NBM in vivo.

The rest recordings served to demonstrate the spectrum of resting state activity in the human NBM for the first time: this was characterised by high power in the delta band, as well as the theta and alpha bands, all significantly greater than that recorded in neighbouring GPi. These results therefore confirm our hypothesis above that resting state NBM activity is significantly different to resting GPi activity.

However, the NBM degenerates significantly in LBDs (Gratwicke et al., 2013) and see Section 1.3.1.5 above), and therefore its resting state activity described here cannot be taken to be representative of that seen in the healthy human NBM in vivo. Interestingly, both EEG and MEG studies have shown that cognitive decline in Lewy body diseases is associated with increasing cortical delta oscillatory power alongside a relative decline in faster beta and gamma activities (Bosboom et al., 2006; Caviness et al., 2007; Franciotti et al., 2006; Ponsen et al., 2012). It is therefore possible that the high delta power observed in the NBM of our LBD patients is the subcortical correlate of this, especially since evidence suggests that cortical activation rhythms are driven by ascending control from NBM (Buzsaki et al., 1998; Kalmbach et al., 2012; Lee et al., 2005; Metherate et al., 1992; Nguyen and Lin, 2014a).

Therefore, our resting state observations can only be taken as relating specifically to resting NBM activity in the Lewy body dementia state. Furthermore, a limitation to these recordings is the fact that they were conducted only two to five days after neurosurgery and administration of anaesthetic agents, and therefore depressive after-effects of these factors on physiological functioning of the NBM cannot be excluded.
With regard to the Posner task LFP recordings, the results were heavily confounded by the difficulty that six of the eight patients had in correctly completing the task, evidenced by the generally very slow group reaction times and insignificant ‘Posner effect’ (Figure 25). Despite this, t-contrasts at whole group level showed significant main effects of both valid and invalid targets on NBM activity in the delta band, at 600-767 ms before target presentation. The fact that these changes in delta activity occurred prior to presentation of either target, when the patient cannot have known the upcoming target validity, suggests that they represent general anticipatory activity to an upcoming target rather than an effect of target validity per se.

When the t-contrast was repeated including only the two patients who demonstrated good behavioural performance a similar anticipatory effect on NBM activity in the delta band was again seen to invalid targets, but not to valid ones. This seems difficult to account for if the change in delta band activity was truly anticipatory to an upcoming target, as one would have expected to see it in relation to both target types regardless of validity. However, scrutinising the individual results from these two patients they completed a total of 306 valid trials and 102 invalid trials between them, and therefore the apparent anticipatory delta activity to invalid trials only here may simply be a product of artefact due to the relatively low trial count taken for averaging.

It is difficult to make any clear inferences from the results of the Posner task LFP recordings. The main limitation to this task was its length, as it took roughly 40 minutes for the patients to complete all ten blocks (with necessary rest breaks). Bearing in mind that these were patients with dementia who had undergone invasive neurosurgery and a general anaesthetic only several days beforehand it seems likely than many of them were too fatigued to concentrate properly for this period of time. Indeed, from subjective observation of the individual patients during their
performance of this task recording it was clear that many of them experienced significant lapses of attention and found it progressively more difficult to concentrate as the task continued in length.

Finally, with regard to the SART task LFP recording it is interesting to note that behavioural performance was generally better for this task than for the Posner task. This may be because the task was shorter in duration (taking roughly 20 minutes to complete all ten blocks with necessary breaks) or because it was a simpler paradigm to perform. Four of the seven patients tested completed the task to an acceptable level, evidenced by a higher rate of commission errors than omission errors combined with a faster reaction time during commission errors than on correct Go trials (Figures 27 and 28).

t-contrasts performed at group level (4 patients) showed a significant main effect of cue type on NBM delta band power, with a desynchronisation at 1.1 Hz seen 567 ms following a NoGo cue, but not following a Go cue (p<0.001 corrected, Figure 29 Plot C compared to Plot A). This suggests that NBM activity in the immediate post-cue period reflected the fact that the pre-potent response required inhibition in NoGo trials. The same contrast showed a similar main effect of cue type on delta band power in GPi, with a significant desynchronisation at 0.95 Hz seen 600 ms following presentation of NoGo cues but not not Go cues (p<0.001 corrected, Figure 29 Plot I compared to Plot G). No significant difference between activity in the two nuclei was seen following NoGo cues, and this combined with the similar frequencies and timeframe of the post-presentation evoked responses in both nuclei suggests that activity in both reflected the need to inhibit the pre-potent response in equal measure. A possible volume conduction effect from one nucleus to the other cannot be excluded in the present analysis.
Comparing NoGo trials with correct and incorrect outcomes (purported to reflect maintenance of sustained attention and attentional lapses respectively), there was a significant main effect of correct outcome on NBM delta band power, with a further desynchronisation at 1.5 Hz seen 533 ms post-cue in correct trials, but not in incorrect trials (p<0.001 corrected, Figure 29 Plot C compared to Plot D). Combined with the previous finding this suggests that NBM activity in the immediate post-cue period reflected the requirement to inhibit the pre-potent response and was further modulated by the background level of sustained attention. The same contrast performed in the GPi LFPs also showed a significant main effect of correct outcome on delta band power, but this was at a lower frequency (0.71 Hz) and 114 ms later (at 667 ms) than the main effect seen in NBM. Furthermore, the reverse contrast in GPi also showed a significant main effect of incorrect outcome on delta band power at two different timepoints; a desynchronisation at 1.58 Hz 900 ms pre-cue (p<0.001 corrected), and a desynchronisation at 0.71 Hz at 300 ms post-cue (p=0.029).

These GPi findings were different to those found in NBM, and suggest that post-cue delta activity in GPi reflected not only the need to inhibit the pre-potent response, but also reflected the outcome of this by differential timings of desynchronisation at the 0.71 Hz frequency, with a longer latency post-cue (around 667 ms) when the response was successfully inhibited, and a shorter latency (300 ms) when it was not.

Finally, comparing activity locked to the motor response between the two nuclei; GPi showed a greater increase in delta power locked to the response itself (1.19 Hz, 33 ms post-response, p<0.001 corrected), while NBM showed increased delta power at two timepoints, 333 ms pre-response (1.19 Hz, p=0.03 corrected) and 533 ms post-response (1 Hz, p=0.006 corrected).

Overall, therefore, evidence from the SART task suggested that NBM delta activity locked to the cue reflected both the need to inhibit the prepotent response, and the background level of
sustained attention, while GPi delta activity locked to the cue reflected the need to inhibit the pre-potent response, and whether or not this was successfully achieved. Furthermore, as expected, GPi delta activity locked to the response appeared to reflect the motor response itself. The relevance of the weaker changes in NBM delta activity pre- and post- the motor response are unclear, but may reflect anticipation and confirmation of execution of the response respectively.

Taking both attention tasks into account, the significant modulation of NBM activity in the SART task, combined with the apparent lack of modulation in its activity during the Posner task might support the idea that the nucleus plays a role in sustained attention, but not in orienting of attention. This would be in line with our hypothesis above that activity in the nucleus preferentially reflects one mode of attention over the other. However, the fact that the Posner results are so heavily confounded by the difficulty the patients had in completing the task means that it would be premature to make that conclusion based upon the current dataset. Furthermore, there are a number of other limiting factors to these task recordings, which restrict our ability to draw such inferences.

First, a particular limitation to the SART task must be borne in mind when interpreting our results. Although performance of the task certainly demands sustained attention to action, correct performance is also highly dependent on the participant’s ability to actually inhibit the pre-potent response upon the appearance of the NoGo cue, i.e. their capacity for response inhibition, a core executive function (Dirnberger and Jahanshahi, 2013). Response inhibition has been described in terms of a horse-race model, whereby go and stop processes are stochastically independent and race to completion, with the winner determining whether the response will be inhibited or not (Logan, 1994). Therefore, during performance of the SART task, sustained attention will be necessary in maintaining a strong task set in the inter-cue intervals, and response inhibition will
be required to resolve the conflicting response tendencies. A deficiency in either cognitive ability can therefore potentially lead to errors (Braver and Barch, 2006). LBD patients are known to suffer impairments in both these domains (see Section 1.2 and (Gratwicke et al., 2015a)), and indeed our patients demonstrated marked impairments in both at baseline (Tables 4, 14 and 20). Therefore it is not currently possible to determine whether the commission errors made on the SART task were due to impairments in sustained attention or response inhibition, and therefore whether the main effect of outcome on NBM LFP activity was a reflection of lapses in the former or the latter.

Our data are also limited by the small numbers of patients who managed to complete the tasks successfully, which resulted in relatively small numbers of rare event trials (invalid trials in the Posner and NoGo trials in the SART respectively) being recorded relative to the large number of common trials (Valid trials and Go trials respectively). As discussed above, averaging across low trial numbers makes the occurrence of artefacts in the electrophysiological data more likely, which will further confound the results obtained. We tried to counter this problem by applying robust averaging methods to the dataset to boost the signal-to-noise ratio (Litvak et al., 2012), however this can have the side-effect of introducing artefacts into the data itself if patients were not performing the task correctly (as in the case of the Posner task recordings).

In addition, as discussed above, the NBM degenerates significantly in LBDs ((Gratwicke et al., 2013) and see Section 1.3.1.5 above), and therefore the task-related evoked activity in NBM described here cannot be taken to be representative of that which might be seen in the healthy human NBM during the same task paradigms. In addition, confounding effects on NBM activity due to the recent invasive neurosurgery and administration of a general anaesthetic cannot be excluded.
In summary, there are many potential confounding influences and limitations to the data presented here, which restricts our ability to make sound inferences regarding the normal physiological function of the NBM. Nevertheless, this was a unique opportunity to directly investigate the physiological properties of the human NBM in vivo for the first time. In this context the present results provide preliminary evidence that spectral activity in the nucleus is distinct from neighbouring GPi, both at rest and during active behaviour, and may reflect the prevailing level of sustained attention as previously postulated.

