Deep Brain Stimulation and memory restoration

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Chapter 1

General introduction

Memory

Memory is the cognitive ability that allows humans to encode, store and retrieve information. This can be classified according to time of retention (short-term and long-term memory) and type of material and encoding (declarative and procedural)[1, 2]. Two theoretical models have been proposed with respect to temporary storage: short-term memory and working memory. Short-term memory refers to a cognitive system that has the ability to retain limited information for a short period of time. In particular it is used for holding sensory events, movements, and cognitive information, such as digits, words, names, or other items [3]. It has been suggested that an average person can hold around seven items in short-term memory.

The term "working memory" became famous through the homonymic model of Baddeley and Hitch [4]. Compared to short-term memory, the working memory model has more intrinsic features. The classic working memory model consists of three parts: central execution system, language material coding system (phonological loop) and visual spatial material coding system (visuospatial loop) [5]. In short, the phonological loop is specialised storage system for speech-based information, and possibly purely acoustic information, whereas the visuospatial sketchpad is critical for the online retention of objects and spatial information. The central executive is not a memory system per se but instead coordinates the processes of the phonological loop and visuospatial sketchpad, and is related to the function of the prefrontal regions [6]. A new component, the episodic buffer, was later added to the model[7].

Long-term memory refers to the storage of information over an extended period of time and can be attributed to the hippocampus, entorhinal gyrus, periolfactory gyrus and parahippocampal gyrus [8]. Long-term memory can be subdivided into two different types: explicit and implicit memory.Explicit memory (also known as declarative memory) needs conscious coding and recall; It includes episodic memory and semantic memories, which are related to the functions of the medial temporal lobe and lateral temporal lobe, respectively [9-11]. Implicit memories are mostly unconscious. This type of memory includes procedural memory which involves conditioning and memories of body movement, which are related to cerebellar function [12].

Memory loss

Memory loss may be caused by many neurological and mental diseases, including delirium from any cause, cerebrovascular disease (ischemic and hemorrhagic), traumatic brain

injury,neurodegeneration, demyelination and mental illness. There are currently almost 47 million people living with dementia around the world, and this number will be expected to increase to 132 million by 2050 [13]. Nearly 60–80% of dementia is caused by Alzheimer's disease (AD). The most common early symptom is memory loss. As the disease progresses, symptoms may include language problems, disorientation, lack of motivation, difficulty speaking and writing and behavioral problems [14]. The incidence rate of dementia is also very common in patients with Parkinson's disease (PD). In PD patients, the prevalence of dementia is between 24% and 31% [15]. Remarkably, the cumulative prevalence shows that at least 75% of PD patients who survive for more than 10 years will develop dementia [16].The cognitive characteristics of Parkinson's Disease Dementia (PDD) patients include impaired executive function, visuospatial function and memory loss [17]. Studies showed that the cholinesterase inhibitors such as donepezil, galantamine, and rivastigmine, and the N-methyl-D-aspartate receptor antagonist, memantine, are accepted medications for the treatment of dementia [18].However, these treatment options are not effective for every patient and only alleviate symptoms temporarily, which indicates the need for new and innovative therapies[19]. Recently, many studies have appeared on deep brain stimulation in memory loss and some researchers have found beneficial effects on memory and cognition[20].

Deep brain stimulation(DBS)

DBS is an invasive surgical method for neurological and psychiatric disorders. During the surgery, stimulation electrodes are stereotactically implanted into particular brain targets of patients who are under local or general anaesthesia. The stimulating electrodes are connected with an internal pulse generator through a subcutaneous wire. With a wireless connected controller, stimulation parameters such as frequency, amplitude, pulse width, the choice of bipolar or monopolar stimulation, and continuous or intermittent stimulation can be adjusted to achieve the best therapeutic effects with little side effects.

Studies with deep brain stimulation

Over 180,000 patients worldwide have undergone DBS surgery and the numbers are increasing each year [21]. In particular, DBS within the basal ganglia network has proven to be safe and effective for movement disorders, whereas the application of DBS to modulate different neural pathways such as the circuit of Papez is considered experimental. The circuit

of Papez is one of the major pathways of the limbic system and is primarily involved in emotional expression, neurovegetative function, and memory [22]. The classical circuit consists of the hippocampal formation, fornix, mammillary bodies, mammillothalamic tract, anterior thalamic nucleus, cingulum, and the entorhinal cortex. Damage to structures within the circuit of Papez can cause anterograde amnesia in patients, i.e., an inability to create new episodic memories.

While DBS applied to limbic targets has been evaluated for patients with treatment-resistant depression [23-25] and obsessive-compulsive disorder [26], recently studies have begun to explore the applicability of DBS in a widening array of conditions such as dementia. In particular, DBS of the fornix and nucleus basalis of Meynert (NBM) is thought to facilitate neuromodulation within memory associated areas of the Papez circuitry. DBS of the fornix/hypothalamus was able to improve memory, reduce cognitive decline [27], and increase hippocampal volume [28] in AD patients. Similarly, NBM DBS induced enhanced memory performance in some patients [29, 30]. The mechanisms mediating these effects are thought to be related to neurochemical changeshippocampus[31], synaptic plasticity[32-34]and neurophysiology[35-37].

Aims of this thesis

The overall aim of this paper is to explore whether DBS can be used as a tool to improve memory and cognitive function and in particular what stimulation parameter produces most beneficial effects. Moreover, I explored the corresponding mechanisms of action.

The specific purpose of each chapter of this thesis is as follows.

In chapter 2, I aimed to provide the up-to-date research on deep brain stimulation techniques and the effects on memory neuromodulation and cognition will be described.

In chapter 3, I aimed to provide the up-to-date research on the effect of fornix deep brain stimulation in brain diseases.

In chapter 4, I aimed to investigate the Deep brain stimulation of the nucleus basalis of Meynert in a scopolamine-induced rat model of dementia. I specifically investigated different stimulation parameters and evaluated potential mechanisms of action.

In chapter 5, I aimed to investigate the cognitive effects of intermittent deep brain stimulation of the subthalamic nucleus in patients suffering from Parkinson's disease dementia.

In chapter 6, I summarize and discuss the major findings of this thesis and make the overall conclusion of the research.

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Chapter2

Deep brain stimulaton and cognition: translational aspects

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Abstract

Many neurological patients suffer from memory loss. To date, pharmacological treatments for memory disorders have limited and short-lasting effects. Therefore, researchers are investigating novel therapies such as deep brain stimulation (DBS) to alleviate memory impairments. Up to now stimulation of the fornix, nucleus basalis of Meynert and entorhinal cortex have been found to enhance memory performance. Here, we provide an overview of the different DBS targets and mechanisms within the memory circuit, which could be relevant for enhancing memory in patients. Future studies are warranted, accelerating the efforts to further unravel mechanisms of action of DBS in memory-related disorders and develop stimulation protocols based on these mechanisms.

Introduction

Cognitive decline has become a commonly observed phenomenon in our time and is caused by ageing, neurological and psychiatric disorders. Dementia is a broad category of brain diseases that cause a long-term and often gradual decline in cognitive functioning, language, problem-solving and other cognitive skills, which have a detrimental effect for patients to perform activities of daily living and thus place significant psychological, social and financial distress on patients and their families. There are different types of dementia, amongst others Alzheimer's disease, vascular dementia, Parkinson's disease dementia and alcohol-related dementia. In these patients, a common mechanism causing cognitive decline is dysfunction of the memory circuit, or the “circuit of Papez”. Damage to structures within the circuit of Papez primarily results in anterograde amnesia, i.e., an inability to create new episodic memories [1-5]. The most common form of dementia is Alzheimer's disease (AD), accounting for 60–80% of all cases. In the early stages, cognition and the ability to acquire new memories is impaired. As the disease progresses, symptoms may include language problems, disorientation, aggression, depression and long-term memory loss [6]. Medical treatments for patients suffering from cognitive decline are marginal and suggest a need for new and innovative therapy [7]. Deep brain stimulation (DBS) has proven to be safe and effective for both hypo- and hyperkinetic movement disorders of basal ganglia origin, while its application to other neural pathways such as the circuit of Papez is under active investigation. The circuit of Papez is considered one of the major pathways of the limbic system and is primarily involved in emotional expression that manifest through neurovegetative signs, and memory [8]. The classical circuit consists of the hippocampal formation, fornix, mammillary bodies, mammillothalamic tract, anterior thalamic nucleus, cingulum, and the entorhinal cortex [8]. While DBS applied to limbic targets has been evaluated for patients with treatment-resistant depression[9-11] , Tourette syndrome[12] and obsessive-compulsive disorder[13] , recently studies have begun to explore the applicability of DBS to alleviate memory impairments.

The introduction of DBS in psychiatry has generated much debate. While good progress has been achieved in DBS for obsessive-compulsive disorder and Tourette syndrome, DBS effects in depression or other mental disorders are inconclusive despite substantial clinical research efforts[14]. This raises the question of DBS’ efficacy for psychiatric disorders, framed by a growing concern for ethics [15]. Key challenges in the treatment of prevalent psychiatric disorders include the complex and heterogeneous clinical manifestations; the multitude of brain circuitries involved in these pathologies; the difficulty in conducting large clinical trials;

and the inconsistent results obtained so far [16]. We believe that a sufficient understanding of disease mechanisms and neuromodulation mechanisms are necessary before experimentation in patients take place.

In the current review, we focus on the translational aspects of DBS in disorders characterized by memory impairment. For this, we discuss the outcome of DBS of different memory-related structures in clinical and preclinical studies. We also discuss the potential mechanisms of action underlying symptom reduction.

Methods

For this review, we searched PubMed for clinical and preclinical studies in English literature with the search terms “deep brain stimulation” and “dementia”. The search yielded 345 studies (Fig.1). Article titles and abstracts were scrutinized for suitability. Only original research articles involving human subjects and rodents were chosen, and then grouped by region of stimulation. Moreover, some studies investigating cognitive outcomes of DBS in other central nervous system disorders, which are not dementia-related were considered. Studies identified in the reference lists of key articles were also included. We summarized the available literature in this field and compared it with the results of relevant preceding research.

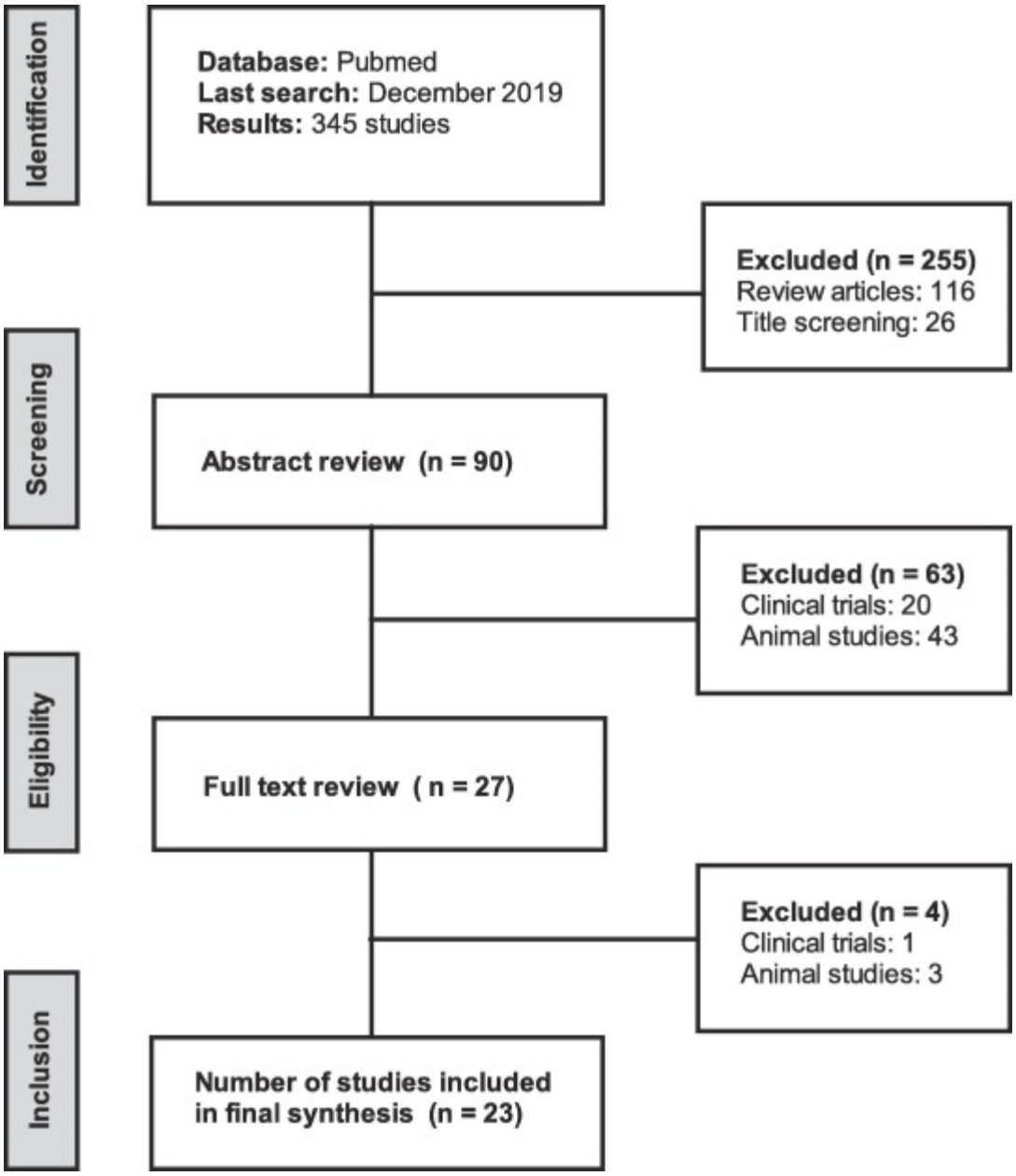

Fig. 1. Flow chart depicting the number of abstracts screened and the full texts retrieved during literature screening.

Classical concept of the neuroanatomy of memory: The circuitry of Papez

One major pathway of the brain which is primarily involved in the cortical control of emotion and in storing memory is the Papez circuit. In 1937 James Papez proposed that the cortical machinery for feelings and memory involves the limbic lobe[8], a region defined by Paul Broca, who originally called it "le grand lobe limbique" [17]. Within the Papez circuit, information is thought to circulate for a certain time while being associated with internal states (emotional as well as motivational) before being transmitted for long-term storage. The classical idea is that the entorhinal cortex projects to the hippocampi, whose efferents are

bundled in the fornix and reach the mamillary bodies. In fact, the fornix is a major input and output pathway of the hippocampus and medial temporal lobe. It provides a source of input from the hippocampal formation to the anterior thalamic nuclei[18, 19], since the mamillary bodies are connected to the anterior nucleus of the thalamus through the mamillothalamic tract. Furthermore, cholinergic fibers from the basal forebrain, including the septal nuclei and the nucleus basalis of Meynert run through the fornix. The circuit is completed by projections of the anterior thalamic nuclei to the cingulate gyrus and via the cingulum to the parahippocampal gyrus and then back to the entorhinal cortex (see Fig.2).

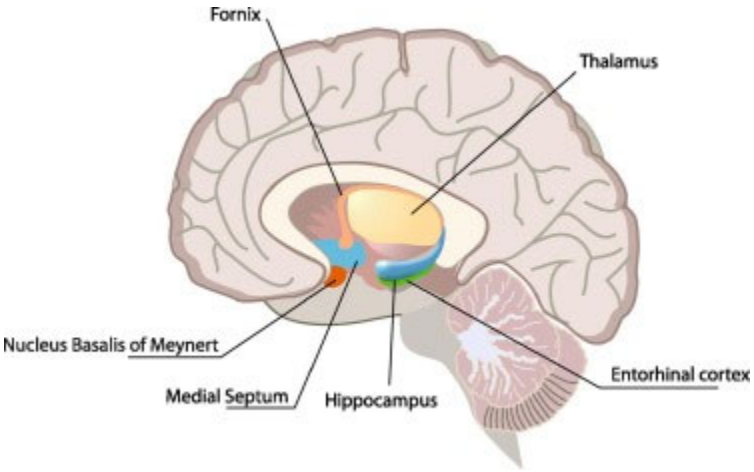

Fig. 2. Anatomical location of the fornix, hippocampus, entorhinal cortex and nucleus basalis of Meynert in the human brain. These structures are important components of the Papez circuit and are considered to be involved in the neuronal basis of memory.

Functionally the paralimbic areas contribute to the activity of different networks[20]. The hippocampal-diencephalic limbic circuit (connected through the fornix and mammillothalamic tract) and the parahippocampalretrosplenial circuit (ventral cingulum), is dedicated to memory and spatial orientation, respectively. The temporo-amygdala-orbitofrontal network (connected through the uncinate fasciculus) is dedicated to the integration of visceral and emotional states with cognition and behavior. The anterior cingulate-medial prefrontal cortex and the posterior cingulate precuneus form the medial default-mode network and are responsible for goal-directed behavior.

Deep brain stimulation (DBS)

DBS is a neurosurgical procedure in which stimulation electrodes are stereotactically implanted into specific brain targets under local or general anesthesia.

The DBS system consists of three components: the implanted pulse generator, the electrode (also known as "lead") and an extension. Stimulation parameters can be adjusted externally with a wireless connected controller to obtain best possible therapeutic effects with no or least side-effects. Nevertheless, since it is an invasive surgical procedure attendant risks include intracerebral hemorrhages, which occur in <2% of patients, while less severe or reversible events, such as infections or lead and pulse generator problems, occur in around 9% of patients [21]. Some important features of DBS therapy are the non-ablative and reversible nature as well as the adjustability of stimulation parameters to the need of individual patients.

Despite considerable research efforts, there is no unified theory on the mechanism by which DBS improves symptoms in patients. However, a number of commonly accepted mechanisms have been identified. These include "functional inhibition" of neuronal cell bodies and the excitation of axonal projections near the electrodes. Supporting these theories, DBS of the subthalamic nucleus (STN) or globus pallidus internus (GPi) at frequencies commonly used in clinical practice (i.e. 130–185 Hz) has shown to suppress firing of neuronal populations surrounding the stimulation electrode [22, 23]. Opposed to that, DBS using the same frequency-range has also shown to increase electrical activity in nearby axonal projections (afferent and efferent projections from targeted regions as well as fibers *en passant*) [24, 25].

In addition to the local electrical effects of DBS, researchers found that DBS can also have profound influences on brain-wide networks. For example, in a rodent model of epilepsy, DBS of the anterior thalamic nucleus caused neurochemical changes through the release of adenosine in the hippocampus[26]. Moreover, DBS has been shown to modify maladaptive plasticity and neurogenesis. In line with this, Gondard and colleagues have demonstrated that acute fornix DBS could modulate neurotrophic factors such as brain derived neurotrophic factor (BDNF) as well as synaptic plasticity markers such as growth associated protein 43, α-synuclein and synaptophysin[27]. Evidence for hippocampal neurogenesis has been found in a group of adult rats after thalamic DBS[28].