Future studies of NBM LFPs in LBD patients would benefit from a longer interval period between surgery and recordings so that patients can recover to a greater extent before attempting cognitive tasks. This might lead to better task performance. In addition, long task paradigms, which cause gradual exponential fatigue in this patient population and thereby deteriorating performance over time, should be avoided if possible. However, unless NBM DBS implantation is attempted in less severely demented and younger patients (who have less degenerate nuclei and are also less vulnerable to the after-effects of surgery and anaesthetics) then future electrophysiological studies may find it difficult to avoid many of the limitations described above.

Finally, to help determine whether evoked activity in NBM is due to sustained attention or response inhibition future studies would benefit from using a different version of the SART, called the SART Fixed. The SART Fixed is identical to the SART apart from the fact that stimuli are presented in a predictable, fixed sequence from 1 to 9. It is hypothesized that this predictable target sequence facilitates preparation of the no-go (withheld) response to such an extent that the race between the go and stop process is largely eliminated, thereby minimising demands on response inhibition (Manly et al., 2003). Simultaneously, by making the appearance of targets entirely predictable, the test is rendered more monotonous and less exogenously alerting, thereby
increasing the demands on sustained attention. Therefore, evoked activity recorded from NBM during performance of the SART Fixed would be much more likely to reflect sustained attention than response inhibition.
Chapter 5: Simultaneous recording of cortical magnetoencephalography and local field potentials from the nucleus basalis of Meynert in patients with Lewy body dementias

5.1 Introduction

Magnetoencephalography (MEG) is a non-invasive functional neuroimaging technique that relies on recording the magnetic fields which are generated by neuronal activity. Any electrical current will produce a magnetic field perpendicular to its direction of transmission (this is described by Maxwell’s equations. See Figure 30, panel A), and this phenomenon is the basis of the magnetic signals recorded by MEG. Post-synaptic ionic currents flowing through neural dendrites produce such a magnetic field, and the MEG signal is based upon the temporal and spatial summation of these fields across a population of neurons. Since the magnetic field generated by synaptic activity in an individual neuron is of a very small order of magnitude, simultaneous activation of approximately 50,000 neurones is required to generate a detectable signal (Okada, 1983). However, if directions of current flow vary significantly amongst the neuronal population then the differing vectors of the magnetic fields generated will cancel each other out. Therefore, in order to generate a signal sufficient to be detected by MEG, neurones and their dendrites must have similar orientations such that they generate magnetic fields of a common direction, which reinforce each other. In this regard, the activity of cortical pyramidal cells is most likely to contribute to the MEG signal. Amongst this neuronal population MEG is most sensitive to activity originating in cortical sulci, as dendrites here travel parallel to the scalp, thus meaning that the perpendicular direction of their magnetic fields passes straight towards the MEG sensors.
overlying the scalp (Figure 30, Panel B). Conversely, pyramidal cells in gyri lie perpendicular to the scalp, thus their magnetic fields travel parallel to the scalp and are not picked up by MEG. It is this selective sensitivity to activity in different neuronal sub-populations that gives MEG its superior spatial resolution compared to modalities such as EEG, which picks up both tangential and radial components of current sources and is thus less selective (Cohen and Cuffin, 1983).

![Figure 30: The physiological basis of the MEG signal. Panel A illustrates the physical phenomenon that the direction of the magnetic field generated by an electrical current is perpendicular to its direction of transmission (as described in Maxwell’s equations). Following on from this, Panel B illustrates the fact that MEG can only detect magnetic signals generated in pyramidal cells in cortical sulci, as their physical orientation (blue arrow) means that the direction of magnetic fields generated from current flow in their dendrites crosses the skull. Pyramidal cells in gyri will generate magnetic fields parallel to the skull, which cannot be detected by MEG.](image)

MEG systems record magnetic fields using superconducting quantum interference devices (SQUIDS) which are set in a helmet-shaped enclave overlying the participant’s head. However, even when summated across thousands of neurones, magnetic activity recorded from the brain is orders of magnitude lower than background activity due to the earth’s magnetic field, the
movement of nearby ferromagnetic objects (e.g. traffic, underground trains) and other
electrophysiological activity (e.g. muscle activity and the cardiac cycle). The effect of these
external interferences is therefore reduced by enclosing the MEG system within a magnetically
shielded room. Furthermore, since sources of noise are typically much further away than the brain
signal of interest, it is possible to achieve noise suppression by using the gradient between two
neighbouring sensors. Noise from distant sources will be the same at both sensors and is therefore
suppressed by subtraction. However, for closer sources (i.e. brain sources), the activity recorded
by both sensors will be subtly different, giving rise to a differential signal. Modern MEG systems
therefore use such combinations of sensors, termed axial gradiometers, to suppress distant
magnetic interference.

Functional imaging using MEG is therefore a highly sensitive method for measuring cortical
neural activity. As with other modalities (EEG, fMRI) it has limited ability to resolve activity in
deeper brain structures (sensitivity loss is proportional to the squared distance between source and
MEG sensor), but it has the advantage that it can be combined with simultaneous deep brain LFP
recordings, allowing functional connectivity (coherence) between the subcortical structure and
cortical areas to be measured. This is not easily feasible using fMRI, due to both the image
artefact caused by DBS electrodes and the substantial metallic recording equipment that is
currently needed for LFP measurement, which poses a safety risk in the magnetic field of the
scanner. It is also difficult to perform with EEG given that neural electric fields are distorted by
the presence of burr holes (van den Broek et al., 1998) and scalp recording sites are limited in the
presence of post-operative surgical wounds and dressings. However, magnetic fields are less
distorted by burr holes and MEG can be recorded post-operatively with a large amount of sensors
around the head without direct skin contact (Litvak et al., 2010), which adds weight to its utility
in this situation.
Evidence from combined STN LFP and MEG recordings in PD patients undergoing DBS for motor symptoms have previously demonstrated the existence of two spatially and spectrally distinct cortico-STN networks involved in movement processing (Hirschmann et al., 2011; Litvak, Jha, et al., 2011). Such recordings from patients undergoing DBS therefore represent a powerful tool to study interactions between distant brain regions and thereby characterise diffuse functional networks.

As discussed above, the exact physiological function of the NBM remains unknown. However, our LFP recordings in the previous chapter provided some potential insights on this issue, demonstrating significantly higher delta power in the resting state nucleus compared to neighbouring GPi, and suggesting that this activity may be differentially modulated by the subject’s level of sustained attention. The period of post-operative electrode externalisation also afforded us the unique opportunity to record simultaneous resting NBM LFPs and cortical MEG in our awake patients, and we were therefore able to directly investigate interactions between activity in the nucleus and the cortical mantle more widely to gain further insight into its possible function.
5.2 Materials and Methods

5.2.1 Patients

As mentioned above all PDD and DLB patients who participated in the clinical trials were planned and consented to undergo combined NBM LFP and MEG recordings in the period of electrode externalisation on the ward, between electrode implantation and IPG implantation (a period of 4-7 days on average). Clinical characteristics of the patients and stereotactic co-ordinates of the most ventral electrode contacts in each hemisphere are detailed in Tables 1 and 11 above. Figures 10 and 14 above show anatomical locations of most ventral DBS contacts in the PDD and DLB patients respectively. Ethical approval and consent for combined NBM LFP and MEG recordings were included in the main ethics applications and consent forms respectively for the clinical trials (as described above in Sections 2.1.3 and 3.1.3).

5.2.2 Experimental Paradigm

Patients attended our research MEG suite during the daytime having taken their usual medications (including their usual doses of both levodopa and AChEI medications). We only conducted resting state recordings, in order to attempt to characterise the cortical connectivity and coherence of NBM. While sitting comfortably in the MEG scanner, patients were instructed to remain still with their eyes open for 3 minutes. They were instructed to focus their gaze on a fixation point projected onto a screen in the scanning room using MATLAB (The Mathworks Inc, Natick, MA, USA) and a custom script based on the Cogent toolbox (http://www.vislab.ucl.ac.uk/cogent.php). A neurologist was present in the magnetically shielded room during the experiments to monitor the patients’ wellbeing and also to ensure that they
remained awake throughout the recording. A single rest recording session lasted about 1h (most of that time being for experimental set-up and preparation).

5.2.3 Magnetophysiological and electrophysiological data acquisition

MEG recordings were performed with a 275 channel CTF system (VSM MedTech Ltd., Vancouver, Canada). A strength of this particular system is the high dynamic range of its sensors and their robustness to strong external interferences. MEG data were sampled at 2400 Hz and stored to disk for subsequent offline analyses.

Head location in the MEG scanner was monitored using three head position indicator (HPI) coils attached to the subject’s nasion and both pre-auricular points. For each subject head locations were recorded continuously throughout the experiment. Loss of head tracking occurred intermittently in some patients, possibly due to metal artefacts disrupting the head tracking function of the sensors. Three fiducials were attached to the patient on the nasion, and on the left- and right- pre-auricular points to enable later offline co-registration of the MEG sensors to the patient’s structural brain MRI scan for source-level analysis (see Section 5.1.6 below).

Bilateral NBM and GPi LFPs, electro-oculographic (EOG) and electromyographic (EMG) signals were simultaneously recorded using a battery-powered and optically isolated BrainAmp system (Brain Products GmbH, Gilching, Germany). As this is a separate recording system to the main MEG system the challenge that this approach poses is the fusion of the LFP and MEG data with minimal timing distortions. To facilitate this, a common synchronisation signal was recorded on both systems – the signal used was random white noise because it can only be matched in a unique way. Note that connecting the noise generator with cables to both the MEG and the LFP amplifier would create a breach in the patient’s optical isolation from the mains power supply,
which would pose a potential safety concern. However, the BrainAmp system incorporates two optically isolated amplifiers into one system with synchronous sampling – accordingly one of these amplifiers was used to record the noise signal, whilst the other was used to record the electrophysiological signals (see Figure 31).

Eight intracranial LFP channels representing contacts 0-3 of each DBS macroelectrode were converted using bridge connectors into a bipolar montage, between adjacent contacts. Six separate DBS channels (R01, R12, R23, L01, L12, L23) were therefore recorded referenced to the left clavicle. As in the LFP experiments in the preceding chapter, sampling LFPs in a bipolar fashion served to limit the effects of volume conduction from distant sources. EMG data were recorded from tendons of the right and left first dorsal interosseous muscles to serve as references for movement artefact in MEG. Recorded electrophysiological signals were amplified (X 50,000), hardware filtered (1.0 – 300 Hz), sampled at a common rate of 2400 Hz and stored to disk on an acquisition laptop.

Figure 31 below shows a schematic illustration of the experimental set-up, which was approved by the MEG safety board of the Wellcome Trust Centre for Neuroimaging, following extensive in-house safety testing.
Figure 31: Schematic representation of the set-up for simultaneous NBM LFP and MEG recordings. All LFP bipolar channels from the left and right electrodes (grey lines) and reference lead from the patient’s left clavicle (black line) are recorded by the BrainAmp bipolar headbox. Black dashed arrows depict optic fibre cables, which serve to optically isolate the patient from the mains power source (see section 5.1.3 above). The timings of signals recorded from the MEG and the LFP acquisition laptop are synchronised through the independent recording of a single white noise source on the two systems (see section 5.1.3 above for further details). Figure modified from a figure in Oswal et al., 2016 by kind permission of the authors.
5.2.4 Data pre-processing

The data were imported into MATLAB (The Mathworks, Inc, Natick, MA) and analysed using custom scripts in conjunction with the SPM (http://www.fil.ion.ucl.ac.uk/spm/) and FieldTrip (http://www.ru.nl/neuroimaging/fieldtrip/) toolboxes (Litvak, Mattout, et al., 2011; Oostenveld et al., 2011). LFP data had been recorded in a bipolar fashion at hardware level and therefore there was no need for offline conversion to a bipolar montage as data was already in this format. Therefore, the LFP file for each patient contained six different channels of data: Right NBM, Right NBM/GPi, Right GPi, Left NBM, Left NBM/GPi, Left GPi. Both LFP and MEG data had been sampled at a common rate of 2400 Hz. The next step was to fuse the two datasets using the white noise recordings which had been recorded alongside both as the common reference in the time domain. This was performed by dividing each dataset into non-overlapping 1s segments and repeatedly cross-correlating the white noise time series until an LFP segment definition was reached which yielded zero-lag when matched to the MEG segment definition (Oswal et al., 2016). The resulting segment definitions for MEG and LFP were then used to epoch the full MEG and LFP data. The epoched datasets were fused and then converted again into a continuous recording (which was straightforward because the segments were consecutive in time). Therefore, in this new dataset MEG and LFP data for the same patient were fused and aligned.

The fused dataset was now divided into arbitrary epochs with duration of 3 ms (1024 samples). To ensure stable head location relative to the MEG sensors for the analysed data the continuous head localisation data from the three HPI coils was analysed: the instantaneous distances between the individual HPI coils at each of the 1024 samples across the three-minute recording was compared to their robust average distances across the whole continuous recording (Litvak et al., 2012). Those data samples where discrepancies of >1cm head displacement were detected were discarded and replaced with linear interpolation based on the other time frames. This method
works well when the tracking is valid for more than half of the recording, which was the case for all recordings reported here. The data were then high-pass filtered at >1Hz and the line noise artefacts at 50Hz and 100Hz were removed using Butterworth notch filters. Trials with artefacts in the LFP recording were rejected by thresholding the peak-to-peak LFP amplitude at 100µV.

5.2.5 Spectral Analysis

All MEG and bipolar NBM/GPi LFP data in the fused dataset underwent spectral analysis using the multi-taper method fast Fourier transform (as previously described in the previous chapter in Section 4.2.5).

5.2.6 Functional connectivity measurement: coherence

The key aim of this experiment is to study possible physiological interactions, or functional connectivity, between the resting NBM/GPi and distant cortical regions. Functional connectivity can be assessed through the statistical relationship of activity signals occurring in two distant brain regions over a discrete time interval. Coherence is one way of measuring this, it provides a frequency-domain measure of the linear phase and amplitude relationships between two signals, bounded between 0 and 1 (Buzsáki and Draguhn, 2004; Thatcher et al., 1986). In other words coherence is the frequency domain counterpart of a cross-correlation in the time domain.

Coherent oscillatory activity between distant neural populations is believed to play an important role in their communication, and implies a functional relationship between the two areas, although it does not provide any information about the directionality of coupling (Bastos et al., 2015; Fries, 2005).

Coherence was first calculated at sensor-level, between each NBM/GPi LFP channel and each MEG channel (which individually represent each physical MEG sensor in space) in order to
define frequency bands of significant coherence within each patient (Litvak, Jha, et al., 2011): Coherence was computed between 5–45Hz (low frequency), with 2.5Hz resolution, and between 60–90Hz (high frequency) with 7.5Hz resolution. Scalp maps of coherence for each frequency bin were linearly interpolated to produce a 2D image. The resulting images were stacked to produce a 3D image with two spatial and one frequency dimension (Kilner and Friston, 2010). This resulted in separate images for high and low frequencies. To determine significant regions within these images, it was compared with null (surrogate) data in which any coherence was destroyed: ten surrogate coherence images were generated from the same MEG data but with the order of the time series for the NBM/GPi LFP channel data shuffled. The original and surrogate images were smoothed with a Gaussian kernel (10mm x 10mm x 2.5Hz for the lower frequencies, 10mm x 10mm x 7.5Hz for the higher frequencies, to ensure conformance to the assumptions of random field theory). They were then subjected to a two-tailed paired samples t-test in SPM (thresholded at P<0.01 family-wise error corrected) to identify significant regions in sensor space and frequency (Litvak, Mattout, et al., 2011). Thereby, for each individual NBM/GPi LFP, this provided frequency ranges where there was significant sensor-level coherence over MEG channels.

With this information in hand, coherence was then analysed at source-level, between each NBM/GPi LFP channel and a 3D grid of points representing spatial locations within the brain, in order to locate coherent cortical sources. This employed the dynamic imaging of coherent sources (DICS) beamforming method (Gross et al., 2001). A summary explanation of this methodology is as follows: MEG data can be directly analysed in relation to the locations of its individual sensors relative to the skull (sensor-level analysis, as above). However, this has limited spatial localising power. Source-level analysis/reconstruction aims to improve spatial localisation by projecting sensor-level data onto 3D brain space. This representation of brain space comprises a large
number of dipolar magnetic sources with fixed locations and the orientations and amplitudes of each of these sources needs to be determined before the sensor-level data can be ‘fitted’ to it correctly and the precise locations of coherent sources determined. This is because sensor-level data could be significant if fitted to a dipole with one particular orientation and/or amplitude, and not significant if fitted to another (see Section 5.1, Introduction, and Figure 30 above for an explanation and graphical illustration of why this might be the case). First, the co-ordinate system of the MEG sensor positions is mapped to the co-ordinate system of the patient’s structural brain MRI in Montreal Neurological Institute (MNI) space according to the three recorded fiducial points: nasion, left and right pre-auricular (‘co-registration’, see (Litvak et al., 2010)). Then an estimation is made of how a brain dipole at a particular location with a particular orientation maps to the individual MEG sensors. This step is known as forward-modelling and is achieved according to a summation of individual Maxwell equations for each dipole, corrected for a spherical volume conductor (the skull). The single shell forward model proposed by Nolte is based on this principle (Nolte, 2003), and we applied this model to an inner skull mesh derived from inverse-normalising a canonical mesh to each individual patient’s pre-operative structural MRI image (Mattout et al., 2007). Thereby a forward model was generated for each individual patient, and was specific to that particular patient alone. In conjunction with the previous co-registration of the patient’s MRI to the MEG sensors, each individualised forward model therefore allows an estimation to be made of how the individual spatially distinct dipole sources in that patient’s brain are likely to map to the MEG sensors. The final step is to generate the inverse model – to predict the original dipolar activity across the brain from the MEG cross-spectral density matrix (source reconstruction). Beamforming achieves this by assuming a linear projection of the signal picked up from each MEG sensor, then applying an adaptive spatial filter to mathematically minimise the variance between the sensor-level data recorded and the predicted
sensor-level data from the forward model (Gross et al., 2001; Litvak, Jha, et al., 2011). The final result is cross-spectral coherence values computed on a 3D grid of the individual patient’s brain in MNI space, with spacing of approximately 5mm and bounded by the inner skull surface. This allows accurate spatial determination of cortical sources coherent with the NBM/GPi LFPs across different frequencies. Values at the gridpoints are then linearly interpolated to produce volumetric images with 2 mm resolution and smoothed with an 8mm isotropic Gaussian kernel. Of note beamforming was the chosen method of source reconstruction here since it has previously been shown to be effective at suppressing artefact generated by the ferromagnetic cables connecting the DBS electrodes to the recording equipment (Litvak et al., 2010).

To identify the cortical areas consistently coherent with the NBM across the group a group mean image was generated from the individual patient images. These images had been normalized prior to averaging (by dividing the coherence at each beamformer grid point by the mean of that image) in order to ensure that each included hemisphere-contact pair contributed equally to the calculation of the average. All images corresponding to left NBM contacts were flipped across the mid-sagittal plane to allow comparison of ipsilateral and contralateral sources to the NBM regardless of original NBM side. The global maximum of the resulting image was defined as the cortical source maximally coherent with NBM across all subjects.