Translational principles of DBS

Since nonsurgical lesions restricted to the circuit of Papez are rare in humans, the study of memory has greatly benefited from animal experiments. Lesions in the Papez circuit have been found to have a profound effect on learning and memory, whereas spatial and non-spatial memory performances should be considered independently. The examples of the different targets described below clearly illustrate how scientifically grounded translational

research can help to bring important laboratory findings to bear on the alleviation of human suffering. In particular, translational research has helped to study the mechanisms that underlie the effects of DBS, the pathophysiological mechanisms and circuitries underlying the disorders and the possible side effects of DBS. A summary of the findings can be found in Table 1.

Table 1. Table of stimulation studies organized by target structures. The table shows the subjects involved, type of stimulation, memory task and the outcome of the surgical intervention. ADAS-cog, Alzheimer's Disease Assessment Scale-cognitive subscale; CDR-SOB, Clinical Dementia Rating Scale Sum of Boxes; DBS, deep brain stimulation; MMSE, mini-mental state examination; OLT, object location task; WAIS, Wechsler adult intelligence scale.

Structure	Subject	Type of stimulation	Memory task	Effect	Reference
Entorhinal cortex and hippocampus	Human (epilepsy patients) *N* = 49	Bipolar, 50 Hz, 0.5 to 1.5 mA, 300 µs pulse width, cycle of 5 s on and 5 s off	Virtual-reality spatial memory task, verbal memory task (free recall task)	Electrical stimulation in the entorhinal region and hippocampus impaired memory performance in both spatial and verbal tasks.	Jacobs et al. (2016)
Entorhinal Cortex	Human (patients with pharmacoresistant epilepsy) *N* = 7	Bipolar, 50–130 Hz, 0.5 to 1.5 mA, 300–450 µs pulse width, cycle of 5 s on and 5 s off	Virtual-reality spatial memory task	Stimulation of the entorhinal region enhanced memory of spatial information when applied during learning.	Suthana et al. (2012)
Entorhinal Cortex	Mice *N* = 25	Bilateral, 130 Hz, 50 µA, 90 µs puse width, for 1 h during surgery	Morris water maze	Water-maze memory was facilitated 6 weeks after stimulation due to hippocampus-	Stone et al. (2011)

Structure	Subject	Type of stimulation	Memory task	Effect	Reference
				dependent neurogenesis.	
CA1 subregion of the hippocampus	Rats (model of experimental dementia) *N* = 10	Bilateral, 100 Hz, 100 µA and 100 µs pulse width	OLT	Acute DBS improved spatial memory performance although also increasing anxiety-related behavior.	Hescham et al. (2015)
Anterior thalamic nucleus	Human (patients with refractory partial epilepsy) *N* = 54	Bilateral, 145 Hz, 5 V, 90 µs pulse width, 1 min on and 5 min off	Neuropsychological testing, e.g. Wechsler Abbreviated Scale of Intelligence (WASI); California Verbal Learning Test, Visuospatial Memory Test; Profile of Mood States (POMS)	Cognition and mood showed no group differences, but participants in the stimulated group were more likely to report depression or memory problems as adverse events.	Fisher et al., 2010, Tröster et al., 2017
Anterior thalamic nucleus	Rats *N* = 12	Bilateral, 130 Hz or 20 Hz, 90 µs pulse width, and either 500 µA or 100 µA	Contextual fear conditioning, spatial alternating test	High frequency stimulation of 500 µA disrupted the acquisition of contextual fear conditioning and impaired spatial memory.	Hamani et al. (2010)
Anterior thalamic nucleus	Rats *N* = 16	Bilateral, 2.5 V, 90 µs pulse width, and variable frequencies (10, 50, 130 Hz),	N/A	High-frequency stimulation of the ANT restores corticosterone-suppressed hippocampal	Toda et al. (2008)

Structure	Subject	Type of stimulation	Memory task	Effect	Reference
		duration 1 h during anesthesia		neurogenesis.	
Anterior thalamic nucleus	Mice *N* = 28	Bilateral, 2.5 V, 90 µs pulse width, and either 10 Hz or 130 Hz	N/A	High frequency DBS increased hippocampal neurogenesis.	Encinas et al. (2011)
Fornix	Human (morbid obesity patient) *N* = 1	Bilateral, 3–5 V, 130 Hz and 60 µs pulse width, continuous for 3 weeks	Neuropsychological tests, e.g. verbal learning test, WAIS attention index, spatial associative learning, etc.	Significant improvements on the California Verbal Learning Test and Spatial Associative Learning Test.	Hamani et al. (2008)
Fornix	Human (AD patients) *N* = 6	Bilateral, 3.0–3.5 V, 130 Hz, and 90 µs pulse width, continuous for 12 months	ADAS-cog, MMSE	Possible improvements and/or slowing in the rate of cognitive decline at 6 and 12 months in some patients.	Laxton et al. (2010)
Fornix	Human (AD patients) *N = 6*	Bilateral, 3.0 V, 130 Hz and 90 µs pulse width, continuous for 12 months	ADAS-cog, MMSE	Local volume increase in parahippocampal gyri, right superior temporal gyrus, left parietal lobule and bilateral precuneus as well as thalamus and superior frontal gyrus.	Sankar et al. (2015)
Fornix	Human (AD	Bilateral,	ADAS-Cog-13,	Participants	Lozano et

Structure	Subject	Type of stimulation	Memory task	Effect	Reference
	patients) *N = 42*	3.0–3.5 V, 130 Hz and 90 µs pulse width, continuous for 12 months	CDR-SOB, California Verbal Learning Test-Second Edition (CVLT-II), the Alzheimer's Disease Cooperative Study Activities of Daily Living scale (ACDS-ADL)	aged ≥ 65 years show less cognitive decline while there was possible worsening in patients below 65 years with stimulation.	al. (2016))
Fornix	Rats (model of experimental dementia) *N* = 10	Bilateral, 100 and 200 µA, 10 and 100 Hz, 100 µs pulse width, acute stimulation	OLT	Memory enhancement in high current densities (frequency-independent).	Hescham et al. (2012)
Fornix	Rats *N* = 19	Bilateral, 100 Hz, 100 µA and 100 µs pulse width for 1 h	N/A	C-Fosincrease in CA1 and CA3; extracellular hippocampal acetylcholine levels peaked after 20 min of stimulation.	Hescham et al. (2016)
Fornix	Rats (transgenic rat model of AD, tgF344) *N* = 6	Bilateral, unipolar, 130 Hz, 80 µs, 100 µA, permanent for 42 days	N/A	Amyloidosis, inflammatory responses, and neuronal loss decreased in both cortex and hippocampus after fornix DBS.	Leplus et al. (2019)
Fornix	Mice (transgenic mouse model of	Bilateral, monophasic, 100 Hz,	Morris water maze	The acute DBS treatment improved	Gallino et al. (2019)

Structure	Subject	Type of stimulation	Memory task	Effect	Reference
	AD, 3xTg) *N* = 17	100 µs pulses, 100 µA, 1 h during anesthesia		learning and long term memory in a delayed, sex specific, and transient manner relative to sham-stimulated controls.	
Nucleus basalis of Meynert	Human (AD) *N* = 1	Unilateral, 3 V, 50 Hz and 210 µs pulse width, cycling between 15 s on and 12 min off throughout the day and night, repetitive for 9 months	N/A	No clinical effect, but increased cerebral glucose metabolism.	Turnbull et al. (1985)
Nucleus basalis of Meynert	Human (Parkinson patient) *N* = 1	NBM: bilateral, 1 V, 20 Hz, and120 µs pulse width STN: bilateral, 3.5–4.2 V, 130 Hz and 60 µs pulse width	Neuropsychological tests, e.g. clock drawing, letter-number-span, auditory verbal learning, etc.	Dramatic improvement in neurocognitive evaluations with regard to attention, concentration, alertness, drive, and spontaneity.	Freund et al. (2009)
Nucleus basalis of	Human (Parkinson	Bilateral, monopolar	California Verbal Learning Test-II,	No improvements	Gratwicke et al.

Structure	Subject	Type of stimulation	Memory task	Effect	Reference
Meynert	patients) *N* = 6	, 20 Hz, of 60 µs pulse width for 6 weeks	WAIS-III digit span, verbal fluency, Posner covert attention test, simple and choice reaction times	in the primary cognitive outcomes. Other neuropsychiatric symptoms, such as visual hallucinations were alleviated.	(2018)
Nucleus basalis of Meynert	Human (AD patients) *N = 6*	Bilateral, 2.0–4.5 V, 10–20 Hz and 90–150 µs pulse width, 2 weeks on and 2 weeks off or vice versa, followed by continuous stimulation for 11 months	ADAS-cog, MMSE, CDR	Stable or improved cognitive function in 4 patients and increased glucose metabolism in 3 patients. QoL ratings improved in 2 patients, 2 noticed no change and 2 patients reported a decrease in their QoL.	Kuhn et al. (2014)
Nucleus basalis of Meynert	Human (AD patients) *N* = 2	Bilateral, 2.0–4.5 V, 10–20 Hz and 90–150 µs pulse width, 2 weeks on and 2 weeks off or vice versa, followed by continuous stimulation for	ADAS-cog, MMSE	Favorable long-term effects of DBS in 2 younger and early stage AD patients.	(Kuhn et al., 2015)

Structure	Subject	Type of stimulation	Memory task	Effect	Reference
		11 months			
Nucleus basalis of Meynert	Rat (basal forebrain lesion) *N* = 5	Unilateral, 1 V, 120 Hz, 90 µs pulse width for 1 h per day for 1 week.	Morris water maze	Improved spatial memory performance is related to changes in glutamic acid decarboxylase and glutamate transporter levels in the medial prefrontal cortex.	Lee et al. (2016)

Hippocampus and entorhinal cortex

Clinical and preclinical evidence suggests that the hippocampus serves a critical role in learning and memory. Neurogenesis occurs throughout the human life span in the hippocampus and likely contributes to memory formation. It is therefore not surprising, that impaired neurogenesis compromises hippocampal function and plays a role in cognitive deficits of AD mouse models[29].

So far, contradictory results have been reported in literature regarding the effects of hippocampal and entorhinal cortex DBS on memory. In a multisite clinical study, 49 epileptic patients were subjected to hippocampal and entorhinal region DBS and their performance in spatial and verbal-episodic memory was assessed. DBS at 50 Hz, 0.5–1.5 mA (depth contacts) and a balanced biphasic stimulation pulse of 300 µs per phase significantly impaired spatial and verbal memory encoding in these patients[30]. Contrary to this, in another study, 7 pharmacoresistant epilepsy patients showed superior memory performance in a virtual spatial memory task when DBS was applied in the entorhinal cortex region [31]. Although, the stimulation parameters were identical to the previously mentioned study (50 Hz, 0.5–1.5 mA, biphasic stimulation pulse of 300 µs), this study had key methodological differences. Most notably, only a small number of patients was included and the spatial memory task differed. In particular, Suthana et al. [31]had a visible target destination and a fixed starting location, which could allow the use of non-allocentric strategies, while Jacobs et al. [30]used a task

similar to the Morris water maze, including an open arena with distant landmarks, hidden target locations and randomized starting positions. This design encouraged subjects to encode spatial memories allocentrically. Finally, the duration of stimulation was different in both studies. In Suthana's study a variable stimulation was applied according to the length of time that the patient spent navigating each trial, whereas Jacobs and colleagues applied stimulation for exactly 10 s per trial. As a result of this difference, patients might have been stimulated for a longer total duration in the study of Suthana et al.

Suthana and colleagues found that enthorhinal stimulation with 50 Hz led to a theta phase resetting measured through hippocampal depths electrodes. The entorhinal cortex is strongly connected to the dentate gyrus via the perforant pathway. In a preclinical study, it could likewise be demonstrated that electrical stimulation of the perforant pathway triggers a theta phase resetting in rodents, thereby creating favorable conditions for long term potentiation[32]. Moreover, the beneficial effect of entorhinal cortex stimulation has been suggested to be mediated via neurogenesis. In rodents, the effects of entorhinal cortex DBS on spatial memory was assessed either 1.5 or 6.5 weeks after stimulation[33]. DBS was performed for 1 h at 50 μA, 130 Hz and 90 μs pulse width (while being under general anesthesia). With the help of the proliferation marker BrdU, the authors found that DBS increased proliferative activity in the dentate gyrus after 6.5 weeks, which in turn resulted in the enhancement of spatial memory. Of note, the concept of adult neurogenesis has been shown to exist in animals, however, there is insufficient evidence that adequately supports its existence in adult humans [34]. Additional studies exploring the dynamic changes of neurogenesis in the known regions of the human brain, with reference to the physiological and diseased conditions are needed in order to understand whether the beneficial effects of entorhinal cortex DBS may indeed be mediated via neurogenesis.

Hippocampal stimulation was re-investigated in a later study using a rat model of experimental dementia. Acute DBS of the CA1 subregion of the hippocampus at 100 Hz, 100 μA and 100 μs improved performance in an object location task, although also increasing anxiety-related behavior[35].

Anterior thalamic nucleus

Neurotoxic anterior thalamic nuclei lesions have shown to disrupt performance in spatial memory tasks, e.g. T-maze alternation, radial-arm maze, Morris water maze, object location [36-39]. These tests depend on allocentric rather than egocentric processing.

With regard to DBS, the anterior nucleus of the thalamus (ANT) has been targeted in the SANTE study[40]. When examining the incidence of memory and depression adverse events in the blinded phase and their relationship to objective neurobehavioral measures, no significant differences were found. However, ANT DBS was associated with subjectively reported depression and memory deficits[41].

nterestingly, DBS of the ANT was performed in rats and also induced memory impairment. Hamani et al. [42]showed that ANT stimulation at relatively high current (500 μA) disrupted the acquisition of contextual fear conditioning and impaired performance on a spatial alternating task (four-arm maze) in rats. This has not been observed at parameters generating a charge density that approximated the one used in clinical practice (100 μA), but memory performance of DBS rats was not enhanced with either current density. The authors suggest that stimulation with too high current density causes a depolarization block. In another study, corticosterone-treated rats were stimulated in the ANT with 2.5 V, 130 Hz and 90 μs pulse width while being under general anesthesia[43]. The authors have found that with high frequency stimulation, cell division in the subgranular layer of the hippocampus has significantly increased 28 days after the last BrdU injection. This was not observed in non-stimulated control groups and suggests that DBS might enhance neurogenesis in certain areas which are involved in memory formation[43, 44].

Nevertheless, when applying ANT DBS in patients, monitoring and neuropsychological assessment of depression and memory are recommended.

Fornix

The fornix arises from output fibers of the hippocampus located in the medial temporal lobe below the base of the lateral ventricle[45, 46]. The fornix is imperative to the function of formation and consolidation of memory in rodents and primates[47, 48]. It is known that lesions of the fornix lead to various amnestic syndromes[49].

Serendipitously, it was found that fornix DBS at 130 Hz, 3–5 V and 60 μs pulse width generated detailed autobiographical memories in a patient suffering from morbid obesity [47]. Following this, a phase I trial was launched investigating the effects of fornix DBS in 6 AD patients[50]. Patients received chronic high frequency DBS for a period of 12 months. The authors found that the application of DBS in the fornix vicinity was safe and triggered neural activity in the memory circuit, including the entorhinal and hippocampal areas. PET scans showed a striking reversal of the impaired glucose utilization in the temporal and parietal

lobes that was maintained after 12 months of continuous stimulation. Evaluation of the Alzheimer's Disease Assessment Scale cognitive subscale (ADAS-cog) and the Mini Mental State Examination (MMSE) suggested possible improvements and slowing the progression of memory loss at 6 and 12 months, especially in patients that were less severely affected at the time of surgery. After 1 year of continuous DBS, the functional connectivity analysis demonstrated increased cerebral metabolism in cortical-subcortical and cortical-hippocampal networks. In similar cortical regions, both a higher baseline metabolism and an increase after 1 year of DBS were correlated with less decline or improvement in global cognition, memory, and quality of life [51] Surprisingly, 2 patients demonstrated a hippocampal volume increase following DBS[52].

Because of the promising preliminary results, researchers proceeded with a phase II study of a yearlong, randomized, double-blind trial of fornix DBS in 42 mild AD patients. Patients stimulated in the fornix exhibited increased metabolism at 6 months, but not at 12 months. The post-hoc multivariate regression analysis showed that age and treatment interacted significantly; contrary to patients < 65 years old (n = 12) whose clinical outcomes trended to be worse with DBS ON, patients ≥ 65 (n = 30) with DBS ON demonstrated not only increased cerebral glucose metabolism but also benefits on clinical outcomes[53]. The authors hypothesized that this interaction in age and treatment might be related to greater brain atrophy and metabolic deficits, or a more malignant course in younger AD patients. Another conclusion of this trial was that the stimulation parameters applied to AD patients were not disease-specific[53] and retrospectively the trial can be ventured as pre-mature. Developing AD-specific stimulation parameters is likely to improve the current approach of DBS in AD.

In rodent research, bilateral DBS of the forniceal region, improved spatial memory performance in the object location task in a rat model of experimental dementia [54] In other words, DBS reversed the memory impairing effects of scopolamine when compared to sham rats in high current densities (both 200 μA and 100 μA were effective and independent of frequency). C-Fos immunohistochemistry data revealed that fornix DBS selectively activated cells in the CA1 and CA3 sub-region of the hippocampus. Moreover, microdialysis sampling was performed in the dorsal hippocampus during fornix DBS. Extracellular neurotransmitters such as acetylcholine substantially increased 20 min after the initiation of stimulation while hippocampal glutamate levels were not significantly different when compared to the baseline[55]. Remarkably, the release of acetylcholine was substantial when DBS was initiated, but then declined over time despite ongoing DBS. Notwithstanding, the authors

investigated these extracellular neurotransmitters with only the stimulation paradigm of 100 Hz, 100 μA and 100 μs. Further studies should investigate whether an optimal release of acetylcholine can be achieved through different stimulation parameters of the fornix and lead to long-term therapeutic effects. The first preclinical study that reported about chronic fornix DBS in a transgenic rat model of AD, found beneficial effects of DBS on amyloid burden, inflammation, and neuronal loss in both cortex and hippocampus[56]. Researchers applied permanent, bilateral, and unipolar stimulation (130 Hz, 80 μs, 100 μA) 10 days after implantation surgery [56]. In another translational study, fornix DBS was applied in an AD mouse model[57]. The authors combined brain imaging and behavior by a proof-of-concept methodology in longitudinal assessments. After 1 h of fornix DBS at 100 Hz, 100 μs pulse width and 100 μA, mice were assessed in the Morris water maze. The authors found that DBS treatment improved learning and long-term memory 3 and 6 weeks later, in a delayed, sex specific, and transient manner relative to sham, with significant differences driven mostly by males. Females tended to perform well irrespective of stimulation status. Significant, persistent, volumetric changes were seen in diverse brains structures, such as the bilateral cingulate cortex areas. In particular, DBS induced higher final volumes in males and lower final volumes in females. In contrast, the fimbria, alveus and external capsule displayed the opposite, in which stimulation resulted in higher final volumes for females, and lower volumes for males. The greatest volumetric changes were found in the colliculi, which are not part of the circuit of Papez and are related to visual and auditory processing. It is thus possible that differences in visual/auditory processing and coordinated movements could affect the latencies to reach the target in the Morris water maze. While the exact mechanism of DBS in causing volumetric changes remains controversial, the authors hypothesized that DBS acts through neurogenesis and/or the modulation of plasticity factors. The pronounced sex differences highlight the importance of conducting trials with both sexes, since experiments with only male animals, can lead to false conclusions about the effectiveness and safety, and significantly limit generalizability of treatments under investigation in preclinical trials[57].