5.2.7 Statistical analysis

Statistical analysis was performed using a general linear model (GLM) based approach, implemented in the SPM12 software package, identical to that described in Section 4.2.7.2 in the preceding chapter. All the reported findings are significant with family-wise error correction \( (p<0.05) \). In the results I also report the peak t statistic with the corresponding degrees of freedom and p value.
5.3 Results

All six PDD patients and four of the DLB patients underwent combined resting NBM LFP and MEG recording in the immediate post-operative period. DLB Patient C was too fatigued to complete the MEG recording, and DLB patient D was too unwell in the post-operative period (due to his contraction of antibiotic-associated C.Difficile diarrhoea) to undergo MEG recording. Clinical details and contact locations of the included patients are as previously documented in Chapters 2 and 3. All completed recordings were included in the analysis.

5.3.1 Resting NBM and GPi power spectra

Figure 32 below shows the grand average resting NBM and GPi LFP power spectra averaged over all respective hemispheres in all patients (20 hemispheres per nucleus). As in Chapter 4 resting spectral peaks can again be seen in the delta band, and to a lesser extent in the theta band, in both nuclei. Significantly greater low frequency power was again found in NBM compared to GPi in the delta, theta, and alpha bands (p<0.001 for all). These results were generated from an entirely separate set of NBM and GPi LFP recordings to those used in Chapter 4, and therefore serve to confirm those results. Again, the relatively low activity in the beta band in the GPi spectra is consistent with the fact that all patients had taken their usual levodopa medication prior to LFP recording, which has previously been shown to suppress GPi beta activity (Brown and Williams, 2005).
Interestingly, when separate grand average resting power spectra were computed for the PDD patients (N = 6) and the DLB patients (N = 4) separately (Figure 33), PDD patients showed higher power in the delta and theta bands than their DLB counterparts (although these differences were not significant). PDD patients also showed higher power in the beta band (15-25 Hz) in both NBM and GPi compared to their DLB counterparts, which reached significance in the latter (t=9.0, df=8, p<0.001). It is now clear that beta peaks had not been visible in the resting power spectra when both groups were combined for analysis (Figure 32 above) due to the lack of beta power in the DLB patients obscuring the beta power in the PDD group. The results obtained here
are very similar to the resting LFP recordings in Chapter 4 (Figure 22), reinforcing the validity of the former. However, spectral peaks in the beta band were not seen in those recordings in either PDD or DLB patients. This could be due to methodological differences, or the fact that the microlesion effect in the GPi was greater when the LFP recordings were conducted (often only one or two days after DBS surgery), but had recovered to a greater extent by the time LFPs were recorded in MEG (which tended to be performed several days later) meaning that beta activity in GPi was more readily detectable. In addition, normalised LFP power across the low-frequencies in both NBM and GPi appeared slightly higher in the Chapter 4 recordings (Figure 22) compared to the respective recordings here (Figures 32 and 33). This could be due to the fact that the Chapter 4 recordings were averaged over 11 patients (as opposed to 10 patients here), or differences in hardware filtering of the original signals due to differences in the experimental set-up between the two sets of recordings.

Figure 33: Group average resting NBM and GPi normalized power spectra for PDD and DLB patients. Spectral peaks are evident in the delta and theta bands in both nuclei in both patient groups, with higher power seen in both frequency bands in the NBM compared to the GPi (note the difference in Y axis scales). Spectral peaks can also be seen in the beta band in both the NBM and the GPi in the PDD patients, but not the DLB patients. All power spectra are normalised to the mean power across the 55-95 Hz frequency range. The shaded regions represent standard errors of the mean.
5.3.2 Spatial location of cortical sources coherent with NBM

In total, cortico-NBM coherence was estimated for 20 NBMs from all ten patients. Each of the NBM bipolar channels was used as a reference to calculate the location and frequency range over which significant cortical coherence existed. Although the frequency ranges spanned 5–90 Hz, none of the sources had a scalp pattern typical of a focal cortical source.

5.3.3 Spatial location of cortical sources coherent with GPi

In total, cortico-GPi coherence was estimated for 20 GPs from all ten patients. Each of the GPi bipolar channels was used as a reference to calculate the location and frequency range over which significant cortical coherence existed. Although the frequency ranges spanned 5–90 Hz, none of the sources >45Hz had a scalp pattern typical of a focal cortical source. These high-frequency sources were therefore excluded from further analysis. This resulted in a total of 46 cortically coherent sources in the delta, theta, alpha and beta ranges. The peak frequencies and spatial locations of coherent cortico-GPi sources are displayed in Figures 34 and 35 below. In the frequency range 5-45 Hz, sources fell into two broad bands, which we will term the delta-theta-alpha band at 1–12Hz and the beta band at 15–25 Hz. These ranges formed the basis of the fixed frequency bands used for group analysis. Sources in the beta range clustered around medial motor/premotor areas ipsilateral to the pallidal nucleus, whilst delta-theta-alpha range sources clustered in two areas - ipsilateral temporo-occipital regions and bilateral frontal regions, with ipsilateral predominance.
Figure 34: Peak frequency distribution of potential cortical sources of cortico-GPi coherence. Ten patients (20 GPis) were recorded from (6 PDD and 4 DLB patients). We searched for coherent sources between 5–45Hz and 60–90 Hz, although after subsequent visual lead field inspection we excluded sources >45Hz. Yellow highlight denotes frequencies in the delta-theta-alpha band (1-12 Hz), red highlight denotes frequencies in the beta band (15-25 Hz).

Figure 35: The variation in location and peak frequency of significant cortical sources coherent with GPi in the 5–45Hz frequency range. Results from 20 pallidal nuclei. The images are 'glass brains' (inner boundary of skull marked with grey mesh) viewed from the above, right and front. All left pallidal nucleus sources are reflected across the middle sagittal plane to allow comparison of ipsilateral (right) and contralateral (left) sources. The peak frequency of the coherence is represented by a colour scale where warmer colours reflect higher frequencies.
5.3.4 **Topography of cortical activity coherent with Gpi activity is frequency dependent**

We tested for the effect of frequency on coherence through a further group analysis. We computed DICS images with our two standardized frequency bands (delta-theta-alpha 1–12Hz and beta 15–25 Hz). To allow a balanced comparison these DICS images were normalized by dividing by the mean value over voxels, thereby allowing us to compare differences in the topography of cortico-GPi coherence between the two bands. This confirmed a temporo-occipital preponderance for delta-theta-alpha band coherence with Gpi and a primary motor cortex/premotor area preponderance for beta band coherence with Gpi (Figure 36).

**Figure 36:** Differences in the topography of cortico-GPi coherence between the two bands. Mean of the normalized DICS images. Unthresholded delta-theta-alpha (yellow) and beta (red) coherence is superimposed onto a T1-weighted canonical MRI. Coronal, sagittal and axial sections through the image are displayed, oriented to the respective local maxima for each set of images: Image A shows regions of cortico-GPi coherence in the delta-theta-alpha band, which centre on temporo-occipital cortex. Image B shows regions of cortico-GPi coherence in the beta band, which centre on primary motor cortex/premotor areas. The colour scale is coherence normalized to individual image global values (arbitrary units). A value greater than 4 units means that the activity in that voxel is consistently greater than the mean across the image.
Differences in cortico-GPi coherence between these two frequency bands were then directly compared by analysis in SPM. This confirmed a significant main effect of frequency across these two distinct brain regions (Figure 37, left): Regional cortico-GPi coherence was greater in the delta-alpha-theta band than the beta band in the ipsilateral lateral occipital area (t=12, 8 df, P<0.001). Meanwhile regional cortico-GPi coherence was greater in the beta band then the delta-theta-alpha band in ipsilateral primary motor cortex (t=11, 8df, P<0.001).

To explicitly illustrate the relative differences in frequency of coherence between the two distinct brain regions and GPi, the mean absolute (non-normalized) coherence spectra were computed between the peak values at their respective regional maxima (identified in SPM) and the grand average GPi (and NBM) dipole data (Figure 37, right). These spectra clearly confirm that delta-theta-alpha band coherence was higher than beta band coherence between the lateral occipital area and GPi, and vice-versa between the primary motor cortex and GPi. The spectra also confirm the lack of coherence between NBM and either of these cortical regions across these frequency bands.
Figure 37 (left): SPMs confirming differences in the relative topography of delta-theta-alpha and beta band coherence between cortex and the GPi. Top: Voxel where regional delta-theta-alpha coherence with GPi is significantly greater than beta coherence. A regional maximum was identified in the lateral occipital area \([t=12, 8\text{df}, P<0.001, \text{red arrowhead}]\). The area of higher relative delta-theta-alpha coherence is represented on the 3D brain model. Bottom: Voxel where regional beta coherence with GPi was significantly greater than delta-theta-alpha coherence. A local maximum was identified in the primary motor area \([t=11, 8\text{df}, P<0.001, \text{red arrowhead}]\). The area of higher relative beta coherence is represented on the 3D brain model. Colour bars indicate the t-statistics.

Figure 37 (right): mean absolute (non-normalised) coherence spectra between the respective regional maxima identified on the left, and grand average GPi (and NBM) dipole data. Top: delta-theta-alpha band coherence is higher than beta band coherence between the lateral occipital area and GPi. Bottom: beta band coherence is higher than delta-theta-alpha band coherence between primary motor cortex and GPi. Note the lack of coherence between either of these brain regions and NBM across these frequency bands.
Finally, the respective cortico-GPi coherence spectra at both frequency bands were computed separately for the PDD (N=6) and DLB patients (N=4) and compared. Interestingly, coherence in the delta-theta-alpha band between GPi and lateral occipital cortex was higher in the DLB patients than the PDD patients (p=0.042, Figure 38 below). Meanwhile, coherence in the beta band between GPi and the primary motor area was similar in both patient groups.
Figure 38: Cortico-GPi coherence spectra for PDD and DLB patient groups. The top panel shows coherence spectra between the lateral occipital area and GPi in the delta-theta-alpha band for both patient groups. Note that there is significantly greater coherence in this band in the DLB patients compared to the PDD patients, which represents a dissociation between power and coherence effects in this frequency band in the GPi between the two groups (see the individual resting PDD and DLB power spectra for this nucleus above in Figure 33, right plot). The bottom panel shows coherence spectra between the primary motor area and GPi in the beta band for both patient groups. There was no difference between the two groups here.
5.4 Discussion

The combined NBM/GPi LFP and MEG experiments revealed a number of interesting findings, both with regard to the general physiological functions of NBM and GPi, and with regard to physiological differences between these nuclei in PDD and DLB patients.