Nucleus basalis of Meynert

Almost 90% neurons of the nucleus basalis of Meynert (NBM) are cholinergic[58]. A decline in the number, size, or function of cholinergic neurons of the NBM may be associated with impaired cognition and memory in dementia[59-61]. The projecting fibers originating from

the NBM are mainly found in the hippocampus, amygdaloid nuclei, hypothalamus, thalamus and prefrontal cortex, which are all structures associated with memory and cognition[62].

In 1984, unilateral NBM DBS was applied in a patient suffering from AD[63]. Stimulation parameters were set to 3 V, 50 Hz, and 210 µs, cycling between 15 s on and 12 min off. Even though the patient's cognition did not improve after being stimulated for 8 months, DBS had an effect on cerebral glucose metabolism. Using the patient's unstimulated contralateral hemisphere as a control, FDG-PET scans of the right hemisphere showed that glucose metabolism in the frontal, temporal, parietal, and occipital lobes decreased by 21%, 24%, 10%, and 7.5%, respectively. In contrast, glucose metabolism in the stimulated left hemisphere had decreased by only 12% in the frontal lobe and 4.1% in the occipital lobe and increased by 1.5% in the temporal lobe. There are many limitations to the study. First, the NBM was targeted indirectly using atlas coordinates. Second, it is unclear why the authors chose to target the NBM unilaterally, since AD affects both hemispheres. Third, the stimulation parameters seem to be somewhat arbitrary and no rationale was provided by the authors for choosing these parameters.

Some years later, a patient suffering from Parkinson's disease dementia underwent NBM DBS in combination with bilateral subthalamic nuclei (STN) DBS. The parkinsonian symptoms responded well to conventional 130 Hz STN stimulation and bilateral stimulation of the NBM at 20 Hz, 1 V, and 120 µs resulted in sustained improvement in various aspects of cognitive functioning, such as concentration, attention, alertness, apraxia, ataxia and memory[64].

Based on these positive findings, a randomized, double-blind, crossover clinical trial with six patients with Parkinson's disease dementia was launched in the United Kingdom[65]. After surgery, patients were assigned to receive either active stimulation (bilateral, monopolar, 20 Hz with a pulse width of 60 µs) or sham stimulation for 6 weeks, followed by the opposite condition for 6 weeks. There was no significant change in cognitive outcomes across the group with stimulation, but there was a suggestion of improvement in neuropsychiatric symptoms, particularly visual hallucinations.

In a different, double-blind clinical trial, 6 mild AD patients were subjected to NBM DBS. The clinicians opted for an elaborate study design of two phases: phase I) a randomized sham-controlled DBS phase of one month where patients underwent two weeks of stimulation

followed by two weeks off stimulation (sham); phase II) an open stimulation phase of eleven months, where stimulation settings were adjusted according to individual needs. Using the ADAS-Cog as the primary outcome measure, the authors concluded that four out of six patients responded with cognitive improvement. No significant side effects were observed[66]. Interestingly, it was noted that disease severity and age predicted response to stimulation on the primary outcome measure. To further explore these prediction factors, the same group performed NBM DBS on two younger patients, aged 61 and 67 respectively, with mild AD. They subsequently provide evidence that NBM DBS performed at an earlier stage of the disease and at younger age may have a favorable impact on disease progression and cognitive functions, probably due to the modulation of cholinergic processes [67, 68].

With regard to neurochemical effects of NBM stimulation in rats with basal forebrain cholinergic neurons degeneration, gamma-aminobutyric acid (GABA), and glutamate seem to play a role in restoring memory loss[69]. The degeneration of basal forebrain cholinergic neurons is preferentially vulnerable in AD and is associated with spatial learning and memory impairment. Stimulation was unilateral, bipolar and parameters were 1 V, 90 µs at 120 Hz for 1 h per day for 1 week. In a spatial memory test, the DBS group with basal forebrain lesion showed an equivalent performance to controls without lesion, while sham animals performed significantly worse. Moreover, NBM DBS seemed to regulate levels of glutamic acid decarboxylase, which is involved in the synthesis of GABA and glutamate. In sham animals glutamic acid decarboxylase decreased in the medial prefrontal cortex, while expression of glutamate transporters increased in the medial prefrontal cortex and hippocampus.

All in all, neuromodulation of ascending basal forebrain projections of the NBM may represent a new and complementary strategy for enhancing the residual nucleus basalis output. This can be achieved by using low-stimulus rates (20 Hz). There is converging evidence from different experimental conditions that low-frequency stimulation has excitatory rather than inhibitory actions. Furthermore, NBM neuronal discharge rates at approximately 20 Hz are typically observed during active behavior in rats.

Discussion

In animal models, DBS has long been used to probe the behavioral and cognitive roles of various brain structures. The technique, however, lacks specificity. Despite improving motor disability in Parkinson patients, for example, DBS can induce severe mood disorders such as depression, impulsivity and suicidal ideation[70-72]. These mood-related side-effects often mitigate the positive effects on motor symptoms and negatively influence the quality of life of patients and their caregivers[73]. The undesired psychiatric side effects are thought to be caused by current spill to adjacent non-motor regions from the target[74, 75]. These side effects may also be caused by inadvertent stimulation of limbic circuit elements far away from the target, since DBS can have a circuit-wide effect. Moreover, DBS does not only affect neurons in this network, but also glial cells, which in turn can have profound effects on network activity patterns[76].

Another shortcoming of DBS used in cognitive disorders is that the stimulation parameters are selected mostly based on movement disorder trials and similar mechanisms are considered when the outcomes are discussed.

Nevertheless, despite similar stimulation settings compared to movement disorders, the mechanisms behind the therapeutic effects in cognitive disorders may be quite different. This can be largely attributed to differences in stimulation targets, but also to differences in neuropathologies/circuitopathies. In many movement disorders continuous abnormal burst firings disrupt the regular activity in the basal ganglia[77, 78]. Moreover, at the level of local field potentials the burst activity of neuronal population is synchronized and oscillates with a frequency at beta range [79]. The stimulation settings commonly used in clinical practice are likely to reduce spontaneous firing of neuronal populations and drive axonal projections near the electrodes. This may result in presynaptic release of inhibitory transmitters or congestion of the neural network[22, 80-82]. Through these, DBS modulates the pathological activity in the basal ganglia and replaces it with regular pattern of discharges, which was termed *neuronal hijacking*. Other factors, such as pattern, oscillation, and synchronization, as well as changes in the network dynamics have also been discussed to play a role. DBS in basal ganglia regions has shown to attenuate these pathological changes [83-89].

In cognitive disorders, however, the exact circuitopathy is largely unknown, e.g. precise electrophysiological and neurochemical perturbations in the network have not been identified.

In line with this, it is important to note that unlike movement disorders, cognitive decline does not seem to have a pathognomonic oscillatory abnormality due to a known neurodegenerative process. Thus far, stimulation targets in cognitive disorders were selected within the circuit of Papez and related limbic structures. Studies have shown that the improvement in cognitive symptoms following DBS appears in stages, with immediate changes in memory predictably evoked by initial stimulation in the operating room [47]. Thereafter, more gradual changes in cognitive symptoms occur with ongoing chronic stimulation[50], and the full clinical response generally further evolves over months[53]. One interpretation of the slow time course is that different local and remote neural elements in different stages can be influenced by the application of electrical current, some rapid and some slow.

Rodents and human studies have suggested that that chronic stimulation delivered to white matter in and around the fornix causes changes in neural activity, synaptic integrity and metabolic function in memory related areas such as the hippocampus[27, 52]. This hypothesis is also supported by the time course of metabolic and blood flow changes in regions with direct connections to and from the fornix over the course of months of DBS therapy[50]. It also fits well with the effect of electrical stimulation of fornical white matter in rodents, which is associated with increased neurotransmitter release in the hippocampus[55].

Altogether, a better understanding of these mechanisms will help to improve current applications and develop new ones for patients with neurological and psychiatric disorders.

Conclusion

DBS has shown to exert pro-cognitive effects through a number of mechanisms, e.g. neurochemical changes, synaptic plasticity and neurogenesis. Future studies should identify stimulation protocols based on these mechanisms in order to promote hypothesis-driven research in this field.

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Chapter 3

The effect of fornix deep brain stimulation in brain diseases

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Abstract

Deep brain stimulation is used to alleviate symptoms of neurological and psychiatric disorders including Parkinson's disease, epilepsy, and obsessive-compulsive-disorder. Electrically stimulating limbic structures has been of great interest, and in particular, the region of the fornix. We conducted a systematic search for studies that reported clinical and preclinical outcomes of deep brain stimulation within the fornix up to January 2019. We identified 12 studies (7 clinical, 5 preclinical) that examined the effects of fornix stimulation in Alzheimer disease (n=8), traumatic brain injury (n=2), Rett syndrome (n=1), and temporal lobe epilepsy (n=1). Overall, fornix stimulation can lead to decreased rates of cognitive decline (in humans), enhanced memory (in humans and animals), visuo-spatial memorization (in humans and animals), and improving verbal recollection (in humans). While the exact mechanisms of action are not completely understood, studies suggest fornix DBS to be involved with increased functional connectivity and neurotransmitter levels, as well as enhanced neuroplasticity.

Introduction

The circuit of Papez is considered one of the major pathways of the limbic system and is primarily involved in emotional expression, neurovegetative function, and memory [1]. The classical circuit consists of the hippocampal formation, fornix, mammillary bodies, mammillothalamic tract, anterior thalamic nucleus, cingulum, and the entorhinal cortex [2]. Damage to structures within the circuit of Papez can result in anterograde amnesia in patients, i.e., an inability to create new episodic memories [3-7].

Deep brain stimulation (DBS) within the basal-ganglia network has proven to be safe and effective for movement disorders, while its application to other neural pathways such as the circuit of Papez is under active investigation. While DBS applied to limbic targets has been evaluated for patients with treatment-resistant depression [8-10] and obsessive-compulsive disorder [11], recently studies have begun to explore the applicability of DBS in a widening array of psychiatric conditions and have demonstrated it to be a cognitively safe procedure, for Parkinson's desease[12], essential tremor[13], epilepsy[14], Alzheimer's disease[15] and other brain diseases. DBS has shown to have a positive effect on long-term structural plasticity as well as neurotransmitter release, but despite the long history, the basic neural mechanisms underlying DBS are still debated [16]. Nevertheless, a number of key mechanisms have been demonstrated [17] and preclinical research has demonstrated [18, 19] that different stimulation parameters can be selected in animal models to discover optimal settings that have greater therapeutic benefits. Potential side effects and underlying mechanisms of fornix DBS can also be investigated in animal models.

The fornix is essential in memory function, supported by reports that lesions in the fornix in experimental animals and humans are known to cause memory deficits [20-23]. In the current review we focus on the effects of fornix DBS on brain diseases, discuss advances within DBS systems and the potential mechanisms of action underlying symptom reduction, and briefly describe preclinical and clinical studies with regard to AD, Rett syndrome, traumatic brain injury, and temporal lobe epilepsy to elucidate their potential within future research. Lastly, we highlight the use of fornix DBS to restore memory loss and discuss overall considerations.

Methods

For this review, we searched PubMed for clinical and preclinical studies in English literature with the search terms "deep brain stimulation", "fornix", "Alzheimer disease", "Rett syndrome", "dementia", "traumatic brain injury", and "temporal lobe epilepsy". By using

various Key words both independently and in different combinations a total of 416 results was obtained. Relevant articles were chosen from review papers, original research articles, and book chapters about DBS and the fornix ranging from basic research to clinical applications. We screened the abstracts and included researches, if original data were presented, the manuscript had been published in English and involved with human or rodent research. We summarized the available literature in this field and compared it with the results of relevant preceding research. After investigating all relevant studies we evaluated 13 (7 human; 6 animal studies) studies for further details.

What is DBS?

Deep brain stimulation is a minimally invasive surgical method in which stimulation electrodes are stereotactically implanted into specific brain targets. The implantation of DBS electrodes can be performed under local or general anaesthesia. The most commonly used DBS system uses a multi-contact stimulating electrode that is connected with an internal pulse generator through a subcutaneous wire. The DBS device and the settings can be accessed externally with a wireless connected controller. Stimulation parameters can be adjusted to obtain the best possible therapeutic effects with little or no side effects. Different stimulation parameters such as frequency, amplitude, pulse width, the choice of bipolar or monopolar stimulation, and continuous or intermittent stimulation can be adjusted. Some DBS systems also allow for steering, meaning that a specific part of the circular contact can be activated or de-activated. Severe adverse effects related to the surgical procedure are intracerebral haemorrhages that occurs in 1%–2% of patients while less severe or reversible events such as infections, lead, and pulse generator problems occur in a vast minority of the patients [24].

Advances in DBS technology

Although DBS is an established treatment for many neurological disorders such as Parkinson's disease, tremor, epilepsy, and dystonia, there are still limitations in terms of efficacy, side effects, and battery consumption. In order to accommodate these limitations, advances in DBS technology have focused on stimulation procedures, electrodes, and pulse generator design.

With regard to limited efficacy and the occurrence of side effects, researchers found that these challenges may be due to modulating not only pathological but also physiological neural activity [25, 26]. For this reason, adaptive DBS (aDBS) where stimulation is only applied when necessary might be advantageous. In aDBS, a device records local field potential activity (or other physiological signals) from the implanted DBS electrode and delivers

simultaneous stimulation through the same electrode based on the recorded signal. The recorded physiological signals can then be fed back to dynamically alter and optimize stimulation parameters [27]. Clinical implementation of aDBS has been limited due to a range of challenges in optimizing each component of the feedback [28], but the approach promises substantial benefits in the future.

Another refinement for DBS is called coordinated reset (CR) DBS which aims towards therapeutic reshaping of neuronal connectivity by harnessing synaptic plasticity (e.g., spike timing-dependent plasticity) [29, 30]. In this method,brief high-frequency pulse trains are given through the different contacts of the stimulation electrode in treatment blocks for a few consecutive days resulting in the disruption of pathologically synchronized oscillations. The goal of CR-DBS is to decrease synaptic weights thereby debilitating pathological connectivity and synchrony [31]. In a non-human primate model of parkinsonism, CR-DBS of the STN for 5 consecutive days resulted in acute motor improvements and, in contrast to traditional DBS, showed benefits persisting up to two weeks after stimulation [32].

The advent of directional leads is another technological advancement in DBS that allows targeting to be made more accurately with the goal of avoiding side effects [33]. Unlike conventional DBS leads which use cylindrical electrodes, directional leads are comprised of radially segmented electrodes that allow the stimulation field to be moved in the plane perpendicular to the lead, or shaped using anodes and cathodes to steer stimulation in a particular direction [34]. Given the novelty of this approach, however, there is currently no firm clinical evidence.

Finally, the use of rechargeable implantable pulse generators (rIPG) pretense another innovation in the field and have been proven effective and applicable in Parkinson's disease, essential tremor, and dystonia [35]. These rlPGs have a longevity of at least 15 years in contrast to the non-rechargeable IPGs showing a mean longevity of 3-5 years. The major advantage is that patients need fewer replacement surgeries while a disadvantage is that patients must charge the rIPGs a few times a week [36].

Mechanisms of DBS

Initial hypotheses about the mechanism of DBS were based on observed similarities between DBS and lesion therapy on the alleviation of symptoms in Parkinson's disease. For example, internal globus pallidus (GPi) DBS [37-39] and pallidotomy [40] both produce similar effects on parkinsonian motor symptoms. Thus, DBS was initially believed to generate a

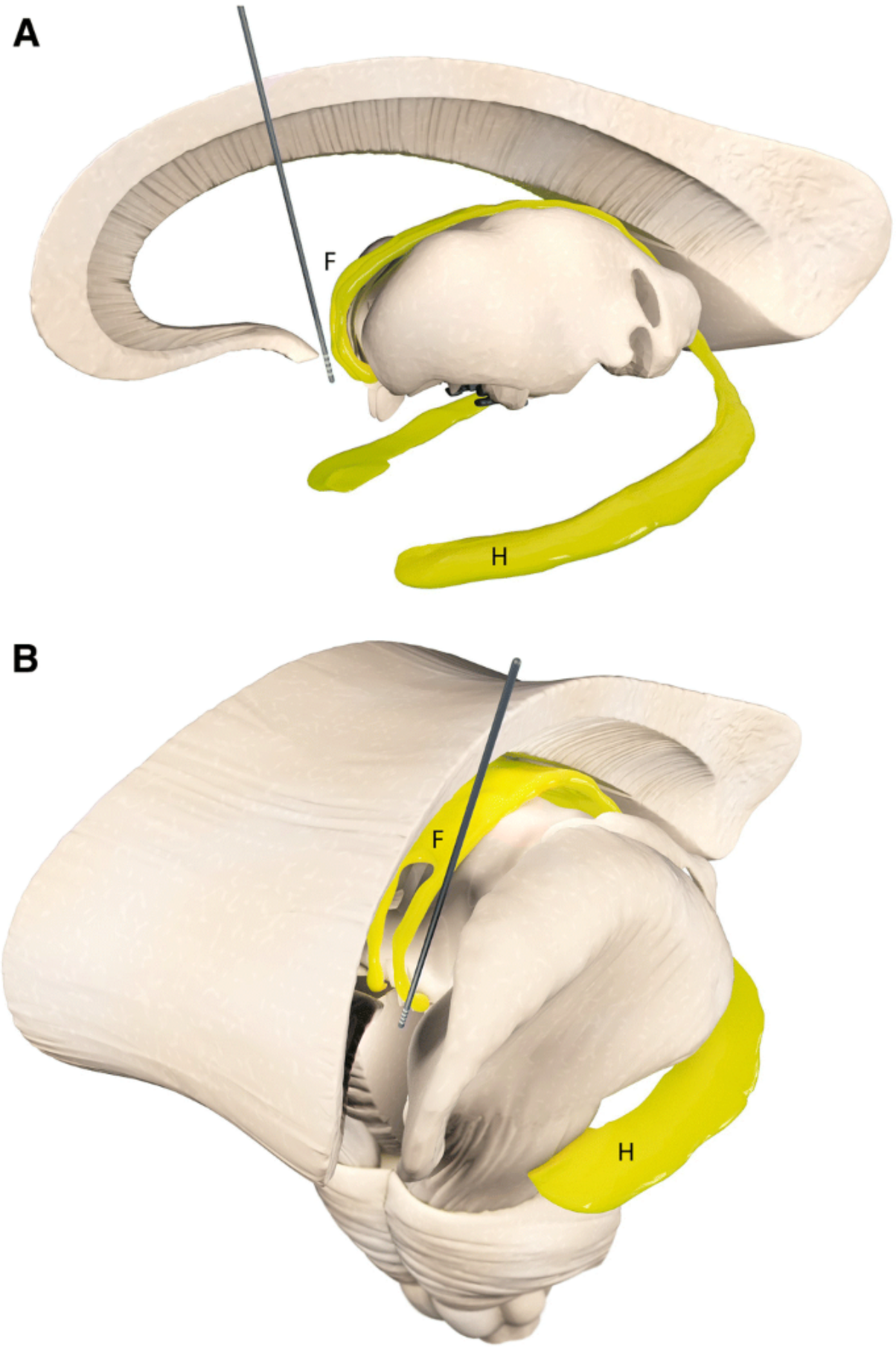
depolarization block of neurons around the stimulating electrode [41, 42]. Later, it was shown that DBS might also have an effect on neuronal firing patterns. These changes in firing patterns are thought to prevent transmissions of pathologic bursts and oscillatory activity, resulting in the reduction of disease symptoms through compensatory processing of sensorimotor information.