First, we failed to identify a specific cortical network coherent with the NBM. This is not necessarily surprising however considering the major afferent and efferent connections of the nucleus: most of its inputs come from deep limbic and brainstem areas, while its connections to the cortex are almost exclusively efferent projection fibres, with little reciprocal cortical input to NBM (see Sections 1.6.3 and 1.6.4, Figure 6 and (Gratwicke et al., 2013)). This anatomical evidence suggests that NBM acts as an input nucleus to the cortex, possibly to boost the signal to noise ratio of processing on demand (Goard and Dan, 2009; Pinto et al., 2013), but that it is unlikely to participate in a cortico-subcortical processing loop in the same way as the GPi, which may explain why cortical coherence was not seen. Meanwhile, MEG cannot reliably resolve activity in deep brain structures which input to NBM since its sensitivity to signals reduces in proportion to the squared distance between source and sensor (Hillebrand and Barnes, 2002), hence we are unable to determine if any coherence with NBM activity might exist there.

An alternative explanation for the lack of cortical coherence with NBM could be due to the fact that the NBM degenerates by up to 70% in LBDs (Gaspar and Gray, 1984; Hall et al., 2014; Perry et al., 1985; Whitehouse et al., 1983), which is closely associated with equally severe cortical cholinergic binding reductions (Bohnen et al., 2006b; Hilker et al., 2005; Shimada et al., 2009). These factors combined suggest that there is severe cortical deafferentation from NBM in LBDs, and this loss of direct connections will consequently reduce the likelihood of finding a cortical network coherent with the nucleus. However, the GPi also degenerates severely in PD (Fearnley
and Lees, 1990; Huot et al., 2007), yet its coherence with cortex was maintained. Therefore, nuclear degeneration alone seems unlikely to account fully for the lack of cortical coherence observed with NBM.

Another possibility is that cortical cells groups can become coherent with NBM activity, but only when specifically activated by discrete corticopetal projections from the nucleus. We recorded LFPs and MEG in the resting state, without a task to place any specific motor or cognitive processing demands on cortical areas. In the absence of such demands there may have been no stimulus to engage the ascending NBM cholinergic system to modulate cortical activity, and hence the nucleus, and any potential coherence with cortex that it might be able to induce, remained quiescent. However, the GPi displayed coherence with two spectrally and spatially distinct cortical networks in the absence of any specific task, therefore a lack of task dependent activation of NBM seems a less likely explanation for its lack of cortical coherence here.

The finding of two spatially and spectrally distinct cortical networks coherent with activity in the resting GPi is in line with previous work. Using the same experimental set-up in PD patients, Litvak and colleagues previously demonstrated that a frontal premotor network is coherent with the subthalamic nucleus (STN) in the beta band (15-35 Hz) (Litvak, Jha, et al., 2011), similar to our finding of a frontal/primary motor area network coherent with GPi in the beta band (15-25 Hz) (Figure 37, upper panel). The similarity in these results is expected, since the STN and GPi are anatomically distinct yet heavily functionally interconnected subcortical nuclei within the same cortico-basal ganglia motor network (Albin et al., 1989; Nambu, 2008), and so would be expected to both be coherent with the same cortical motor regions in the same spectral band. Beta activity in this cortico-basal ganglia network has been shown to be modulated by dopaminergic medication, which increases coherence, and movements (whether personally executed, imagined,
or observed in others), which reduced coherence, and it is therefore proposed that this network is involved in motor planning (Alegre et al., 2010; A. A. Kühn et al., 2006; Lalo et al., 2008; Litvak, Jha, et al., 2011).

Litvak and colleagues also simultaneously demonstrated a separate temporo-parietal network coherent with the STN in the alpha band (7-13 Hz)(Litvak, Jha, et al., 2011). The cortical topography of this network is similar to that of the lateral occipital-temporal network coherent with G Pi in our study, although the frequency band was different (delta-theta-alpha band (1-12 Hz, Figure 37, upper panel). As discussed above, these findings are again likely to reflect different sub-components of the same cortico-basal ganglia network. However, the finding in our experiments that the spectral band of this network extended to lower delta and theta frequencies may reflect the fact that our patients had concurrent dementia as well as motor parkinsonism (whereas the patients in the Litvak study were not demented): as discussed in previous chapters, worsening cognitive decline in LBDs is associated with an increasing shift of resting cortical oscillatory power away from faster activity towards lower frequencies (Bosboom et al., 2006; Caviness et al., 2007; Franciotti et al., 2006; Ponsen et al., 2012). The functional relevance of alpha coherence in this posterior cortico-basal ganglia network is under debate, however it has been shown to be differentially modulated by dopaminergic medication and the dynamics of upcoming/executed movements (Oswal et al., 2013), as well as visuospatial attention (Gould et al., 2011; Thut et al., 2006). Considering the latter, a shift towards coherence at lower frequencies in this posterior cortico-basal ganglia network in our LBD patients compared to non-demented subjects may reflect the relative atrophy in posterior regions of the fronto-parietal attention network in this group, and consequently their impaired clinical ability to orient attention.
However, caution must be exercised when attempting to ascribe physiological function to precise anatomical regions on the basis of evoked spectral activities. Whether the delta-theta-alpha activity in the GPi LFP originates within the GPi itself or from a broader brainstem region is unclear (Hirschmann et al., 2011; Litvak, Jha, et al., 2011). For example, prominent alpha activity has been reported in LFPs recorded from the pedunculopontine nucleus in PD patients (Androulidakis et al., 2008; Thevathasan et al., 2012), and given the upward connections of this nucleus to basal ganglia structures (Pahapill and Lozano, 2000) one cannot exclude the possibility that the low frequency activity seen is actually transmitted from this deep brainstem generator. Furthermore, even despite the improvements in artefact control offered by beamforming methods, MEG recordings are not immune to spatial noise arising from the metal artefacts and head movements in our patients, and therefore the results and discussion above have been limited to cortical regions/areas rather than precise cortices.

Comparing our grand average resting GPi LFP (Figure 32) and the respective resting coherence spectra for this nucleus (Figure 37) an interesting finding is that cortico-GPi coherence in the delta-theta-alpha band was focussed in theta frequencies (4-5 Hz). LFP power in the GPi, on the other hand, was greater in delta frequencies. A similar dissociative pattern was previously found for beta coherence and beta power in the STN of PD patients withdrawn from medication, with cortical coherence focussed in the upper beta frequency and LFP power in the lower beta frequency (Litvak, Jha, et al., 2011). Our results therefore lend further support to the hypothesis proposed in that paper, and previously by Fogelson that the difference in frequencies between peak basal ganglia power and peak cortico-basal ganglia coherence are not simply passive phenomena, but may have functional significance: ‘frequency of synchronization may be exploited as a means of marking and segregating processing in the different functional subloops, over and above any anatomical segregation of processing streams.’ (Fogelson et al., 2006).
Of note, we did not observe the same dissociation of frequencies in the beta range in our patients as those seen in the work of Litvak and colleagues. However, unlike their patients ours were on dopaminergic medication, which is known to suppress low beta oscillations (Brown and Williams, 2005), and this may be why such a dissociation was not evident in this frequency band. Interestingly however, cortico-GPi coherence in the beta band was evident in all our patients (Figure 37, lower right plot and Figure 38, lower plot), in agreement with previous results showing that coherence in the upper beta band is relatively less modulated by dopaminergic therapy (Litvak, Jha, et al., 2011).

Our comparisons of low frequency activity in the delta-theta-alpha band in GPi between the PDD and DLB groups revealed an interesting double dissociation between resting power and cortical coherence in this band: PDD patients demonstrated higher mean low frequency power in GPi (Figure 33, right plot), whereas DLB patients demonstrated significantly higher mean low frequency coherence between GPi and lateral occipital cortex (Figure 38, upper plot). The relevance of this is not certain, however, a dissociation between local alpha band power and alpha band coherence in the same network within a group of PD patients has previously been shown (Oswal et al., 2013): local alpha band power in the STN was suppressed 2 seconds before movement onset, and was not modulated by dopamine, whereas regional cortico-basal ganglia alpha band coherence was suppressed only after movement onset and was modulated by dopamine. This therefore suggests that local alpha band power and regional alpha band coherence in the basal ganglia may be functionally distinct. If this is the case, then the observed double dissociation in our patients may also have functional relevance: our PDD patients suffered a greater level of motor impairment than their DLB counterparts (mean UPDRS part III off scores at baseline were 46.67 and 33.5 respectively, Tables 6 and 16), and therefore their higher mean low frequency power in GPi may be a reflection of this, especially since suppression of local
alpha power in the basal ganglia appears to be necessary for the initiation of movement (Oswal et al., 2013). However, whereas local alpha power in the basal ganglia is unaffected by dopamine, cortico-basal ganglia coherence in the alpha band appears to be suppressed by dopamine, and therefore the fact that our PDD patients had taken a higher mean daily equivalent dose of levodopa than the DLB patients prior to recordings (646.88 mg and 348.96 mg respectively, Tables 1 and 11) may account for the lower mean level of low frequency coherence observed in this group.