In addition to the local electrical effects of DBS, researchers found that DBS could also induce neurochemical changes locally and through the stimulated network. For instance, DBS of the anterior thalamus for the treatment of epilepsy in a rodent model induces the release of hippocampal adenosine [43]. Moreover, DBS has shown to induce plastic changes with regard to synaptic plasticity and neurogenesis. In line with this, Gondard and colleagues have shown that acute fornix DBS can modulate neurotrophic factors such as brain derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) as well as synaptic plasticity markers such as growth associated protein 43, α-synuclein and synaptophysin [44]. Hippocampal neurogenesis has additionally been induced following thalamic DBS in a group of adult rats [45]. The authors concluded that an involvement of the Papez circuitry is necessary in mediating the effects of DBS and in the treatment of cognitive and behavioral disorders.

The anatomy, connections, and functions of the fornix

The fornix arises from output fibers of the hippocampus located in the medial temporal lobe below the base of the lateral ventricle. Under the ependymal surface of the lateral ventricle is a thin layer of efferent fibers known as the alveus that mainly ascend from the pyramidal cells of the hippocampus and form a fringe of fibers known as the fimbria. Beneath the splenium of the corpus callosum the white matter of the fimbria separates from the hippocampus and becomes the crus of the fornix [46, 47]. Sometimes the fimbria and fornix are referred to as the fimbria-fornix complex to highlight its functional unity and anatomic connections. The left and right crura then converge in the medial plane beneath the trunk of the corpus callosum to form the body of the fornix. The lateral portions of the body of the fornix are joined by a thin triangular lamina that contain some commissural fibers that connect the two hippocampi known as commissure of the fornix or commissure of the hippocampus. The body of the fornix travels anteriorly and divides again near the anterior commissure. The left and right parts separate into the anterior pillars, and there is also an anterior/posterior divergence. The posterior fibers (called the postcommissural fornix) of each side continue through the hypothalamus to the mammillary bodies and then to the anterior nuclei of thalamus which

project to the cingulate cortex. The anterior fibers (precommissural fornix) end at the septal nuclei and nucleus accumbens of each hemisphere. An anatomic illustration of the fornix can be found in Fig 1.


A
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H

Simplified illustration of anatomical targeting for fornix deep brain stimulation in clinical studies. The fornix (F) and the hippocampus (H) are depicted in yellow. Efferent fibers of the hippocampus known as the alveus join together to form the fimbria. Beneath the splenium of the corpus callosum, the fimbria separates from the hippocampus and becomes the crus of the fornix. The left and right crura then converge to form the body of the fornix. The body of the fornix travels anteriorly and divides again near the anterior commissure. The left and right parts separate into the anterior pillars, and there is also an anterior/posterior divergence. The posterior fibers (called the postcommissural fornix) of each side continue through the hypothalamus to the mammillary bodies. The anterior fibers (precommissural fornix) end at the septal nuclei and nucleus accumbens of each hemisphere. a Sagittal view of fornix DBS electrode location. b Frontal view of fornix DBS electrode location in one hemisphere

The most common types of neuroglia cells in the fornix are oligodendrocytes, followed by astrocytes, and microglial cells [48]. The primary function of these neuroglia cells is to form myelin, maintain homeostasis, and provide support and protection for neurons amongst others. Neuroanatomical and axonal tract tracing studies reveal that fibers in the fimbria-fornix fall into two categories, thin unmyelinated and thick myelinated [49]. In particular, it was shown that a major source of cholinergic innervation of the hippocampus comes from the medial septum via the fimbria-fornix pathway and contains axons that are unmyelinated or thinly myelinated [50]. GABAergic septohippocampal axons also project to the hippocampus via the fimbria-fornix pathway and contain thickly myelinated fibers [51]. The cholinergic neurons synapse onto all hippocampal cell types while the GABAergic neurons terminate on hippocampal GABAergic neurons [50].

The fornix is an integral part of the classical Papez circuit. When considering the rodent and primate Papez circuits, the core connections of the hippocampal-diencephalic-cingulate network are respectively homologous. One of the major differences is in the connections of the cingulate cortices in rodents and primates (for review see [52]). The fornix is imperative to the function of formation and consolidation of memory in rodents and primates [53, 54] as it has been shown that lesions of the fornix lead to various amnestic syndromes [55].

Studies on fornix DBS

We identified 12 studies that examined the effects of fornix DBS in Alzheimer disease (n = 8), traumatic brain injury (n = 2), Rett syndrome (n = 1), and temporal lobe epilepsy (n = 1). A

summary of these studies can be found in Table 1. In the following, we will review each disorder separately.

Subject	Type of stimulation	Memory task	Effect	References
Human (patients with TBI) $N = 4$	Continuous DBS using a burst pattern (200 Hz in 100 ms trains, 5 trains/s, 100 µs pulse width, 7 mA, bipolar and bilateral stimulation)	Rey auditory verbal learning test, Medical College of Georgia Complex Figure Test, Boston Naming Test	Burst stimulation of the fornix was associated with a robust reversible improvement in immediate and delayed performance on the Medical College of Georgia Complex Figure Test	Miller et al. [58]
Rats (model of TBI) $N = 21$	Low-frequency (5 Hz), high-frequency (130 Hz), and theta-burst stimulation (200 Hz in 50 ms trains, five trains per second; 60 µA biphasic pulses)	Swim T-Maze, Morris Water Maze	Deficits in learning and memory after TBI are improved following DBS of the fornix	Sweet et al. [60]
Human (patients with intractable epilepsy) $N = 11$	Bilateral, high amplitude, low pulse width,	MMSE	An increase of MMSE scores during stimulation.	Koubeissi et al. [63]

Subject	Type of stimulation	Memory task	Effect	References
	low frequency (8 V, 0.2 μs, 5 Hz), for 4 h		Hippocampal spikes were significantly reduced during and outlasting each stimulation session. Seizure odds ($n = 7$) were reduced by 92% in the 2 days that followed stimulation	
Mice (model of RTT) $N = 21$	Bilateral, 130 Hz, 60 μs pulse duration, for 1 h per day for 14 days	Fear conditioning, Morris water maze, open field, light–dark box, wire hang and dowel walk, accelerating rotarod, three-chamber interaction, and pain threshold	Forniceal DBS in RTT mice rescues contextual fear memory as well as spatial learning and memory	Hao et al. [65]
Human (morbid obesity patient) $N = 1$	Bilateral, 3–5 V, 130 Hz and 60 μs pulse width, continuous for 3 weeks	Neuropsychological tests, e.g., verbal learning test, WAIS attention index, spatial associative learning, etc	Significant improvements on the California Verbal Learning Test and Spatial Associative Learning Test	Hamani et al. [50]
Human (AD patients) $N = 6$	Bilateral, 3.0–3.5 V, 130 Hz, and 90 μs pulse width,	ADAS-cog, MMSE	Possible improvements and/or slowing in the rate of cognitive decline at 6	Laxton et al. [72]

Subject	Type of stimulation	Memory task	Effect	References
	continuous for 12 months		and 12 months in some patients	
Human (AD Patients) *N* = *6*	Bilateral, 3.0 V, 130 Hz and 90 µs pulse width, continuous for 12 months	ADAS-cog, MMSE	Local volume increase in parahippocampal gyri, right superior temporal gyrus, left parietal lobule and bilateral precuneus as well as thalamus and superior frontal gyrus	Sankar et al. [73]
Human (AD patient) *N* = 1	Bilateral, 2.5 V, 130 Hz and 210 ms pulse width, continuous for 12 month	ADAS-cog, MMSE, Free and Cued Selective Reminding Test	Cognitive scores worsened after 6 months but returned to baseline after 12 months of chronic DBS	Fontaine et al. [74]
Human (patients with mild AD) *N* = 42	Bilateral, 3.0–3.5 V, 130 Hz, with a pulse width of 90 microseconds at the top, or second from top, of the 4 electrode contacts, continuous for	ADAS-Cog-13, CDR-SB, California Verbal Learning Test-Second Edition (CVLT-II), the Alzheimer's Disease Cooperative Study Activities of Daily Living scale (ACDS-ADL)	Participants aged ≥ 65 years show less cognitive decline while there was possible worsening in patients below 65 years with stimulation	Lozano et al. [75]

Subject	Type of stimulation	Memory task	Effect	References
	12 month			
Rats (model of experimental dementia) $N = 10$	Bilateral, 100 and 200 µA, 10 and 100 Hz, 100 µs pulse width, acute stimulation	OLT	Memory enhancement in high current densities (frequency-independent)	Hescham et al. [78]
Rats $N = 29$	Bilateral, 100 Hz, 100 µA and 100 µs pulse width for 1 h	N/A	Fornix DBS induced a selective activation of cells in the CA1 and CA3 subfields of the dorsal hippocampus, a substantial increase in the levels of extracellular hippocampal acetylcholine	Hescham et al. [79]
Rats (transgenic rat model of AD, tgF344) $N = 10$	Permanent, bilateral, and unipolar stimulation (130 Hz, 80 µs, 100 µA)	N/A	Amyloidosis, inflammatory responses, and neuronal loss decreased in both cortex and hippocampus after DBS in the fornix	Leplus et al. [76]
Mice (transgenic mouse model of	Monophasic, 1 h (100 Hz,	Morris water maze	Fornix DBS improved learning	Gallino et al. [77]

Subject	Type of stimulation	Memory task	Effect	References
AD, 3xTg) $N = 50$	100 µs pulses, 100 µA)		and long- term memory after 3 and 6 weeks with significant differences driven mostly by males	

The table shows the subjects involved, the type of stimulation, memory task, and the behavioral outcome of the surgical intervention

ADAS-cog Alzheimer's Disease Assessment Scale-cognitive subscale, CDR-SB Clinical Dementia Rating Scale Sum of Boxes, OLT object location task, DBS deep brain stimulation, MMSE mini-mental state examination, WAIS Wechsler Adult Intelligence Scale

Traumatic brain injury (TBI)

Traumatic brain injury (TBI) is one of the world's most devastating causes of morbidity and mortality. TBI affects more than 1.5 million patients in the Europe and 1.7 million people in the United States every year. TBI is considered to be an injury to the head which is related to symptoms or signs caused by injury, i.e., skull fracture, amnesia, decreased or altered levels of consciousness, neurological or neuropsychological abnormalities, or intracranial lesions [56].

Many TBI patients experience significant functional deficits, e.g. somatic disorders (such as headaches or dizziness), emotional sickness (such as sleep disturbance, anxiety, or depression), impaired executive function, and memory loss [57]. Based on past TBI studies memory dysfunction is common and results from abnormal hippocampal activity [58]. Memory abnormalities caused by TBI are most likely to have a complicated underlying mechanism involving synaptic dysfunction, cell death, changes in hippocampal connectivity, and neural pathway dysfunction. While hippocampal theta oscillations may be associated with learning and memory, especially in spatial memory [59, 60], it is important to note that hippocampal theta oscillations have been reported to be decreased after TBI [61].

In a recent study, theta burst stimulation of the dorsal fornix was reported to induce memory improvement in patients with TBI [62]. Because of this, it was hypothesized that the

modulation of neural activity via the hippocampus by fornix DBS may improve cognitive recovery after TBI. Stimulation electrodes were thus implanted in the proximal fornix and dorsal hippocampal commissures of four TBI patients. Three patients received their electrode on their language dominant side and one patient received it on their non-dominant side. A diffuse evoked potential was generated by the electrode in the head and body of the ipsilateral hippocampus.

Memory tests were performed once a day for at least two consecutive days with different test forms each day such as verbal memory via Rey Auditory-Verbal Learning Test (RAVLT), visual-spatial memory via the Medical College of Georgia Complex Figure Test, and visual confrontational naming via the Boston Naming Test Short Form (BNT). All fornix electrodes were continuously stimulated using a burst pattern (200Hz in 100ms trains, 5 trains/s, 100µs pulse width, 7mA). Results showed that the burst stimulation of the fornix was correlated with an improvement in the Medical College of Georgia Complex Figure Test. It was hypothesized that the stimulation on the language dominant side may improve verbal memory while on the non-dominant side it may improve visual memory. However, results showed that the stimulation of either side improved visual spatial memory and reflects the role that both sides of hippocampus have in spatial memory, especially in spatial relationships [63]. Results suggest that the hippocampus plays an important role in spatial learning and memory and that the spatial processing might be more susceptible to stimulation than the processing of verbal memory. While this study had a small sample size and only explored limited mechanisms of action, theta burst stimulation of the fornix may prove to be a therapeutic method to improve visual-spatial memory in TBI.

Recently, different parameters of fornix stimulation in how they affect cognitively demanding tasks after TBI were investigated in male rats. Researchers implanted electrodes into the fornix and separated rats into a fluid-percussion injury group and a sham-operated group. A 60-s delayed non-match-to-sample (DNMS) swim T-maze was serially performed using four stimulation parameters: no stimulation (no stim), low frequency (5Hz), high frequency (130Hz), and theta-burst stimulation (TBS, 200Hz in 50ms trains, five trains per second; 60mA biphasic pulses). In the cognitively demanding DNMS swim T-maze and a water maze there was a significant difference in performance between TBI+no stim and TBI+TBS groups but no significant difference between sham+no stim and TBI+TBS. The TBI+TBS group performed significantly more platform crossings in the probe trial and exhibited improved search strategy starting on day 3 when compared to TBI+no stim, demonstrating that fornix DBS with TBS improved memory after TBI. While there are limitations in this study, such as

the low sample size and the stimulation settings being different from previous human studies, these results indicate that the modification of neural activity in the hippocampus induced by fornix TBS may constitute a new therapeutic method for memory deficits after TBI [64].

Temporal lobe epilepsy (TLE)

Temporal lobe epilepsy (TLE) is the most common form of intractable epilepsy. The prevalence of TLE in developed countries ranges from 4 to 10 cases per 1,000 [65]. Mesial TLE usually arises in the hippocampus, an area of the brain known for its involvement in memory. The efficacy and safety of DBS for epilepsy has been demonstrated by the SANTE trial where the anterior nucleus of the thalamus (ANT) was targeted [66]. Based on this trial, the U.S. Food and Drug Administration granted approval for DBS therapy for epilepsy. Although ANT-DBS was able to produce beneficial effects on seizure frequency, complaints of memory impairment occurred in 27% of patients over the course of the trial. For this reason, researchers have investigated whether the fornix can be used as alternative DBS target. In one study, two epileptic patients were implanted with electrodes in the fornix, and nine were implanted in anterolateral to the splenium of the corpus callosum where the crus of the fornix has fibers that travel to the dorsal hippocampal commissure (the fornodorsocommissural tract). Low-frequency stimulation (bilateral, 5Hz, 8mA, 0.2ms pulse width) in the fornix was given in 4 hours blocks while a video-electroencephalography unit was monitored simultaneously. Results indicated that the hourly Mini-Mental Status Examination (MMSE) scores trended to increase during the stimulation period compared to pre-stimulation period, suggesting substantial memory improvement. Hippocampal spikes were additionally decreased in and after each low-frequency stimulation, and seizure odds (n = 7) were reduced by 92% in 2 days after the stimulation. Although this study did not use an originally planned control with sham stimulation sessions, and could have confounders including possible interference of antiepileptic drugs with the spike and seizure analysis, results show that low-frequency stimulation of the fornix reduced interictal epileptiform discharges and seizures in patients with intractable mesial TLE without affecting memory [67].

Rett syndrome (RTT)

Rett syndrome (RTT) is a progressive neurodevelopmental disorder caused by a loss of functional mutations in the methyl-CpG-binding protein 2 (MECP2) gene [68]. The main clinical symptoms include developmental deterioration of movement, loss of language and coordination skills, stereotypical hand movements, and microcephaly. Recently, it has been

reported that high-frequency fornix stimulation in a RTT mouse model could improve cognitive deficits related to the dysfunction via regulating neural circuits involved in memory and learning development [69].

This research is the first application of potential therapeutic methods of a childhood intellectual disability disorder in a mouse model. Researchers implanted electrodes in the fimbria-fornix in female MeCP2+/− (RTT) and wild type (WT) mice. After biphasicfornix DBS (130Hz, and 60µs pulse width 1h per day for 2 weeks) mice were tested with behavioral tests including fear conditioning, water maze, open field, light–dark box, wire hang, dowel walk, accelerating rotarod, three-chamber interaction, and pain threshold. Results indicated that fornix DBS significantly improved spatial learning and spatial memory as well as contextual fear memory in WT and RTT mice but did not enhance locomotion, anxiety, pain threshold, motor learning, coordination, social behaviour, or body weight in RTT mice. Moreover, results showed that fornix DBS increased hippocampal neurogenesis and synaptic plasticity, which could improve learning and memory functions [70, 71].

Alzheimer's Disease (AD)

More than 40 million people in the world have Alzheimer's disease (AD). AD is a neurodegenerative disorder characterized by various pathological processes including regionally specific and sequential brain atrophy, amyloid plaques, neurofibrillary tangles, synaptic dysfunction, and neuronal cell death [72]. Patients suffer from progressive memory impairment and dementia leading to the worsening of everyday life [73]. So far, there are no clear effective treatments available to slow down the progression of AD. Equally, pharmacological therapeutic methods only alleviate symptoms temporarily and are not effective for all patients [74, 75].

In 2008 when a patient underwent DBS to treat obesity, the treatment did not have an influence on the patient's appetite, but uniquely evoked a "déjà vu" experience leading to the hypothesis that bilateral stimulation of the fornix may help to improve memory [15]. Following this study, a Phase I research trial of bilateral fornix DBS was conducted in six mild to moderate AD patients and no sham control group. Bilateral stimulation of the fornix proved to be feasible and safe, having no serious adverse events [15]. The principle outcomes were that 4 out of 6 patients showed an improvement in their Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) scores 6 months after surgery, and 5 out of 6 patients showed a reduced decline in their Mini Mental State Examination (MMSE) one year after surgery. Moreover, a sustained partial reversal of hypometabolism was observed. It was

shown in structural magnetic resonance imaging (MRI) that fornix DBS not only decreased the mean hippocampal atrophy but also increased the hippocampal volume in 2 patients one year after treatment, indicating the possibility for long-term structural plasticity driven by fornix DBS [76].

An additional prospective study was conducted to assess the safety and feasibility of fornix DBS in mild AD patients. During a 1 year study, recently diagnosed AD patients (n=110) with predominant impairment of episodic memory were recruited, but only 8.2% of patients (n=9) achieved all the criteria for inclusion and in the end only one patient accepted to be operated upon and accomplished the study [77]. Using permanent stimulation (bipolar, 130Hz frequency, 210ms pulse width, 2.5V) in the fornix for one year, the patient was measured via their memory test scores (ADAS-Cog, MMSE, FCSRT (Free and Cued Selective Reminding Test)), and compared to their baselines. Results showed that the memory test scores were stabilized and the mesial temporal lobes metabolism increased. This study suggested that fornix DBS is feasible, safe, and could act through antidromic stimulation of the hippocampus, even though only one AD patient was involved [77].

Because of the promising preliminary results, researchers embarked on a Phase II study of a yearlong, randomized, double-blind trial of fornix DBS in 42 mild AD patients. During the stimulation of the fornix, patients exhibited increased metabolism at 6 months but not at 12 months. The post-hoc multivariate regression analysis showed that age and treatment interacted significantly; patients < 65 years old (n=12) trended to be worse with DBS ON versus OFF while patients ≥ 65 (n=30) with DBS ON demonstrated not only increased cerebral glucose metabolism but also benefits on clinical outcomes [78]. The authors concluded that this interaction in age and treatment might indicate that younger patients have a tendency towards a more malignant course of the disease. Another conclusion of this trial was that the stimulation parameters applied to AD patients were not disease-specific [78] and, retrospectively, the trial can be viewed as pre-mature. Developing AD-specific stimulation parameters is likely to improve the current approach of DBS in AD.

In a preclinical study that was the first to report about chronic fornix DBS in a transgenic rat model of Alzheimer's disease, the effects of chronic fornix stimulation on amyloid burden, inflammation, and neuronal loss were investigated [79]. Researchers applied permanent, bilateral, and unipolar stimulation (130Hz, 80μs, 100μA) 10 days after implantation surgery [79]. Results showed that amyloidosis, inflammatory responses, and neuronal loss decreased in both cortex and hippocampus after DBS in the fornix.

In another study, researchers applied bilateral fornix DBS with different stimulation parameters in a scopolamine-induced rat model of dementia. Scopolamine is a muscarinic acetylcholine receptor antagonist that mimics memory deficits. After being tested in different behaviour paradigms at different frequencies (10 and 100Hz), different amplitudes (50, 100 and 200μA), and with 100μs pulse widths, it was found that fornix DBS improved spatial memory deficits and had no side-effects on anxiety and general motor activity [80].

Researches then performed c-Fos immunohistochemistry in the hippocampus as well as microdialysis sampling to investigate the neural mechanisms of fornix DBS in association with the memory improvement. It was found that fornix DBS selectively activated cells in the CA1 and CA3 sub-region of the hippocampus. Moreover, extracellular neurotransmitters such as acetylcholine in the hippocampus substantially increased 20 min after the stimulation while hippocampal glutamate levels were not significantly different compared to the baseline [81]. Interestingly, the release of acetylcholine was substantial when DBS was initiated with clear-cut behavioral effects, but declined over time despite ongoing DBS. However, the authors investigated these extracellular neurotransmitters with only the stimulation paradigm of 100Hz, 100μA and 100μs. In continuing this research, it will be crucial to see whether an optimal release of acetylcholine could be achieved through different stimulation parameters of the fornix and lead to long-term therapeutic effects.

Recently Gallino et al. designed an experiment of fornical DBS in an Alzheimer's mouse model. It combined brain imaging and behaviour by a proof-of-concept methodology in longitudinal assessments. After 1 h (100 Hz, 100 μs pulses, 100 μA) fornix deep brain stimulation, mice were measured in water maze tests. DBS treatment improved learning and long term memory 3 and 6 weeks later, especially sex specifically. It has been reported that deep brain stimulation cause the proliferation of dentate gyrus granule cells [82], and neurogenesis and/or the modulation of plasticity such as expression, splicing, methylation and overall protein levels of genes [83]. There are also persistent and volumetric changes in the diverse brains structures, especially mediated by sex, which may be influenced by hormonal profiles [84, 85].

Modulating memory loss with DBS

Effectively any neurological, neurodegenerative, toxic, or traumatic damage to brain structures within the circuit of Papez, especially the hippocampus, may lead to deficits in episodic memory that may resemble or precede AD. This holds true particularly in the absence of other neurological or neuropsychological symptoms or signs indicative of an

alternative cause.The diagnostic procedure of memory impairment is based on a comprehensive clinical investigation (comprised of detailed medical histories, neurological, and psychiatric examination, etc). Additional investigations to support the diagnosis of AD include biomarkers such as reduced $A\beta_{42}$, increased tau in the cerebrospinal fluid, typical patterns in ^{18}F-FDG-PET, and disproportionate atrophy involving medial, basal, and lateral temporal lobes and medial and lateral parietal cortices. Besides neuroanatomical alterations, synaptic degeneration, cell loss, neurotrophic failure, cellular genetics, and neuronal selective vulnerability are evident [86]. Circuit-wide neurochemical and electrophysiological changes also occur in AD, such as acetylcholine depletion [87] and abnormal alpha and theta rhythms [88]. Furthermore, neuroinflammation has been suggested to play a central role in the pathogenesis of AD [89]. In the course of the disease, microglia and astrocytes start to produce cytokines and pro-inflammatory mediators leading to chronic inflammation, the long-lasting and intense activation of which is thought to cause further neurodegeneration[89]. It is apparent that the pathophysiology of AD is complex and multifaceted. Some aspects like the initial causes of the disease, the abnormal formation of Aβ plaques, the mechanisms by which it affects neurons, the relation between the disruption of cholinergic pathways, and the cognitive deficits of AD are to date not fully understood.
Clinical and preclinical DBS studies targeting the fornix have shown to counteract some of the aforementioned pathological features. The phase I and phase II trials of fornix DBS for AD have indicated that fornix DBS is a feasible and safe methodology in AD patients, displaying inspiring early results for cognitive improvement. Moreover, fornix DBS can reverse some of the temporoparietal hypometabolism seen in AD [15].

In preclinical studies, it has been shown that DBS of the fornix improves impairs spatial memory and enhances neuronal activities in the hippocampus. In line with this, bilateral fornix DBS in the rat for 1h induced expression of c-Fos, an immediate-early marker of neural activation, in the hippocampus [81]. High-frequency fornix DBS was found to enhance levels of synaptophysin, a synaptic marker, in the hippocampus of normal rats [44]. BDNF and VEGF were also significantly increased 2.5h after stimulation, suggesting that neurotrophic and proliferating factors are associated with electrical stimulation [44]. Chronic fornix DBS was performed in transgenic AD rats and showed $A\beta_{42}$ plaque clearance in the cortex and hippocampus [79]. Moreover, it decreased astrogliosis and microglial activation and partly rescued neuronal loss in both cortex the hippocampus. Another study has indicated that fornix DBS can lead to enhanced acetylcholine levels in the hippocampus [81].

To summarize, DBS has been found to exert beneficial effects in neuropathological hallmarks, molecular expression, and behavior in AD. So far, whether the effects on these biochemical markers will continue to improve with DBS until they reach a stable plateau or whether these markers will show natural fluctuations under various stimulation parameters, is not well understood and warrants further investigation.

Discussion

The fornix is essential in memory function, supported by reports that lesions in the fornix in experimental animals and humans are known to cause memory deficits [20-23]. In the current review we focus on the effects of fornix DBS on brain diseases, discuss advances within DBS systems and the potential mechanisms of action underlying symptom reduction, and briefly describe preclinical and clinical studies with regard to AD, Rett syndrome, traumatic brain injury, and temporal lobe epilepsy to elucidate their potential within future research. Lastly, we highlight the use of fornix DBS to restore memory loss and discuss overall considerations.

In the Phase II study, increased glucose metabolism observed at 6 months instead of 12 months with direct continuous stimulation of the fornix. This result seems to be adverse to the natural history of AD, and similar to the progress to use DBS in the PAG/PVG to treat pain. As researches showed, it is most frequently delivered by Deep brain stimulation (DBS) in PAG/PVG as the preferred target for chronic neuropathic pain[90]. Experiences reported by Rasche et al showed that pain relief was perceived after PAG/PVG stimulation, however, patients' quality of life didn't seem to improve because of the continuous burning and discontinuous pain[91]. What's more, it's reported that none of the 8 implanted patients used PAG/PVG DBS for chronic neuropathic pain in the long-term follow-up[92]. This maybe due to the small patients numbers and lacking time point data. Futher researches of its mechanisms and the evaluation of long-term effects of DBS-f should be investigated.

For the treatment of memory loss, DBS studies have exposed two regions of interest: the fornix and the nucleus basalis of Meynert (NBM). The NBM has wide cholinergic projections to the neocortex and some to the hippocampus. When applying DBS to these structures we are able to enhance memory in animals as well as in humans. It has been hypothesized that the beneficial memory effects following NBM-DBS are due to neuroprotective properties (for review see [93]). The current hypothesis for the fornix states that this effect is accomplished by driving fornix activity, both orthodromically as well as antidromically. This is supported by the view that large myelinated axons produce excitatory responses upon electrical

stimulation [94]. Electrically stimulating the fornix proves to be effective in symptom reduction including decreasing rates of cognitive decline [15, 77], enhancing memory [15], aiding visuo-spatial memorization facilitation [95], improving verbal recollection [15], reducing Aβ42-related plaques and neuroinflammation [79], decreasing astrogliosis and migroglia levels [79], and increasing metabolism [15][76].

The fornix composes a important afferent and efferent pathway from the hippocampus and medial temporal lobe. It contributes a direct afferent source from the hippocampus to the anterior thalamic nucleus. Stimulation of the fornix produces an evoked response of the hippocampus in brain diseases such as TBI and TLE, deficits in learning and memory are also improved with fornix DBS in the RTT and AD. The DBS of fornix is able to regulate memory processes, which might represent a novel therapeutic method for patient who's suffering from these memory and cognitive disorders.

As directional leads and technological advancements improve, it would be meaningful to see whether stimulation parameters and sites (pre- or post-commissural fornix) can be tailored for the different indications. In addition, fornix DBS has only been performed so far in an open-loop manner in which stimulation is delivered continuously regardless of the physiological signals. However, it has been hypothesized that the timing and rhythmicity of neuromodulation may be crucial for functional activation of memory circuits that lead to long-term effectiveness [17, 96]. It has been shown in mice that DBS can enhance encoding and retrieval functions through theta phase-specific manipulation of the hippocampus [97] because they encompass different neurophysiological phenomena [98]. Likewise, another study has reported that patterned electrical stimulation of the fimbria-fornix increased theta-gamma comodulation in amnestic rats and partially rescued memory performance during the water maze [99]. Interestingly, synaptic correlates of memory, such as long-term potentiation (LTP), have been shown to be sensitive to precisely timed electrical stimulation in behaving rats [100].

The role of animal experiments in the history of DBS is well grounded and useful in clinical research. Animal models mimic human pathologies to verify the safety and effects of DBS as well ashelp to discern the anatomy and physiology of brain structures and the physiopathology of disorders where DBS is to be implemented. Additionally, DBS animal experiments lead to the better understand of mechanisms underlying therapeutic effects. Furthermore, animal models help determine optimal stimulation parameters for treatments that have already been tested on humans.

As research progresses, a number of important issues will need to be addressed. Firstly, new discoveries that contribute to the understanding of the molecular pathogenesis of AD and its relations are crucial as they allow for the greater development of tailored DBS. Secondly, applications of DBS in psychiatric disorders have been modeled after those used in movement disorders and might need modification accordingly. Therefore, the effects of unilateral versus bilateral stimulation as well as various stimulation parameters should be carefully considered and tested. Thirdly, interpretation of animal studies should be taken with caution, as models of disease for psychiatric disorders are naturally imperfect.

Conclusion

In the past two decades great advances in fornix DBS in both human patients and rodent models have led to multiple potential therapeutic methods for the treatment of brain diseases. As reviewed above, using different stimulation parameters in the fornix has shown therapeutic promise in both human patients and rodent models of brain diseases such as AD, RTT, TBI, and TLE. Researches indicated that fornix DBS can be a feasible and safe approach.

Although the mechanisms underlying DBS are still uncertain, these collective results contribute to the understanding of how fornix DBS effects memory function, Stimulation of the fornix produces an evoked response of the hippocampus in brain diseases such as TBI and TLE, and deficits in learning and memory have shown to improve with fornix DBS in the RTT and AD. While studies of fornix DBS have also shown promising results, they have been done with small sample sizes, so further work should include an aging population and an increase sample size of patients with memory impairments from different disorders.

Nevertheless, it is still unclear which stimulation patterns are most optimal within treatment methods of fornix DBS. These have typically been selected by experience based on a transcendental knowledge of neuroanatomy and clinical cases with DBS in other brain diseases. Therapeutic fornix DBS research is still in a period of infancy because of the inherent complexities within diverse disease processes, the challenging progression of preclinical models, and because of heterogeneoussymptoms within patients. To propel future studies of fornix DBS forward, research needs to strengthen animal models, progress the refinement of patient selection, and continue to explore different stimulation parameters.

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Chapter 4

Deep brain stimulation of the nucleus basalis of Meynert in an experimental rat model of dementia: stimulation parameters and mechanisms

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Abstract

Background/Objective: Deep brain stimulation (DBS) of the nucleus basalis of Meynert (NBM) has gained interest as a potential therapy for treatment-resistant dementia. However, optimal stimulation parameters and mechanisms of action are yet to be elucidated.

Methods: First, we assessed NBM DBS at different stimulation parameters in a scopolamine-induced rat model of dementia. Rats were tested in the object location task with the following conditions: (i) low and high frequency (20 Hz or 120 Hz), (ii) monophasic or biphasic pulse shape (iii) continuous or intermittent DBS (20s on, 40s off) and 100 µA amplitude. Thereafter, rats were stimulated with the most effective parameter followed by 5-bromo-2'-deoxyuridine (BrdU) administration and perfused 4 weeks later. We then evaluated the effects of NBM DBS on hippocampal neurogenesis, synaptic plasticity and on cholinergic fibres in the perirhinal and cingulate cortex using immunohistochemistry. We also performed in-vivo microdialysis to assess circuit-wide effects of NBM DBS on hippocampal acetylcholine levels during on and off stimulation.

Results: Biphasic, low frequency and intermittent NBM DBS reversed the memory impairing effects of scopolamine when compared to sham rats. We found that acute stimulation promoted proliferation in the dentate gyrus, increased synaptic plasticity in the CA1 and CA3 subregion of the hippocampus and increased length of cholinergic fibres in the cingulate gyrus. There was no difference regarding hippocampal acetylcholine levels between the groups.

Conclusion: These findings suggest that the potential mechanism of action of the induced memory enhancement through NBM DBS might be due to selective neuroplastic and neurochemical changes.

Introduction

Dementia is a general term for a group of brain diseases, which is characterized by the degradation of neurological, behavioural, emotional, and psychological cognition and function. There are different types of dementia, including Alzheimer's disease (AD), vascular dementia, Parkinson's disease dementia (PDD), Huntington's disease, alcohol-related dementia and Creutzfeldt–Jakob disease. In 2015, 47 million people around the world suffered from dementia, this is expected to increase to 132 million by 2050. The most prevalent cause of dementia is AD, which accounts for 60–70% of cases. The most common early symptom is loss of recent memory. As this disease progresses, language problems, disorientation, mood swings, lack of motivation, inability to manage self-care and behavioural problems might occur [1].

Previously performed clinical trials have demonstrated slight improvements in measures of cognition and activities of daily living with pharmacological treatments including *N*-methyl-D-aspartate (NMDA) receptor antagonist and acetylcholinesterase inhibitors. However, these treatment options are not beneficial for all patients and symptoms are only alleviated temporarily [2]. Moreover, side-effects such as gastrointestinal symptoms (diarrhoea, nausea, vomiting), weight loss, etc. can occur after using drugs [3].

Researchers have found that deep brain stimulation (DBS) may regulate neural activities in memory circuits of the brain, which has encouraged the initiation of clinical trials in dementia patients [4]. It has been shown that DBS of the nucleus basalis of Meynert (NBM) can influence activity in pathologic neural circuits that underlie AD, although optimal stimulation parameters and mechanisms of action are yet to be elucidated [5].

In the present study, we therefore first tested the effects of NBM DBS using different stimulation parameters in an experimental rat model of dementia and assessed metabolic brain activity by using c-Fos immunohistochemistry. We also evaluated activity-dependent

regulation of hippocampal neurogenesis following NBM DBS. In addition, we assessed the neurochemical effect of NBM DBS on the hippocampus and cortex. Lastly, we assessed whether DBS alters the expression of synaptic markers in the hippocampus. In this regard, synaptophysin, a vesicle-associated glycoprotein, was used as a presynaptic marker of synaptic plasticity [8].

Materials and methods

Subjects

All procedures involving animals were carried out with the approval of the Animal Ethics Committee of Maastricht University. Adult male Sprague Dawley rats (Crl:OFA(SD); Charles River, Écully, France; bodyweight 300-350 g) were individually housed in ventilated cages, with rat chow and water available *ad libitum*. The colony room was maintained at a temperature of 21 ± 1 °C and on a reversed 12:12 h light:dark cycle. All experimental manipulations were conducted during the dark phase under red light when rodents are most active. For the behavioral experiment, rats were randomly assigned to one of the following groups: Sham (n = 9) or NBM DBS (n = 13). For the in-vivo microdialysis experiment, we analysed hippocampal acetylcholine levels of sham (n = 6) and NBM DBS (n = 6) animals.