Finally, despite the suppression caused by dopaminergic therapy, there was still significantly higher beta frequency power in the GPi in PDD patients compared to DLB patients (Figure 33, right plot), again in keeping with the greater overall motor deficits in the former group. However, cortico-GPi coherence in the beta band, which is less affected by dopamine therapy as described above, was similar in both groups. This suggests that beta coherence is less reflective of overall level of parkinsonian motor symptoms, and thereby further supports the proposed functional dissociation between peak basal ganglia power and peak cortico-basal ganglia coherence in the beta band.

There are a number of limitations to this study. First, as with the LFP recordings in Chapter 4, the MEG recordings were performed only two to three days after electrode implantation, and so are highly likely to be influenced by the after-effects of both the invasive neurosurgical procedure and the general anaesthetic (both of which are likely to be significant in these demented patients). However, the fact that cortical networks coherent with GPi were easily resolved by the scanner suggests that any post-operative depressive effects on cerebral function were fairly limited. Second, we did not withdraw the patients from their dopaminergic medications, which means that low frequency beta band power in the subcortical structures was likely to have been suppressed.
However, the patients had significant motor symptoms, which were exacerbated by the after-effects of surgery, and without administering their usual doses of levodopa it is unlikely that many of them would have been able to tolerate the scanning session. Thirdly, we did not withdraw the patients from their AChEI medications, and so brain acetylcholine levels were supplemented during the recordings. It is theoretically possible that this could have partially masked NBM activity by diminishing the demand for corticopetal cholinergic input to cortex. However, as with their levodopa doses, if we had withdrawn AChEI medication from these demented patients after invasive neurosurgery it seems unlikely that many of them would have been in a cognitive state robust enough to tolerate the recording. As mentioned previously, although beamforming methods reduce artefacts arising from metallic DBS hardware and ferromagnetic wiring, it does not make recordings immune to distortions and spatial noise caused by these materials in our DBS patients, and therefore our ability to localise cortical areas coherent with subcortical LFP activity using this methodology must be regarded as a highly sensitive estimation rather than definitive localisation (Litvak et al., 2010). The relatively small total number of patients is not unusual in MEG experiments (Hirschmann et al., 2011; Litvak, Jha, et al., 2011), however, when the group was split into PDD (N=6) and DLB (N=4) for sub-analysis by LBD type then the number of patients in each group was particularly small, and was therefore likely underpowered to detect significant differences in some of the comparisons, such as resting delta power. Although the presence of coherence between brain areas implies a functional relationship between time series, it does not provide any information about the directionality of coupling, and so we cannot determine whether the functional connectivity shown between GPi and cortical areas was driven primarily by the cortical or subcortical components of that network. Finally, as with the LFP studies in Chapter 4, both the NBM and GPi are known to degenerate
significantly in LBDs, and therefore the resting state activity described here cannot be taken to be representative of that seen in the healthy human NBM in vivo.

Future investigations combining NBM/GPi LFPs and cortical MEG would benefit from scanning patients after withdrawal of both levodopa and AChEI medications. If performed using externalised electrodes this could be done by waiting longer after electrode implantation before performing the recordings, so that hopefully patients have recovered to a greater extent and are more likely to tolerate being off medications for several hours. Alternatively, newer DBS technologies may allow transmission of LFP signals from fully implanted systems, and therefore such recordings can be performed some months after full recovery, when patients would be much more likely to tolerate being off their medications. Future studies should also investigate the direction of functional connectivity in these coherent networks using advanced methods such as Granger causality (Granger, 1969) which provide information on the direction of signal transmission. Finally, methodologies have recently been validated for the analysis of simultaneous LFP and MEG recordings during active DBS (Oswal et al., 2016), and this will allow the physiological effects of stimulation of the NBM and any resultant changes in widespread functional connectivity to be directly evaluated in LBD patients.
Chapter 6: General discussion

6.1 Overview of the aims of this thesis

In conducting the work in this thesis I aimed to address a number of key questions: (1) can low frequency NBM DBS offer clinical therapeutic potential for Lewy body dementias, (2) is it a safe intervention in this vulnerable patient group, and (3) through investigation of the above can I gain new insight into the physiological functions and roles of the human NBM.

The principle driver behind my first question lies in the current therapeutic landscape for dementias. There are currently only a small number of symptomatic medications available for treatment of these diseases, all of which are limited in their efficacy and have the potential for disabling side effects (Emre et al., 2004, 2010; Qaseem et al., 2008; Rolinski et al., 2012). This is in the face of a significant and growing economic and societal disease burden (Brookmeyer et al., 2011; Olesen et al., 2012; Wimo et al., 2011). Modern approaches to develop disease modifying biologic agents for dementia have so far produced little tangible effect (Le Couteur et al., 2016; Schenk et al., 2016), possibly reflecting the fact that LBDs, as well as other forms of dementia, are caused by a heterogeneous milieu of underlying cellular and genetic pathologies which are not easily modified by single-ligand targeted drugs (Gratwicke et al., 2015a; Kawas et al., 2015). In this context there is growing recognition of the need to explore alternative avenues of treatment, and neuromodulation of cognitive networks, thus bypassing the varying underlying pathology, represents one possibility. The success of DBS in modulating aberrant neural network processing to relieve symptoms in other neuropsychiatric diseases (Blomstedt et al., 2012; Lozano et al., 2012; Williams et al., 2010) raises the possibility that this might be achievable in dementia. To this end, the NBM is proposed to be a key node in multiple distributed cognitive networks and appears a viable anatomical target for DBS implantation. To date only a single randomised
double-blind trial of low frequency NBM DBS has been carried out in patients with AD (J Kuhn et al., 2015), but none so far in patients with LBDs.

With regard to the second question, the proposition to undertake invasive neurosurgery in elderly patients with severe cognitive impairments and associated neuropsychiatric disturbances raises substantial safety issues. Despite the fact that the aim of the intervention is to benefit those same symptoms, there is both no guarantee that it will do so (due to its exploratory nature) and the possibility that the surgery and anaesthetic agent themselves could instead exacerbate those symptoms. In addition, these patients often have significant brain atrophy and other structural cerebral pathologies, such as white matter plaques and microhaemorrhages, which increase the risk of complications from the DBS procedure. Finally, dementia patients are often elderly and physically frail, which increases their risk of undertaking a general anaesthetic. All these factors are usually contraindications to DBS surgery (Foltynie and Hariz, 2010), therefore the risk of NBM DBS surgery for LBDs is inherently high. It is therefore imperative to determine the safety of the procedure in these patients, as even if there was a strongly beneficial response to NBM DBS, the delicate risk-benefit ratio of the surgery might still preclude further practical clinical use if it is determined to be unsafe.

With regard to the last question, despite being implicated as a key node in several cognitive processes in animal studies (Pinto et al., 2013; Voytko, 1996), the physiological role of the NBM in the human brain remains unclear. This is because studying the physiological activity of the nucleus in human subjects in vivo has proven difficult with current imaging techniques - fMRI does not resolve activity well in subcortical structures, while PET studies are limited in their practical application during cognitive tasks. Therefore, human studies of the NBM have so far largely been restricted to post-mortem neuropathological examinations (Mesulam and Geula,
Although helpful in anatomical and biochemical characterisation, the NBM has little to shed light on its function. In undertaking NBM DBS implantation in these clinical trials and temporarily externalising the leads in the post-operative period, we were afforded a unique opportunity to make direct electrophysiological recordings from the human NBM in vivo. These recordings, both at rest and during performance of dynamic cognitive tasks, allowed us to directly investigate the physiological function of NBM in the awake human brain for the first time. Furthermore, by combining these NBM LFP recordings with concurrent magnetoencephalography recordings, we were able to gain further insight into its physiology by examining the resting functional connectivity of the nucleus with cortical areas. In addition, if we were to find any clinical benefits of NBM DBS in LBD patients, then undertaking these electrophysiological investigations might help to shed light on possible mechanisms of action of the therapy.

We will now address each of these key questions in turn in light of the results obtained in these studies.

6.2 Clinical impact of NBM DBS in Lewy body dementias

The sample sizes in both the PDD trial and the DLB trial were necessarily small for ethical reasons, therefore the results from each should be viewed as exploratory and principally hypothesis generating rather than as absolute evidence of efficacy. However, within that context, the blinded clinical impact of NBM DBS on cognitive and behavioural symptoms in both LBDs was limited, with some patients, but not others, showing apparent improvement in specific symptoms, but no global improvement in cognitive function as previously reported in the single case report of NBM DBS in PDD (Freund et al., 2009). There may be several potential reasons
behind this discrepancy in general cognitive improvement, however above all the previous case report was an open-label study and hence susceptible to significant placebo effects, whereas the double-blind randomised nature of our studies mitigated against this. This view would seem to be supported by the results of the more recent double blind randomised trial of low frequency NBM DBS in AD by the same group (J Kuhn et al., 2015), which also failed to demonstrate a general improvement in global cognitive function, in line with our results.

The overall pattern of results across both trials suggests that low frequency NBM DBS may have caused blinded improvements in verbal memory retention and neuropsychiatric symptoms, particularly visual hallucinations, with the magnitude of these improvements larger in the PDD cohort than in their DLB counterparts. There was also a specific improvement in dyskinesias with low frequency NBM DBS in the PDD patients who suffered these symptoms. However, since none of the DLB patients suffered levodopa induced dyskinesias this effect was not seen in that group.

As discussed above, animal studies suggest that the NBM plays a key role in memory encoding (see Section 1.6.6 and Gratwicke et al., 2013), and the improvements in retention in memory scores suggest that modulation of NBM activity might have improved this. As discussed at the end of Chapter 2, an enhancement of attention functions with stimulation could also underlie improvements on this measure, as greater top down attention to the task would have ensured better encoding of information. However, if this had been the case one would have expected a general uptuning of performance across all tests with NBM DBS, since top down attention is crucial to performance on all of them, yet no global improvement was observed. Therefore a specific effect of NBM DBS on memory encoding appears the more likely possibility. However, the other main test of retention of information in memory in both trials (short recognition memory
for faces) failed to show improvement with stimulation in either group (Tables 4 and 14). Nevertheless it is well known that memory for facial information is encoded uniquely both anatomically and functionally within the brain (Kanwisher et al., 1997), therefore the lack of improvement seen on this test with NBM DBS does not necessarily preclude an effect on retention in memory more generally.