Surgical procedure

DBS electrodes were implanted bilaterally in the NBM region using a rodent stereotact (Stoelting, Wood Dale, IL, USA, model 51653). The surgical procedure as well as more details regarding the DBS electrodes have been published before [6-8]. The coordinates for NBM DBS electrodes implantations were AP -1.3, ML 2.8, DV -7.4 (coordinates from Bregma according to the Rat Brain Atlas of Paxinos and Watson [9]).

Sham rats underwent the same surgical procedure with electrode implantation. They were, however, not stimulated. After DBS surgery, all animals were allowed to recover for 2 weeks.

Drugs

Scopolamine hydrobromide (Acros Organics BVBA, Geel, Belgium) was dissolved in vehicle (saline; 0.9% NaCl) and administered (i.p.) at a dose of 0.1 mg/kg (in 1 ml/kg) 30 min before the first trial of the object location task (OLT) and object recognition task (ORT). BrdU was used to identify proliferating cells after DBS treatment. BrdU (Sigma-Aldrich) was dissolved in 0.9 % NaCl to 8 mg/ml (pH 7.6).

Deep brain stimulation

Rats were tested with the following conditions: (i) with the attachment of a stimulation cable (sham), and (ii) with DBS at low and high frequency (20 Hz or 120 Hz), monophasic (Fig. 1B) or biphasic pulse shape (Fig. 1C), 100 μA amplitude and certain pulse width (100 μs pulse in the monophasic setting and 280 μs in the biphasic setting which included an 80 μs zero time) at different durations (continuous or intermittent with 20s on, 40s off) [10]. Comparable parameters were shown to increase working memory and sustain attention in adult rhesus monkeys [10], as well as improve spatial memory in transgenic Alzheimer rats [11]. In these studies, intermittent stimulation was applied at 60 Hz, while here we chose low-frequency stimulation (20 Hz) in order to induce a potentially excitatory effect [12] on NBM neurons, since this frequency resembles the physiological discharge rate of NBM neurons during motor activity in freely moving animals [13].

Every animal was exposed to eight different stimulation settings with a wash-out period of at least 24 hours between two parameters. These various stimulation parameters were randomized within and between groups. After defining the most optimal stimulation setting in the object location task, all other behavioural tests were conducted with this stimulation

parameter. Other behavioural tests were conducted to confirm effects on memory (object recognition task) and check for anxiety-related side effects (Open Field and Elevated Zero Maze).

Behavioural testing

Object location task (OLT)

Following the first week of recovery, animals were handled daily for one week. The handling involved weighing and procedures of injection. The rats were also allowed to explore the arena of the OLT and its objects in the same week. The OLT was conducted as described previously [14], two tests with vehicle and scopolamine injections but without stimulation were carried out before the actual DBS experiment. Sham animals were attached to cables, but not stimulated.

During the first trial (T1) the apparatus contains two identical objects (samples) at the midline of the arena. After the first exploration period of 3 min, the rat is placed back in its home cage. Subsequently, after an interval of 60 min, the rat is placed back in the apparatus for the second trial of 3 min (T2).

Discrimination performance (d2) was calculated as follows (time at object at novel position – time at objects in old position)/exploration time in T2.

Object Recognition Task (ORT)

The ORT is a modified version of the OLT to test recognition memory. During T1 the rat is allowed to explore two identical objects and in T2 one of these objects is replaced with a novel one as described here [15]. DBS with the most optimal stimulation parameters derived from the OLT (20 Hz, biphasic pulse shape, 100 μA amplitude and 280 μs pulse width,

intermittent stimulation with 20s on, 40s off) was performed 5 min before testing as well as throughout T1 and T2.

Open field

The open field (OF) was conducted as described previously [14]. In brief, animals were individually placed in the centre of the arena and allowed to move freely for 5 min. DBS at 20 Hz, biphasic pulse shape, 100 µA amplitude and 280 µs pulse width, intermittent stimulation with 20s on, 40s off was performed 5 min before testing as well as throughout the OF session. Sham animals were attached to cables, but not stimulated. The behaviour of each rat was recorded using Ethovision tracking software (Ethovision, Noldus Information Technology, Wageningen, The Netherlands).

Elevated zero maze

The elevated zero maze (EZM) consists of a circular runway (98 cm diameter, 10 cm path width, 70 cm above floor level), which was equally divided into two open and two parts enclosed with high side walls (50 cm). DBS was again applied using the most optimal stimulation parameter from the OLT. Rats were stimulated 5 min before as well as throughout the 5 min trial. Time spent in the open and enclosed parts was recorded with Ethovision tracking software.

BrdU labelling

BrdU was used to identify proliferating cells after DBS treatment. BrdU (Sigma-Aldrich) was dissolved in 0.9 % NaCl to 8 mg/ml (pH 7.6). DBS groups were stimulated for 1 hour, while sham animals were only attached to cables.

Three days after DBS, all rats were injected intraperitoneally twice daily (8 h apart) with 50 mg/kg BrdU for 3 consecutive days. The interval between DBS and onset of BrdU injection was chosen based on a previous study, in which proliferative activity evaluated by BrdU in the dentate gyrus (DG) reached a plateau at 3–5 days after DBS [16]. Animals were sacrificed after 4 weeks by transcardial perfusion. For this, we have stimulated the animals 2x 30 min with 30min rest in between. Rats were sacrificed 1 hour after the last stimulation session to assess acute effects of DBS on cell activity using c-Fos immunohistochemistry.

Tissue collection

At the end of the experiments, an overdose of pentobarbital (ApotheekFaculteitDiergeneeskunde, Utrecht, The Netherlands) was given and rats underwent perfusion-fixation first with Tyrode solution (NaCl, KCl, CaCl2, MgCl2, NaH2PO4, NaHCO3, Glucose, distilled water) followed by Somogyi solution (4% Paraformaldehyde, Picric Acid, PBS, Glutaraldehyde, distilled water).

To prevent the development of postperfusion artefacts, brains were fixed in fresh fixative (same content as in the Somogyi solution but lacking Glutaraldehyde) at 4 °C. After 2 h, the brains were gently removed and stored in 1% NaN3 at 4 °C.

Brains were embedded in 10% gelatine from porcine skin (Sigma–Aldrich, Zwyndrecht, The Netherlands), and cut into 30 μm slices in the frontal plane using a vibratome (Leica®, Wetzlar, Germany). Slices were immediately transferred into 1% NaN3.

Immunohistochemistry

c-Fos, synaptophysin and choline acetyltransferase (ChAT)

Sections of six random animals per group were selected. In brief, sections were incubated overnight with polyclonal rabbit anti-c-Fos (K-25) primary antibody (1:1000; Santa Cruz Biotechnology Inc.), rabbit-anti-synaptophysin (1:1000; Abcam; ab32127) or goat anti-ChAT primary antibody (1:200; Abcam; ab144P). For the c-Fos and synaptophysin staining, sections were then incubated with biotinylated donkey anti-rabbit secondary antibody (1:400; Jackson Immunoresearch Laboratories Inc.) followed by avidin-biotin peroxidase complex (1:800, Elite ABC-kit, Vectastain). The staining was visualized by 3,3′-diaminobenzidine (DAB, synaptophysin staining) or DAB combined with NiCl2 intensification (c-Fos staining). For the ChAT staining, the sections were incubated with donkey anti-goat Alexa Fluor 488 secondary antibody (1:200; Alexa 488, Invitrogen), followed by incubation with DAPI (1:5000) at room temperature. Lastly, the brain sections were mounted and coverslipped with Shandon Immu-Mount (Thermo Fischer Scientific).

BrdU/NeuN

We have also performed a double-immunofluorescent BrdU/NeuN staining. For BrdU detection, DNA denaturation was conducted by incubating for 2 h in 50 % formamide at 65 °C, followed by washing and 30 min in 2 N HCl at 37 °C. After blocking with donkey serum, sections were incubated with mouse monoclonal anti-BrdU (1:1000; Sigma-Aldrich) overnight at 4 °C. Subsequently, a biotinylated donkey-anti-mouse secondary antibody (1:100; Jackson Immunoresearch Laboratories Inc.) was applied, followed by streptavidin 647 (1:1000; Invitrogen). Incubation with mouse anti-NeuN (1:50; MAB377, Millipore) was carried out for 3 days at 4 °C, followed by donkey anti-mouse secondary antibody (1:100; Alexa 488, Invitrogen). Lastly, brain sections were mounted and coverslipped with 80 % glycerol.

Stereology

The number of c-Fos positive cells and BrdU/NeuN double-labelled cells were counted using the stereological procedure, Optical Fractionator, while ChAT positive fibres were analysed using the stereological procedure, Spaceballs. Counts were done using a confocal microscope (DSU, Olympus® BX51W1), a motorized stage, and the StereoInvestigator software (MicroBrightField, Williston, VT). All c-Fos positive cells and ChAT positive fibres in three sections, 300 µm apart, including left and right hemisphere were counted with a 40x objective and the double-labelled BrdU/NeuN cells with a 60x objective.

For the optical fractionator procedure, the counting frame was set to 75 µm x 75 µm, while the grid size was 150 µm x 150 µm.

For c-Fos, the chosen brain sections of the hippocampus extended from bregma -3.60 mm to bregma -4.36 mm, the primary motor cortex from bregma 1.08 mm to bregma 0.60 mm, the entorhinal and perirhinal cortex from bregma -3.36mm to bregma -4.08mm. For the BrdU/NeuN staining, we defined the granule cell layer of the DG as the region of interest. The chosen brain sections extended from bregma −3.12 mm to bregma −4.92 mm. The total number of positive cells was estimated as a function of the number of cells counted and the sampling probability [17].

Concerning the Spaceballs procedure, the fibre length was approximated by counting the intersections of the fibres with the spherical probe [18]. This probe had a 2 µm guard zone on the top and bottom and a 10 µm radius space ball. At each sampling site, the tissue thickness was measured. The grid size was 200 µm x 100 µm. The chosen brain sections of the perirhinal cortex extended from bregma -3.36mm to bregma -4.08mm and the cingulate cortex from bregma 0.72 mm to bregma 0.12 mm.

Synaptophysin immunoreactive presynaptic boutons

The estimation of the density of synaptophysin immunoreactive presynaptic boutons (SIPBs) followed the description of [19]. All measurements were performed on a single focal plane. CellP (Olympus soft imaging solutions) imaging software with an Olympus AX70 microscope was used for the detection of SIPBs with a 100x oil objective (Olympus UplanApo, NA = 1.35).

In-vivo microdialysis

In a next experiment, rats were anaesthetized with 1.3-1.5 g/kg urethane (ethyl carbamate, Sigma-Aldrich) and mounted in a stereotaxic frame. DBS electrodes were implanted at the site of the NBM, and a single cannula microdialysis probe (CMA11, tip length 2 mm, CMA Microdialysis, Kista, Sweden) was implanted into the hippocampus (coordinates from bregma: AP: −4.8 mm; ML: 3 mm; DV: −4.2 mm). Microdialysis probes were perfused with artificial cerebrospinal fluid (141 mM NaCl, 5 mM KCl, 0.8 mM MgCl2, 1.5 mM CaCl2) at a flow rate of 1.5 µl/min for 2 h before dialysate collection started.

Dialysate samples were collected every 10 min, DBS (20 Hz, biphasic pulse shape, 100 µA amplitude and 280 µs pulse width at the duration intermittent with 20s on, 40s off) was performed for 1 h. In total, 18 samples were collected (6 baseline, 6 during stimulation and 6 after stimulation). Samples were immediately frozen on dry ice and later analysed with liquid chromatography/mass spectrometry.

Liquid chromatography/mass spectrometry

All microdialysis samples were analysed at the Interfaculty Mass Spectrometry Center, University of Groningen, The Netherlands. Briefly, a Shimadzu UFLC XR high-performance

liquid chromatography (HPLC) system (Shimadzu, Japan) and a Thermo TSQ Quantum Ultra mass spectrometer (Thermo Scientific, Waltham, MA, USA) were used.

10 µL samples were injected into the LC-MS/MS system at a flow rate of 0.3 mL/min. The chromatographic retention was obtained using Waters Xbridge BEH Amide analytical column (100x2.1 mm, 2.5 µm) at 55 °C. The gradient elution was carried out using acetonitrile and 2 mM ammonium formate, 95:5 and 5:95 (the pH of 3.0 was adjusted with formic acid).

Acetylcholine was detected by monitoring the m/z 406 → 87.1 transition and its D4 analogue internal standard at m/z 150 → 91.1 (100 ms dwell time, 14 V collision-induced energy). Samples were prepared by 1:10 dilution in internal standard and a single acetylcholine calibration curve between 0.10 and 20.0 nM was run at the end of each batch of samples for quantification.

Verification of electrode placements

Sections containing electrode and microdialysis probe trajectories from all animals were mounted on gelatine-coated glass slides. Standard hematoxylin-eosin staining was employed to inspect the sections using bright field microscopy. Additionally sections were stained for ChAT to confirm correct electrode placement.

Statistical analyses

Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL, USA). For the OLT, discrimination performance (d2) for the OFF-stimulation conditions (saline and scopolamine) were analysed using a one-way ANOVA, followed by Fisher's least significant difference post hoc test. For the scopolamine + stimulation sessions, a repeated-measures ANOVA with

frequency, pulse shape and duration as a within-subjects factor and group as a between-subjects factor was used. Since the d2 of a sham group can either be slightly positive or negative and this would lead to a Type I or II error, a virtual group was constructed with a mean of zero and a S.E.M. comparable to the experimental groups. This statistical approach has been suggested previously [20, 21]. To determine whether d2 of the DBS group deviated from the virtual group, an independent-samples t-test was used.

Other behavioural tests, as well as immunohistochemical data, were analysed using an independent samples t-test. The assumptions underlying all analyses were checked. Microdialysis data were represented as the percentage of the mean of the 6 baseline samples prior to DBS. The effect of NBM DBS on extracellular acetylcholine levels over time was analysed by repeated-measures ANOVA. Generally, all data were normally distributed and p values <0.05 were considered to be statistically significant.

Results

Histological evaluation of electrode placement

The bilateral electrodes were all implanted within the histological boundaries of the NBM (Fig. 1A) and the unilateral microdialysis probes within the histological boundaries of the dorsal hippocampus. With the current stimulation setting, we found no evidence for histological damage observable with routine hematoxylin-eosin staining.

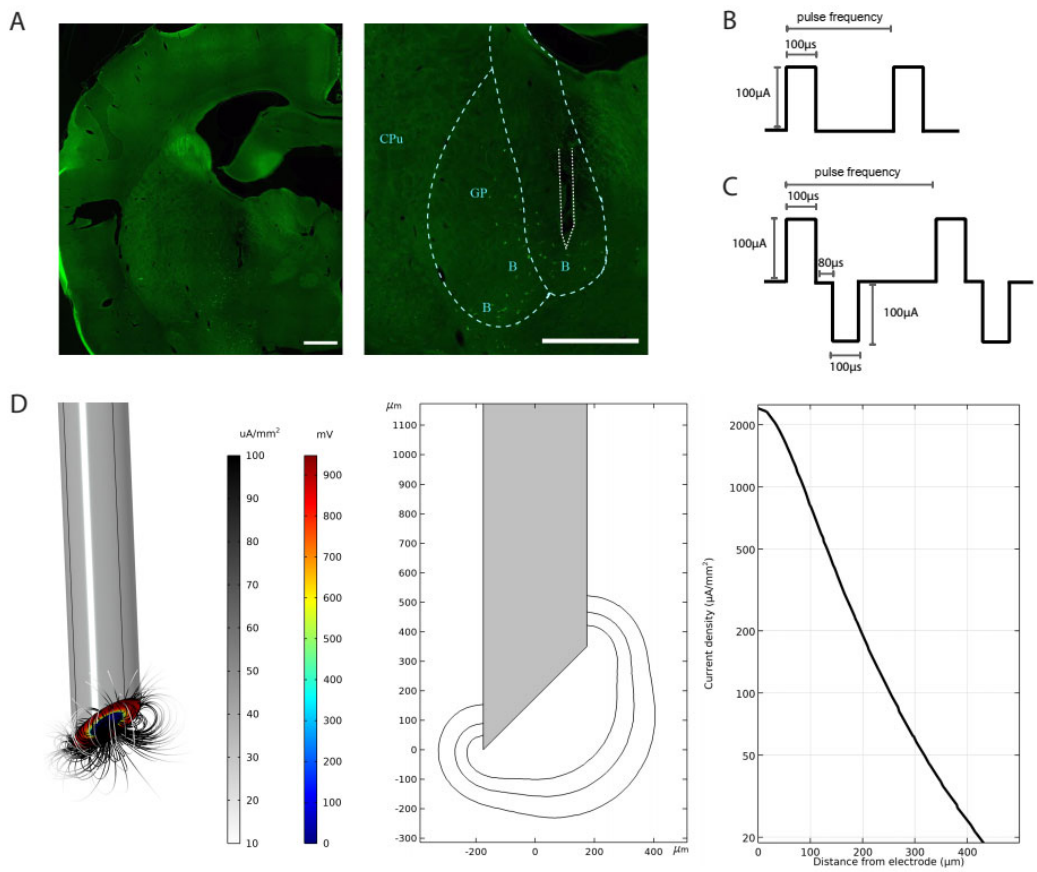

Fig. 1: NBM DBS. (A) Histological evaluation of electrode tips at bregma -1.32 mm [22]. Sites were localized by microscopic examination of histologically prepared tissue. Scale bar 1000 µm. (B) Monophasic pulse shape. (C) Biphasic pulse shape. (D) Estimating the spatial extent of current spread with our rat DBS electrodes using a finite element (FEM) model. Given the geometry of our current DBS electrodes, and assuming brain tissue conductivity of 0.3 S/m, 100 µA stimulation would cause a current spread of about 430 µm (considering a threshold of 18,75 µA/mm2 as in [23]).

Object location task

During off stimulation sessions, all groups significantly differed from the virtual group when saline was injected ($F(2;25) = 5.273$, $p < 0.05$; Fig. 2A), but not when scopolamine was injected ($F(2;27) = 0.067$, n.s.; Fig. 2A).

For the following DBS sessions, rats were injected with scopolamine and stimulated with various stimulation parameters. The repeated measures ANOVA indicated a significant interaction between duration and pulse shape ($F(1;20) = 4.774$, $p < 0.05$). There was no significant effect for individual variables or the interaction between frequency, pulse shape, duration, and groups (all F's < 3.475, n.s.). Analysis of the individual stimulation conditions revealed that 20 Hz, biphasic pulse shape, 280 μs pulse width (which includes 80 μs zero time), 100 μA amplitude at 20s on, 40s off stimulation significantly restored scopolamine-induced memory loss ($t(18) = 3.720$, $p < 0.05$). There was no significant difference for NBM DBS and virtual group for the other stimulation parameters (all t's < 1.789, n.s.; Fig. 2A).