It is interesting to note that although both patient groups demonstrated an improvement in retention in memory on-stimulation compared to baseline, this reached significance in the PDD group (p=0.042, uncorrected) but not in the DLB group. However, this was one case where the cognitive tests used differed between the two groups; the PDD group were tested on the CVLT-II, which has a longer word list and so is more challenging, but also has only two alternate forms, meaning that it is more susceptible to practice effects. The DLB group on the other hand were tested with the HVLT-R, which has a shorter word list and so is easier for demented patients, but has six alternate forms, making it is less susceptible to practice effects. Bearing in mind that each respective memory test was administered eight times to an individual patient across the course of the trial (during each detailed and abbreviated cognitive battery, see Figures 1 and 13), then PDD patients would inevitably have been tested four times on each parallel version of the CVLT-II, whereas DLB patients would only have been tested once on most parallel versions of the HVLT-R. Therefore, the fact that the PDD patients appeared to show a greater improvement in retention in memory scores on-stimulation compared to the DLB group could be due to a confounding effect of task familiarity in the former which was not present in the latter.

An alternate reason for the difference in the magnitude of improvements on these tests between the two groups could be an effect of age, since there is a strong independent association between increasing age and worsening cognitive ability (Wechsler, 1999). The mean ages of the PDD
patient group and the DLB patient group were 65.17 years (SD 10.74) and 71.33 years (SD 3.67) respectively (Tables 1 and 11). However, the mean age of the PDD group is skewed due to the inclusion of one young-onset PDD patient (Patient E). When he is excluded the mean age of the PDD patients becomes comparable with the DLB patients (69.00 years, SD 5.83), and therefore it seems less likely that age played a significant role in the differences in performance observed.

Another possible reason for the difference could be an effect of diagnosis itself. DLB patients are more likely than PDD patients to have significant co-existent AD pathology (Gomperts et al., 2008; Halliday et al., 2011; Lippa et al., 2007), and the presence of multiple cellular pathologies is strongly associated with greater dementia severity (Kawas et al., 2015). However, mean baseline MMSE scores were only slightly different across the two groups (23.67, SD 1.75 for the PDD group, and 22.83, SD 1.17 for the DLB group, Tables 1 and 11) and therefore it seems unlikely that there was a significant difference in dementia severity to account for the differences in improvements seen on-stimulation.

Finally, given the small sample sizes in both trials, and the lack of contemporaneous control groups, it remains entirely possible that the difference in retention in memory scores on-stimulation compared to baseline between the two groups was due to chance alone.

Overall however, the improvements in retention in memory scores in either group did not appear to have any direct impact on daily activities for the patients or the quality of life of either themselves or their carers. Therefore, although intriguing from a cognitive neuroscience point of view, these test score improvements did not translate into tangible improvements from the patient’s point of view, and therefore their clinical value is limited.
The improvements in neuropsychiatric symptoms on-stimulation in both groups are the more salient results of the two trials, especially since they were paralleled by mean improvements in associated caregiver distress scores (Tables 5 and 15), suggesting that they had a real direct impact on patient and carer quality of life. These objective and clinical improvements on-stimulation are all the more significant since both groups also experienced a mean worsening in these symptoms during the blinded off-stimulation period compared to baseline, paralleled by worse caregiver distress scores. In the PDD group the improvement in NPI total scores on-stimulation compared to off-stimulation reached significance (p=0.027 uncorrected), driven in particular by a marked blinded reduction in visual hallucinations on-stimulation in two patients (Table 5 and Figure 12). In the DLB group one patient (Patient D) experienced a similar marked reduction in hallucinations on-stimulation compared to both off-stimulation and baseline (Table 15), but another patient with particularly marked and distressing visual hallucinations (Patient E) did not. However, the latter patient also suffered from severe macular degeneration, and so his hallucinations likely had both a central and a peripheral basis, which may have proven more resistant to the effects of NBM DBS than in the other DLB and PDD patients where they were only centrally (i.e. cerebrally) generated.

As discussed in Chapter 2, the improvement in levodopa induced dyskinesias on-stimulation compared to both off-stimulation and baseline in PDD Patients A, C and D was an unexpected finding (Figure 11). This effect is likely explicable by current spread from NBM to the overlying GPi. However, given that conventional GPi DBS for dyskinesia control in PD is generally delivered at high frequency (130Hz), the finding that low frequency (20Hz) stimulation directed towards the NBM also attenuates dyskinesias warrants further study in its own right.
However, aside from the improvements in these specific measures and symptoms, most of the cognitive and behavioural outcome measures in both trials remained unchanged with stimulation (see in particular Tables 3, 4, 13 and 14). This suggests that low frequency NBM DBS did not have a general augmenting effect on global cognitive ability, as discussed above. Furthermore, one particular outcome measure, simple movement time, consistently worsened with NBM DBS in both groups compared to baseline (Tables 10 and 20). Indeed, in the case of the DLB group their mean simple movement time on-stimulation worsened significantly compared to baseline (p=0.028, uncorrected). However, simple movement time also worsened off-stimulation compared to baseline in this group, and so some of the deterioration observed may have been due to either the surgery itself or disease progression. Alternatively, the reduction in dyskinesias on-stimulation in the PDD patients suggests that NBM DBS at 20 Hz may have influenced motor symptoms through current spread to the overlying GPi, and this might have had other subtle concurrent motor side-effects such as worsening bradykinesia.

In summary, the present results demonstrate that low frequency NBM DBS has only a very limited impact on symptoms in LBDs, and would not be supportive of its adoption for clinical use in its current form. However, the fact that blinded improvements did occur in specific symptoms on-stimulation, namely neuropsychiatric symptoms and retention in memory, would support further evaluation of this therapeutic approach targeted towards these specific cognitive and behavioural outcomes.

### 6.3 Safety of NBM DBS in Lewy body dementias

The safety profile of NBM DBS in both trials was very favourable: Only a single serious adverse event occurred in each trial. In the PDD trial the right electrode cap eroded through the scalp in
Patient C 15 months after implantation. This necessitated explantation of that electrode for safety, but did not cause any long term adverse sequelae. In the DLB trial one patient developed antibiotic-associated *Clostridium difficile* colitis post-operatively, which necessitated a prolongation of his hospital stay by two weeks while he recovered. However, there were no long term adverse sequelae from this either. The other adverse events mainly consisted of transient post-operative confusion or burr hole cap discomfort, all of which resolved fully within two weeks post-operative. Importantly, the surgery itself did not appear to cause any significant worsening of cognitive function, as evidenced by the relatively stable results across primary outcome measures on the cognitive batteries performed at baseline and at one week post-operative (Tables 8 and 18).

Given our prospective concerns regarding the safety of performing NBM DBS implantation in this elderly and vulnerable patient group, it was a welcome surprise to find that the procedure was well tolerated and has a safety profile consistent with other standard clinical applications of DBS (Foltynie and Hariz, 2010). Our safety results are consistent with those of the other studies of low frequency NBM DBS to date, which have similarly showed that both surgery and stimulation are well-tolerated and safe in demented patients (Freund et al., 2009; Kuhn et al., 2015a; Kuhn et al., 2015b).

Of note, our PDD patients received a dual NBM and GPi implant, the latter being available for the treatment of concurrent motor symptoms in the event of little clinical benefit from NBM stimulation. Standard GPi DBS, though not as effective as STN DBS for control of motor symptoms (Foltynie and Hariz, 2010), can still benefit patients in this respect, but dementia remains a contraindication to this surgery. In light of the good safety profile observed with combined NBM/GPi implants in demented patients in these trials this contraindication is now
called into question. Further work to determine whether this restriction should now become relative for demented patients with severe motor impairments who might benefit from GPi DBS appears to be warranted.

6.4 Implications of the electrophysiological findings in NBM for cognitive neuroscience

The LFP recordings from NBM confirmed that its resting spectral activity is significantly different from neighbouring GPi, in particular it showed significantly higher power across low frequencies in the delta, theta and alpha ranges. In light of previous findings from rodent studies that unilateral NBM lesions lead to synchronised delta oscillations in the ipsilateral cortical EEG (Buzsaki et al., 1988), and human MEG studies suggesting a correlation between cortical delta oscillations and worsening cognitive decline in LBDs (Ponsen et al., 2012), we hypothesised that cortical activation rhythms in LBD might be driven by ascending control from NBM. We therefore performed simultaneous resting NBM LFP and MEG recordings in our patients to look for evidence of functional connectivity to cortex. However, no cortical activity coherent with NBM activity was found in the present experiments, meaning that we found no evidence to support this hypothesis.

Our NBM LFP recordings during performance of two different types of attention task shed some light on the possible physiological role of NBM in the human brain: During performance of the sustained attention to response task, NBM activity in the delta band appeared to reflect the need to inhibit the pre-potent response, though whether this was a reflection of sustained attention to
the task or preparation to inhibit the response was unclear. However, the strength of this same delta band desynchronisation in NBM appeared to be greater in trials where the pre-potent response was correctly inhibited compared to those where it was not, suggesting that activity here also reflected the background level of sustained attention to the task. In contrast, there was no clear differential modulation of NBM activity during a task of orienting of attention. Taken together therefore, these task recordings provide preliminary evidence that the human NBM may play a role in the maintenance of sustained attention, but not the shifting of visual attention between stimuli. Such a role would be in line with existing evidence showing that corticopetal cholinergic input from NBM increases the signal to noise ratio for the cortical representations of salient stimuli (Bentley et al., 2011; Goard and Dan, 2009; Pinto et al., 2013; Soma et al., 2013), the possible neurobiological correlate of sustained attention to a stimulus.