Object recognition task

When saline was injected and no DBS applied, there was no significant difference between the NBM DBS group and the sham group ($t(16) = 1.740$, n.s.). When scopolamine was injected and DBS groups were stimulated, we found a significant restoration of memory for NBM DBS rats ($t(16) = 2.520$, $p < 0.05$; Fig. 2B).

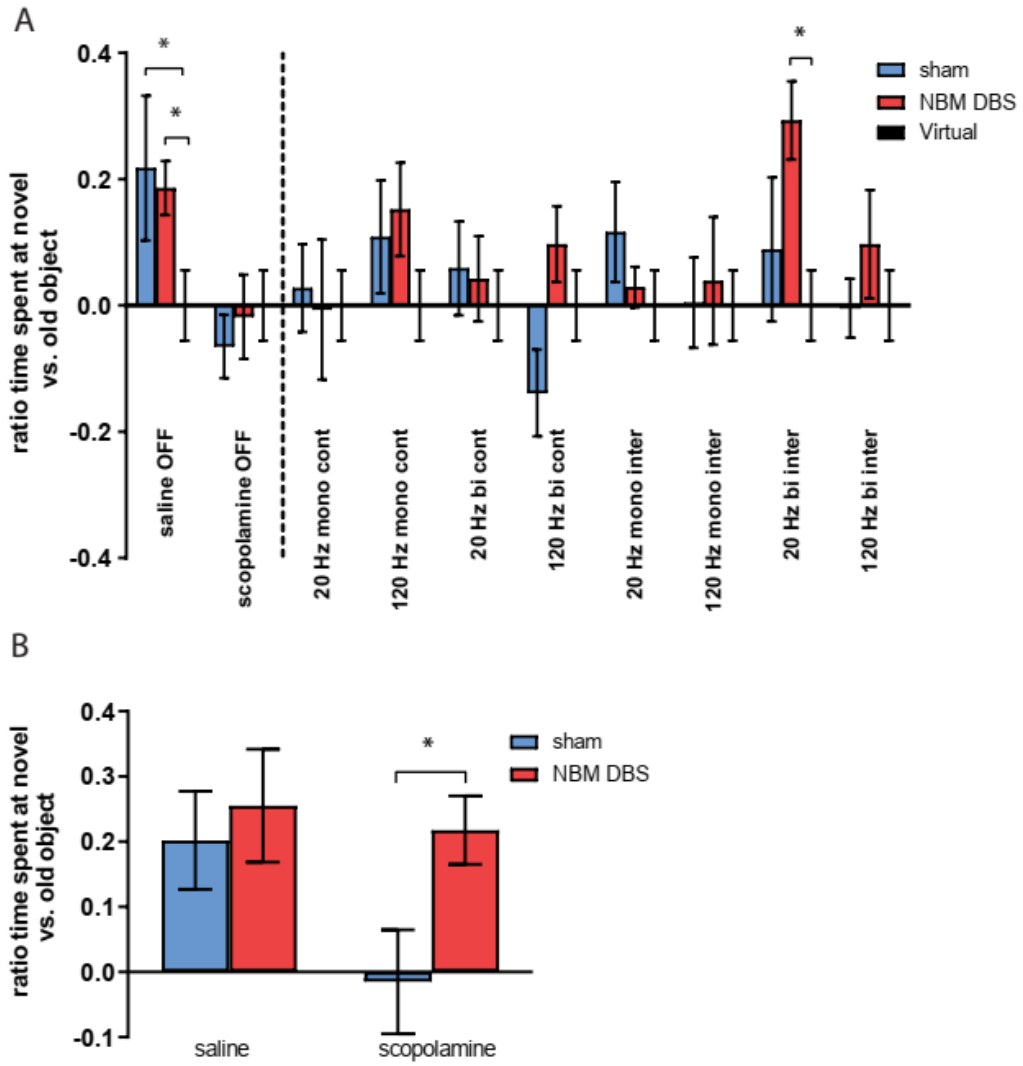

Fig. 2: **NBM DBS at specific parameters enhances spatial and recognition memory**. A) Ratio time spent at new object vs. old object for sham and NBM DBS groups in the OLT. All groups were compared to a virtual group, which has a mean of zero and a S.E.M. comparable to the other groups (always the last column, only S.E.M visible because mean is zero). In the first session, saline was injected, and stimulation was off (all groups differed from zero). In the following sessions, scopolamine was injected and DBS groups were stimulated with the parameters mentioned above. Sham rats were attached to cables, but not stimulated. At 20 Hz biphasic pulse shape, 100 µA amplitude and 280 µs pulse width at intermittent with 20s on, 40s off duration NBM DBS rats significantly differed from the virtual group, suggesting enhanced memory performance. *Indicates $p < 0.05$. Data are represented as mean ± S.E.M. (B) Ratio time spent at new object vs. old object for sham and NBM DBS groups in the ORT. In the first session, both groups were injected with saline and stimulation was off. There was no significant difference between sham rats and rats with NBM DBS. In the following session, scopolamine was

injected and DBS was applied while sham rats were attached to cables, but not stimulated. NBM DBS rats significantly differed from the sham group, suggesting enhanced memory performance. *Indicates $p < 0.05$. Data are represented as mean ± S.E.M.

Open field and elevated zero maze

There was no difference in time spent in the corners, walls, or centre of the OF between DBS groups and sham (t (17) ≤ 1.421, n.s.; Table 1). There was also no difference in time spent in the closed or open arm of the EZM between the DBS groups and sham (t (17) ≤ 0.885, n.s.; Table 1).

Time spent in the different areas of the open field.

	Corners [s]	Walls [s]	Centre [s]
Sham	25±5	153±10	122±13
NBM DBS	37±7	155±10	107±10

Time spent in the open or closed arm of the elevated zero maze.

	Open arm [s]	Closed arm [s]
Sham	89±10	211±10
NBM DBS	100±7	200±7

Table 1. Time spent in the different areas of the open field and elevated zero maze. There was no significant difference

Immunohistochemistry

c-Fos

We found increased c-Fos expression in the CA1 (t (5.32) = 2.783, p < 0.05), CA3 (t (10) =-3.448, p < 0.05), DG (t (5.88) = 2.746, p < 0.05), perirhinal (t (10) = 7.424, p < 0.05) and entorhinal cortex (t (10) = 2.841, p < 0.05) for NBM stimulated animals when compared to sham (Fig. 3). In a brain structure, that is not associated to mnemonic functions, such as the

primary motor cortex, there was no significant difference between NBM DBS and sham (t (10) = 0.926, n.s.; Fig. 3A and B).

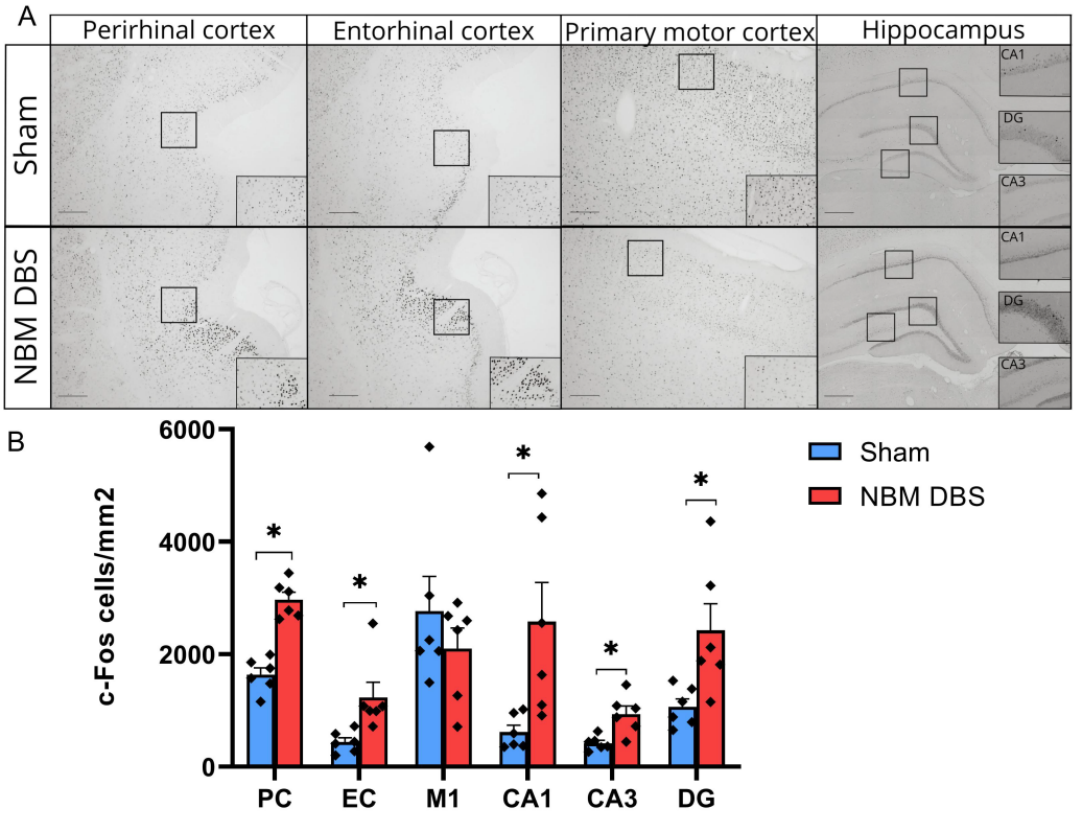

Fig. 3:NBM DBS increases neuronal activity in various brain regions. (A) Representative low-power photomicrographs (scale bar = 500 µm) of coronal brain sections stained for c-Fos (K-25) showing the perirhinal cortex (PC), entorhinal cortex (EC), the primary motor cortex (M1) and the hippocampus of sham and NBM DBS animals. The high-power photomicrograph insets of the PC, EC, M1, and subregions of the hippocampus being the stratum radiatum of the CA1, the stratum lucidum of the CA3 and the stratum moleculare of the dentate gyrus (DG) (scale bar = 50 µm). (B) NBM DBS rats show increased c-Fos (K-25) expression in the PC, EC and the CA1, CA3 and DG of the hippocampus when compared to sham. There was no statistical difference between sham and DBS groups in the M1. *$p < 0.05$, independent-samples t-test for NBM DBS vs. sham rats. Data represent mean ± SEM.

BrdU/NeuN

Next, we counted numbers of BrdU/NeuN double-labelled cells in the DG for NBM DBS and sham animals. We found increased numbers of BrdU/NeuN double-labelled cells for NBM DBS rats when compared to sham ($t(10) = 8.010$, $p < 0.05$; Fig. 4 A and B).

ChAT

The length of ChAT positive fibres in the perirhinal cortex and the cingulate cortex was measured for NBM DBS and sham animals. An increased length of fibres was found in the cingulate cortex in NBM DBS rats when compared to sham ($t(31) = 6.528$, $p < 0.05$; Fig. 4 A and C). In the perirhinal cortex, there was no significant difference between NBM DBS rats and sham ($t(31) = -1.528$, n.s.; Fig. 4 A and C).

Synaptophysin

To assess the effects of NBM DBS on synapses, we measured the density of SIPBs per 100 µm2 in CA1, CA3 and DG subregions of the hippocampus. We found that there were significant differences in the number of SIPBs in the CA1 ($t(10) = -3.903$, $p < 0.05$), and DG ($t(10) = -2.902$, $p < 0.05$), but no significant difference in the CA3 ($t(161) = -0.242$, n.s.; Fig. 4 A and D).

In-vivo microdialysis

NBM DBS for 60 min showed no significant difference in hippocampal acetylcholine levels between the groups (repeated-measures ANOVA: $F(1;8) = 0.062$; n.s., Fig. 4E).

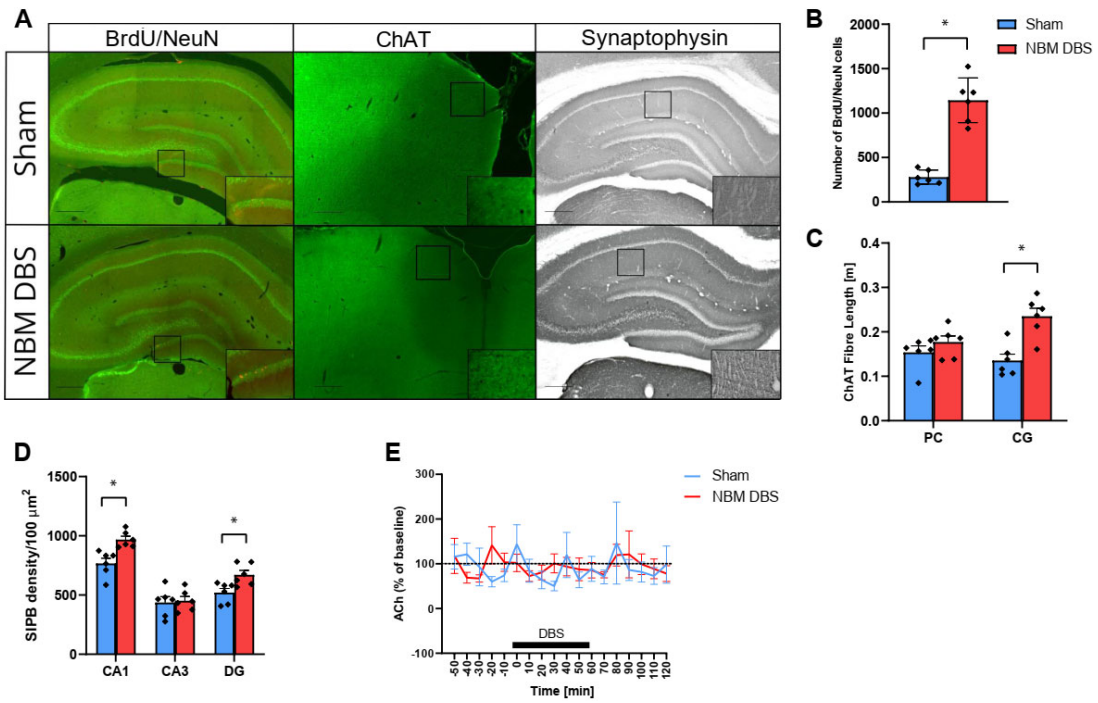

Fig. 4:NBM DBS induces adult neurogenesis, promotion of cholinergic fibres, and synaptic plasticity. (A) Representative low-power photomicrographs (scale bar 500 µm) of coronal brain sections stained for NeuN (green) and BrdU (red), ChAT, and synaptophysin with high-power photomicrograph insets of the DG, the cingulate cortex (CG), and the stratum radiatum of the CA1 (scale bar 50 µm). (B) Graph represents the number of double-labelled BrdU/NeuN cells in the dentate gyrus expressed as the percentage of sham ± S.E.M. There was a significant increase of BrdU/NeuN cells in NBM DBS animals compared to the sham group. (C) NBM DBS rats show increased ChAT expression in the CG when compared to sham. There was no statistical difference between sham and DBS groups in the perirhinal cortex (PC). (D) The density of synaptophysin-immunoreactive presynaptic boutons (SIPB) per 100 μm^2 in CA1, CA3 and DG subregions of the hippocampus. There were significant differences in the number of SIPBs in the CA1 and DG but no significant difference in the CA3. (E)Microdialysate acetylcholine (Ach) levels of the dorsal hippocampus in anaesthetized NBM DBS (n = 6) and sham rats (n = 6). The horizontal bar indicates the stimulation period. There was no statistical difference for hippocampal acetylcholine between NBM DBS and sham. *$p < 0.05$, independent-samples t-test or repeated-measures ANOVA for NBM DBS vs. sham rats. Data represent mean ± SEM.

Discussion

The OLT was used to evaluate which stimulation parameters can restore memory loss in a scopolamine-induced rat model of dementia. It embodies several mnemonic processes that are critical to cognition including attention, stimulus discrimination, encoding, and memory for recent events [24]. Intermittent stimulation consisting of 20 pulses per second delivered for 20 seconds and followed by 40 seconds without stimulation of the NBM led to acute improvement in the task, while continuous stimulation did not show any effects. Recent animal studies in healthy rhesus macaques systematically compared the cognitive effects of continuous versus intermittent burst of 20 s, 60 Hz, ON and 40 s OFF stimulation and also found the intermittent stimulation paradigm to be more beneficial for working memory and attention [25, 26]. The stable effect of intermittent NBM DBS, however, is observed following 10-25 weeks of daily 30-60 min bout of stimulation. The topography and morphological structure of the NBM are different between rodents and primates, including differences in the circuitry distribution of cholinergic neurons within this region and the degree of afferent and efferent fibres from NBM subdivisions, which could account for species-dependent outcome of behavior in experimental settings [27]. Similar to our findings, Koulousakis et al. 2020, has found beneficial spatial memory effects in the modified Barnes maze following acute intermittent NBM DBS using 60 Hz, monophasic pulses, 100 µs and 200 µA in an Alzheimer rat model [11]. Interestingly, the authors did not find any effects on recognition memory. Compared to this study, we employed slightly different stimulation parameters and found that beneficial stimulation parameters entailed 20 Hz stimulation, 100 µA, as well as biphasic pulse shapes. Using the most optimal stimulation parameters derived from the OLT, we found enhanced recognition memory performance of NBM DBS rats when compared to sham. While the OLT mainly relies on the hippocampus for encoding, consolidation and retrieval [28, 29], and is particularly sensitive to manipulations in the dorsal CA1 [30], the ORT requires several different brain regions, including the insular cortex [31],

perirhinal cortex [32], ventromedial prefrontal cortex [33] and to a lesser extent, the hippocampus [34]. Future studies are warranted comparing acute and chronic NBM DBS. This might be particularly relevant, because NBM DBS applied during sleep, disrupts the normal sleep-wake cycle [35], which might affect the physiological process of learning and memory.

When considering the projections of the NBM to the limbic system, we also assessed anxiety-related side effects due to stimulation. In the OFT and EZM noclear evidence for anxiety-related side effects after DBS was found. It has been shown that structures within the limbic system are involved in different experimental models of anxiety and therefore it is our opinion that tests for anxiety should be included when applying NBM DBS in clinical trials [36, 37].

The NBM is the major source of cholinergic innervation to the neocortex. Both in AD and PDD, the NBM degenerates, thereby causing a gradual loss of cholinergic efferents[38, 39]. As a result, the spatiotemporal flow of signals to the cortex is disrupted. The flow of information is primarily based on the anatomical connectivity, synaptic strength of the connections, and the selective intrinsic excitability of the network neurons [40]. Thus, optimal stimulation parameters should be designed to rebuild and reinforce this neocortical neuroplastic network. Additionally, animal experiments should focus on the relationship between partially lesioned NBM and the effect of NBM stimulation, in order to guide clinical patient selection.