These findings could also potentially be in line with our clinical results. Low frequency NBM DBS did not produce any objective improvements on a task of orienting of attention in either trial (Posner’s covert attention test, Tables 3 and 13). However, improvements on a test of sustained attention were seen on-stimulation compared to both off-stimulation and baseline in several of the DLB patients (Sustained attention to response task, Table 14, N.B. PDD patients were not tested on this). Furthermore, the reductions in visual hallucinations observed on-stimulation in particular patients in each trial could also reflect an improvement in levels of sustained attention/alertness, since evidence suggests that impairments in this cognitive faculty contribute to the generation of visual hallucinations in LBDs (Gratwicke et al., 2015a).

However, as discussed previously, the NBM degenerates significantly in LBDs ((Gratwicke et al., 2013) and see Section 1.3.1.5), and therefore the electrophysiological findings described here are
not necessarily representative of the intrinsic activity of the healthy human NBM in vivo. Therefore, it is important that readers interpret the results in this context.

6.5 Limitations of the present work and future directions for DBS for dementia

The trials described here were always meant to be exploratory, to determine relevant outcomes for further exploration in future work. Therefore, even though only limited clinical benefits were found with low frequency NBM DBS, the fact that blinded improvements in specific measures were seen, combined with the fact that the approach is safe, and the finding of a possible mechanistic link between low frequency stimulation and sustained attention levels in LBD patients justifies the further exploration of DBS to this nucleus as a therapy for this patient population, and for dementias in general.

There are a number of limitations of the work presented in this thesis. Many of the limitations specific to each study have already been presented in the relevant chapters, therefore here I will present an overview of general methodological issues, and suggest how they can be addressed in future studies of DBS for dementia.

The major limitation to both clinical trials was the fact that only a small number of patients was recruited in each, meaning that each trial was underpowered to detect statistically significant differences between the on- and off-stimulation periods. This was of course inevitable in these pilot trials, due to the fact that at the outset the safety of NBM DBS implantation in this vulnerable patient group was unknown, necessitating a cautious approach to both ethical approval and recruitment. However, as a consequence, given the multiple comparisons we have performed, our data cannot be interpreted as evidence for efficacy. In light of this, future trials of DBS for
Lewy body dementias should consider combined recruitment of patients with a diagnosis of either PDD or DLB into one trial, in order to increase recruitment numbers. This approach seems valid given the common underlying neuropathological basis of the two conditions and their significant clinical overlap (see Sections 1.2 and 1.3), and in support of this current thinking appears to be moving toward a dissolution of the boundary distinction between PDD and DLB (Berg et al., 2014). However, not everyone ascribes to this view (Boeve et al., 2016), and the small areas of difference between the two conditions might therefore leave such trials open to criticism. However, on balance, at present it would seem preferable that trials with sufficient numbers to determine the full impact of DBS interventions in LBDs are conducted rather than delayed.

A second major issue with the clinical trials was the fact that AChEI medications were continued (at stable dosage) throughout the trial periods. The use of such medications is likely to partially mask any augmenting effects of DBS on the NBM cholinergic system, and will thereby reduce the magnitude of any differential clinical effects seen. However, given the relative safety of AChEI medications in comparison to DBS, we did not feel it was appropriate to expose patients to surgical risks who might gain sufficient cognitive benefits from the use of medications alone, therefore patients had to be taking such medication already prior to recruitment. Once recruited, the fact that the patients were likely deriving some (albeit sub-optimal) cognitive benefit from their AChEI medication meant that it would have been unethical to withdraw it, and moreover patients and their carers are unlikely to have consented to the trial if this had been a condition. One way in which future studies could address this issue would be through the use of functional imaging using PET, so that brain acetylcholine receptor occupancy could be measured both pre- and post- the usual dose of AChEI, and then again with additional blinded NBM stimulation, to look for a superadded biochemical effect. However, even if this were to clarify the contribution of
NBM DBS in such patients from a mechanistic point of view, the clinical effects might still be masked.

Thirdly, the results of the clinical trials were limited by their relatively short duration; the duration of each blinded period was only six weeks. Although this is sufficient to demonstrate an effect of medium-term NBM DBS, it does not inform on the sustainability of the effects seen over longer periods, or give any indication as to whether the intervention modified the course of the disease. One way to address these two issues in the future would be to have two parallel groups of LBD patients undergo surgery, then a much longer blinded period of one year within which one group receives active stimulation and the other sham. The difficulty with this approach is that patients with disruptive symptomatology from dementia and their caregivers alike may not be willing to undergo invasive neurosurgery in the knowledge that they might not actually receive any active stimulation until one year later. However, trial designs of this type might be possible in patients with milder dementia (Lozano et al., 2016). An alternative could be to conduct a similar blinded crossover study to those detailed here, but to also include a separate, non-operated, control group of LBD patients, who would complete all the same assessments. The only difficulty with this approach is that any difference in the disease course detected between the two groups could be attributed to the effects of the surgery itself rather than the active stimulation.

The lack of functional outcome measures, and the resultant difficulty in interpreting how differences in the performance on neuropsychological tests relate to the patient’s daily life has previously been discussed. Future trials of DBS in dementia should aim to incorporate such measures into their outcomes battery, for example the Alzheimer’s Disease Cooperative Activities of Daily Living Inventory (Galasko et al., 2005), in order to better determine the clinical impact and relevance of the intervention to the patient and their carers. In addition,
another limitation previously discussed in Chapter 2 is that we only stimulated the NBM at 20 Hz, although the existing scientific rationale for this is limited (see Section 1.7.2). Stimulation at a different frequency might produce different results, and future studies should investigate this using a titration schedule, investigating cognitive responses to different frequencies of NBM DBS.

Another limitation is the fact that we did not subdivide the NBM into its constituent subsectors to determine which of these was predominantly receiving stimulation in each patient. The different subsectors of NBM provide topographical innervation to different cortical areas (Gratwicke et al., 2013), and therefore stimulation delivered to one particular subsector in a patient might have different effects to stimulation of another subsector in a different patient. Novel high-field imaging techniques combined with DTI tractography offer the potential to resolve these issues in future studies, which should allow better characterisation of the beneficial effects of discrete NBM subsector stimulation.

Related to the last point, the size of the macroelectrodes relative to NBM means that we cannot be sure which NBM subsector the LFPs were recorded from, or indeed whether they originated from the nucleus or represent volume conduction from nearby sources. However, as I argued in Chapter 4, the use of a bipolar montage for LFP recordings limited the effects of distant volume conduction. Moreover, the fact that statistically significant differences in resting power spectra were found between NBM and its closest neighbouring structure, GPi, further suggests that the recordings we made were NBM specific and not due to volume conduction from neighbouring sources. However, which subsector of NBM the LFP represented cannot currently be distinguished.
A methodological limitation of our electrophysiological results is that the analyses that I employed assume that brain signals are stationary, for example when performing Fourier spectral analysis over long time windows, or when computing covariance matrices for beamformer source localisation estimates. Using these techniques to average over long segments of data increases the signal to noise ratios, which is particularly beneficial under the conditions we faced with patient MEG recordings (Litvak et al., 2010). However, we must bear in mind that resting and task related brain activity is not stationary, but is instead constantly dynamic and complex (Baker et al., 2014; Deco et al., 2008), and therefore our analysis of electrophysiological signals can at best only ever approximate to this. One way for future studies to approach this problem is to reduce the length of the time windows into which the continuously recorded data is epoched before spectral analysis, in effect sampling the true signal on a finer scale. Although this would still not be perfect, it would further approximate the data toward the measured signal, although the computational costs of analysing data on this finer scale increase exponentially, which can cause problems for processing power and data storage.

It is also important to point out that the many of the electrophysiological analyses in this thesis are of a correlative nature (LFP task recordings and MEG coherence recordings). While correlations are a critical step in establishing relationships, they by no means imply causality. In order to demonstrate causal relationships, the behavioural and clinical consequences of specifically modulating oscillatory activities in the NBM need to be established. To this end future studies should take advantage of the newly validated methods for performance of simultaneous LFP and MEG recordings during active DBS (Oswal et al., 2016) to determine the direct effects of NBM stimulation on local and diffuse brain activity and the behavioural effects of this.
A final question is whether NBM DBS might be more effective if performed earlier in the LBD disease course. Since the nucleus is known to degenerate by up to 70% in both PDD and DLB (Gaspar and Gray, 1984; Hall et al., 2014; Perry et al., 1985; Whitehouse et al., 1983) then attempting to modulate its function with NBM DBS at a late stage may not have a significant effect simply because there are too few intact corticopetal cholinergic fibres left to stimulate. Future trials could therefore evaluate whether earlier intervention, at a stage when the NBM is less degenerate, might yield a greater response. Indeed such evaluations are already being undertaken in AD using NBM DBS (Kuhn et al., 2015b).

Cognitive networks are both multiple and diffusely distributed within the brain (Gratwicke et al., 2015a; Seeley et al., 2009; Stam, 2014), and possibly even more complex in their nature than motor networks. Therefore attempting to directly modulate cognitive functions using DBS constitutes a considerable challenge, and the lack of marked beneficial effects in the trials described here should not deter future investigations of this therapeutic approach. These are, in effect, some of the first steps, and they needed to be taken in order to provide initial guidance in a research field where there are currently a multitude of possibilities. The application of DBS technology in its current form to cognitive networks may be a rather blunt instrument for fine and complex systems. Advancements and refinements to the technology may be needed before neuromodulation can be applied to cognitive networks on an appropriate scale, and possibly across the correct number of distributed simultaneous sites. Nevertheless, further studies in this field should continue at the present time, especially since the increasing global burden of dementia represents an ever more pressing problem to our modern society.
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