When comparing the NBM DBS to the sham group, we found an increase in cholinergic fibre length in the cingulate cortex. Cholinergic neurotransmission is essential for many forms of learning and memory and a deterioration of the number and length of cholinergic fibres in cortical areas have been associated with memory loss in dementia [41, 42]. The anterior

cingulate cortex is involved in emotional processing and the posterior cingulate cortex has outputs to the hippocampal memory system [43]. An alternative, less likely, explanation for the cholinergic fibre length difference, is an activity-dependent change. However, since the difference was consistent between the groups, it is likely to be related to DBS. NBM DBS might thus be able to slow down the deterioration of cholinergic fibres in this region in AD and PDD, although further studies are necessary to confirm this hypothesis. Nonetheless, an alternative explanation for the cholinergic fibre length difference, could also be related to an activity-dependent change. ChAT expression is well known to be modulated by cell activity, so it is possible that ChAT expression itself in the fibers could be altered without any overt morphological change.c

Contrary to this, hippocampal acetylcholine levels were not affected by NBM DBS, since there is no direct hippocampal cholinergic input from the NBM. It has been claimed that the anticholinergic action of scopolamine may more readily impact the hippocampus than other structures [44-47]. Previously, we have found that fornix DBS is able to reverse scopolamine-induced dementia by increasing hippocampal acetylcholine levels [8]. Although it has been shown that cholinergic neurons in the medial septum, rather than the NBM, regulate hippocampal circuits, we wanted to investigate the possibility of a circuit-wide effect of stimulation, in which NBM DBS would indirectly modulate the medial septum and the hippocampus. The memory-enhancing effects of NBM DBS might therefore be related to enhanced cholinergic modulation of the neocortex. Several microdialysis studies of other groups were able to show that NBM DBS, applied both continuously and intermittently, enhanced the release of acetylcholine in the frontoparietal cortex [48, 49].

Immunohistochemical c-Fos (K-25) results revealed evidence of enhanced neural activity in the entorhinal and perirhinal cortex. The entorhinal cortex plays a significant role in spatial

memory, while the perirhinal cortex is involved in recognition memory. This is in line with our findings of improved memory performance in both the OLT and ORT following NBM DBS [50].

Additionally, we found enhanced neural activity in the three main hippocampal fields, CA1, CA3 and DG, which all play an important role in spatial memory [51-54]. In particular, the DG is crucial for behaviourally discriminating similar spatial memories [54]. The CA3 subregion plays a role in encoding and retrieval of spatial location sequences and the CA1 contributes to memory encoding by binding cues to their temporal context, which in turn also enables retrieval of location sequences [52]. Interestingly, a substantial neuronal loss in the CA1 is observed in AD and PDD [55, 56]. Increased neural activity in the CA1 subfield following NBM DBS might thus compensate for reduced neuronal integrity of this region in AD and PDD.

In addition, we found an increase in the density of SIPBs in the CA1 and DG subregion of the hippocampus in the NBM DBS group when compared to sham. Synaptophysin is an important membrane protein located in the synaptic vesicles, which plays an important role in the release of neurotransmitters [57]. Similarly, another study showed that chronic entorhinal cortex DBS significantly enhanced synaptophysin expression in the CA1-subregion in an AD mouse model when compared to non-stimulated controls [58]. The characteristic of AD is loss of synapses, and the reduced expression of the synaptophysin in the frontal, parietal, occipital and temporal cortex, and hippocampus of patients [59-62]. If DBS can modulate synaptic plasticity, it may improve cognition and counteract synaptic dysfunction in AD. It might be interesting to investigate whether astrocytes play a role as partners with neurons in NBM DBS-induced hippocampal plasticity, since they have shown to mediate NBM-induced cortical plasticity through their direct activation by cholinergic modulation via muscarinic receptors [63].

Lastly, regarding the long-term effects of NBM DBS, we found evidence for adult hippocampal neurogenesis in the granule cell layer of the DG. Most of these adult-generated dentate granule cells are thought to contribute to the formation of hippocampus-dependent memory [64, 65]. Studies have shown before that NBM DBS induces vasodilation in the cerebral cortical parenchyma in rats and cats [66-68], and also increases nerve growth factor (NGF) release into the cortical extracellular fluid [69]. NGF is known to promote survival of new neurons in the adult hippocampus and is down-regulated in the NBM in AD [70]. Whether NBM DBS is able to produce similar effects in an animal model of dementia, in which NGF levels are already affected, remains to be elucidated.

Conclusion

NBM DBS with optimal stimulation parameters offers the potential to improve memory function in conditions characterized by memory impairment. Additionally, there seems to be a causal relationship between the stimulation-induced promotion of cholinergic fibres, adult neurogenesis, neuroplasticity, and spatial memory enhancement.

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Chapter 5

The effects of intermittent subthalamic nucleus deep brain stimulation on cognitive functions and neurotransmitter levels in Parkinson's disease patients

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Liu et al. To be submitted

Abstract

Background and Aim:

Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is mainly used to treat the m otor symptoms in patients with Parkinson's disease (PD). However, the cognitive impairments in PD patients also necessitate extra attention. The primary purpose of this study is to evaluat e the effect of intermittent and continuous STN-DBS on cognitive functions. Moreover, neuro transmitters levels of acetylcholine (Ach) and glutamate (Glu) in the cerebrospinal fluid (CSF) were also investigated.

Methods:

A total of ten patients with PD were randomly assigned into two different DBS treatment groups. Group A was treated with intermittent STN-DBS (20 seconds DBS-on and 40 seconds DBS-off) and group B underwent continuous STN-DBS. The mean unified Parkinson's disease rating scale scores (UPDRS) and the Hoehn and Yahr (HY) staging were assessed before and after the DBS surgery. Furthermore, the simple reaction time (SRT) and complex reaction time (CRT) performance were evaluated in two conditions (medication-off with DBS-off; and medication-off with DBS-on). In addition, lumbar punctures were conducted one-day before and after DBS surgery to obtain CSF samples and measure neurotransmitters levels of Ach and Glu.

Results:

Both continuous and intermittent DBS improved motor symptoms of PD. However, only intermittent STN-DBS showed a significant improvement in the SRT and CRT tasks when compared to continuous STN-DBS. Additionally, intermittent STN-DBS significantly increased the levels of Ach in the CSF, whereas Glu levels were not different between the treatment groups.

Conclusion:

In this study, intermittent STN-DBS has proven to be an effective and safe treatment for cognitive impairments in PD patients, which might be linked to increased Ach levels in the CSF.

Introduction:

Deep brain stimulation (DBS) is stereotactic neurosurgery that involves electrode implantation into specific brain targets to treat several neurological and psychiatric disorders [1]. DBS of the subthalamic nucleus (STN) is particularly effective in treating motor disability in patients with Parkinson's disease (PD) [2-4]. In addition to the specific motor symptoms such as tremor, rigidity, bradykinesia, and gait impairment, PD is also characterized by several non-motor symptoms [5], such as increased reaction time [6] and declined cognitive function [7]. These symptoms are based on attention difficulties, slow mental processing, problem-solving and other executive dysfunction, memory and recall problems, abnormal word retrieval and naming, and visual-spatial impairment [8].

Most of STN-DBS studies have investigated the effects on the motor symptoms of PD. Interestingly, anatomical tracing studies in rodents and primates showed that the STN is connected with associative and limbic circuits [9-11]. Furthermore, the STN has shown to have a role in cognitive function, especially in the ability to select and inhibit a dominant response or sustained response at the appropriate time [12, 13]. Researchers found that STN DBS affects decision-making, which indicates that STN-DBS affects the cognitive aspect of response selection through the indirect pathway of basal ganglia loop [13]. Some studies reported cognitive changes after continuous STN-DBS, such as the decline of overall cognitive and executive function, attention, concentration and verbal memory [14-18].

The reaction time (RT) evaluation is widely used in clinical and experimental neuroscience studies and evaluates cognitive processing speed [19-21]. The most used RT tasks include simple RT (SRT) and choice RT (CRT). In the SRT there is one stimulus requiring only one type of response, whereas in the CRT there are two or more stimuli requiring different responses. In both types of tests, subjects are asked to respond to visual or auditory stimuli as quickly as possible [22-24]. In the present study we employed these tests

to study the time course of cognitive processing [25, 26]. In order to understand potential mechanisms of action of DBS on cognitive function, we also measured changes in neurotransmitters levels of Ach and Glu in cerebrospinal fluid (CSF) before and after DBS. These neurotransmitters are considered to play an important role in learning and memory [27-30].

Materials and methods

Patients

This study included ten patients with PD (65.70 ± 2.055 years; six males) who underwent bilateral STN-DBS at the Shandong Provincial Hospital Affiliated to Shandong First Medical University (mean PD duration=9.10 ± 0.526 years, mean Hoehn and Yahr (HY) score =3.50 ± 0.167) (Table 1). The patients were randomly assigned into two groups (groups A and B). Group A used intermittent STN DBS (pulse width 60 ms, frequency 130 Hz, and amplitude 1.0±0.2 V, 20s on, 40s off), and group B used continuous STN-DBS (pulse width 60 ms, frequency 130 Hz, and amplitude 1.0±0.2 V). All patients met diagnostic criteria for PD based on the United Kingdom Parkinson Disease Society Brain Bank (UK-PDSBB). All patients had been diagnosed by neurologists subspecialized in movement disorders and had no other neurological and mental diseases. All patients provided written consent to participate. The study was agreed upon by the local ethics committee of Shandong Provincial Hospital Affiliated with Shandong First Medical University.

Before the patients participated in the study, they were treated with anti-PD drugs, including levodopa, dopamine agonist, entacapone, and selegiline. Patients did not receive anticholinergic drugs before or during the study. The stimulation parameters of the bilateral

STN-DBS were similar in all cases: pulse width 60 ms, frequency 130 Hz, and amplitude 1.0±0.2 V.

Surgical Procedure

In all patients, the stereotactic procedure was performed under local anaesthesia. Surgery was consistent with established DBS procedures. The quadripolar DBS electrodes (model 3389S-40; Medtronic, Minneapolis, MN) were implanted bilaterally in the STN target with the co-registered stereotactic positioning of a computerized tomography (CT) imaging, magnetic resonance imaging (MRI)-guided targeting, and the Leksell stereotactic frame. Then, the DBS electrodes were connected to a pulse generator (IPG) (37086-60, Medtronic) which was implanted in the subclavian area under general anaesthesia. After surgery, the location of the electrodes was confirmed with CT imaging and fused to the pre-operative MRI scan. The DBS device was turned on immediately after surgery.

UPDRS Motor Scores Evaluation

All assessments (the Unified Parkinson's Disease Rating Score (UPDRS) Part II and PartIII) were carried out when patients were in a medication "OFF" state for at least 12 hours before or after surgery. Patients' characteristics are listed in Table 1.

Cognitive evaluation

The neuropsychological evaluation was conducted in a medication "OFF" state for at least 12 hours before or one day after surgery in ten patients. Global cognitive functions were assessed using the Chinese version of Montreal Cognitive Assessment (MoCA-C) [31], Addenbrooke's Cognitive Examination (ACE) [32], frontal assessment battery (FAB), executive function—verbal fluency (VF)-ACE [32]. The tests were chosen to comply with the published guidelines

for cognitive assessment of patients with PD. The tests were administered by a trained neuropsychologist and required one hour on average for the individual patient.

Reaction time paradigms

Subjects were asked to perform two RT tasks in the following order with the same automated device (HL-0607, Reaction time tester, China):

1. Simple RT task. Subjects placed the index finger of their writing hand on the “start” button to be standby for the trial. After 1000 to 4000 ms, the green light appears directly above the "start" button, and the subjects lift their index finger to touch the target as soon as possible.

2. Choice RT tasks. Subjects placed their left and right index fingers on two separate "start" buttons on the left and right sides of the device. After a delay of 1000 to 4000 ms, a random green light appears above the "start" button on the left or right, and the subject raises the corresponding finger to touch the target as soon as possible.

In these two tasks, the time of releasing the "start" button and pressing the target button was measured as Reaction time (RT) and Motor time (MT), respectively. In addition, no response and error response was measured. SRT and CRT performance evaluation is performed only for correct responses. Compared with traditional response time measurement methods, these methods are reliable and effective and have been proven to be sufficient to detect cognitive changes in PD [26]. In the test phase, 50 tests were completed and averaged (SRT = 25 and CRT = 25).

Measurement of neurotransmitters in CSF

CSF samples were collected in a medication "OFF" state for at least 12 hours or one day before and after surgery in 10 patients. All patients agreed to the lumbar puncture procedures. The CSF samples were stored at−80°C and neurotransmitters such as Ach Glu levels were

analysed. All transmitters were measured using commercial ELISA kits, in accordance with the manufacturer's instructions. All steps were performed in duplicate and at room temperature. Transmitter levels were then calculated plotting the optical density (O.D.) of each sample against the standard curve.

Statistical analysis

SPSS 26.0 software was used for data processing and statistical analysis. Data are presented as means, standard deviation (SD) and standard error of the mean (SEM). Statistical methods included paired-samples t-tests, independent-samples t-tests, correlation analysis, and regression analysis. Statistical significance was defined as a $p < 0.05$.

Results

Patient characteristics

The patients' characteristics (gender, age, disease duration, H&Y, UPDRSII (pre-surgery), UPDRSIII (pre-surgery)) of the patients did not differ between the intermittent and continuous STN-DBS groups (Table 1). The means of patients characteristics and the effect of intermittent vs continuous STN DBS on Hoehn-Yahr grade, UPDRSII and UPDRSIII (pre-, post-surgery). compared between both groups. There were no significant differences between the groups. Statistical significance was defined as a $p < 0.05$ (Table 2).

Table 1: Basic Information About Each Patients in the Intermittent and Continuous STN-DBS Group

Patient	Gender	Age	disease duration	H&Y	UPDRSII (pre-surgery)	UPDRSII (post-surgery)	UPDRSIII (pre-surgery)	UPDRSIII (post-surgery)
I01	M	73	8	4	19	15	40	21
I02	M	70	9	4	22	16	35	19
I03	F	70	6	3	20	16	46	25
I04	M	56	11	3	24	19	40	19
I05	F	58	11	4	22	19	45	17
C01	M	70	8	4	19	15	44	22
C02	M	72	9	3	24	20	33	21
C03	M	68	8	3	21	19	42	29
C04	F	62	10	3	25	21	45	31
C05	F	58	11	4	25	19	31	19
Mean	6M/4F	65.7	9.1	3.5	22.1	17.9	40.1	22.3
SD		6.499	1.663	0.527	2.311	2.183	5.384	4.620

Table 1: Patient characteristics and the individual outcome of intermittent and/or continuous STN DBS on Hoehn-Yahr grade, UPDRSII and UPDRSIII scores (pre-, post-surgery).

Table 2: The means of patients' characteristics and the effect about the Patients in the Intermittent and Continuous STN-DBS Group

Means (SD)	intermittent STN-DBS	Continuous STN-DBS	p-value
Age	65.40(7.797)	66.00(5.831)	0.894
Hoehn-Yahr grade	3.60(0.548)	3.40(0.548)	0.580
Disease duration	9.00(2.121)	9.2(1.304)	0.862
UPDRSII (pre-surgery)	21.40(1.949)	22.80(2.683)	0.373
UPDRSIII (pre-surgery)	41.20(4.438)	39.00(6.519)	0.550

Table 2: The means of patients characteristics and the effect of intermittent vs continuous STN DBS on Hoehn-Yahr grade, UPDRSII and UPDRSIII (pre-, post-surgery). compared between both groups. There were no significant differences between the groups. Statistical significance was defined as a $p < 0.05$.

The effect of STN-DBS on motor symptoms

All procedures were performed without complications. The clinical evaluations of the effects of STN-DBS are listed in Table 1. In both groups, the mean ON phase UPDRS-II scores also significantly decreased from 22.1 ±2.311 to 17.9 ± 2.183 after the surgery (Fig 1). In both groups, the mean ON phase UPDRS-III scores also significantly decreased from 40.1 ± 5.384 to 22.3 ± 4.620 after the surgery (Fig 1).

Both intermittent STN-DBS and continuous STN-DBS resulted in a significant decrease in UPDRS-II scores compared to the OFF state [intermittent – MED OFF, t (4) =8.629, p<0.01; continuous – MED OFF, t (4) =10.634, p<0.01]. Both intermittent- and continuous- STN-DBS resulted in a significant reduction in UPDRS-III scores compared to the OFF state [intermittent – MED OFF, t (4) =6.325, p<0.01; continuous – MED OFF, t (4) =7.738, p<0.01].

Fig 1.

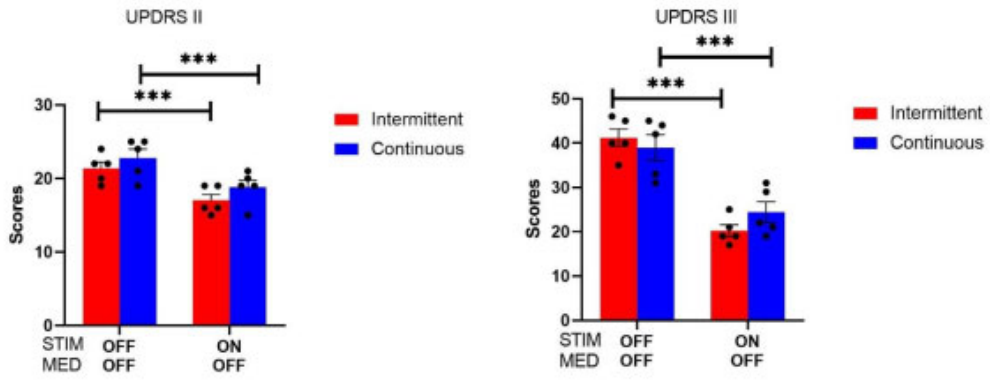


Fig1: UPDRS scores indicate activities of daily living (part II) and parkinsonian motor symptoms (part III). Both STN DBS groups significantly improved motor and UPDRS scores in the STIM ON MED OFF condition. All values are expressed as mean ± SEM. Both intermittent- and continuous- stimulations resulted in a significant reduction in UPDRS-II scores compared to the OFF state [intermittent –MED OFF,t(4)=8.629, p<0.01; continuous –MED OFF, t(4)=10.634, p<0.01]. Both intermittent- and continuous- stimulations resulted in a significant reduction in UPDRS-III scores compared to the OFF state [intermittent – MED OFF,t(4)=6.325, p<0.01; continuous –MED OFF, t(4)=7.738, p<0.01].

The effect of STN-DBS on cognitive functions

The information on the patients and the results of the neuropsychological evaluation are shown in Table 1. There was no significant difference in any of the neuropsychological tests, between the intermittent STN-DBS group and the continuous STN-DBS group before surgery.

All procedures were performed without complications. The total motor score (UPDRS III) improved significantly following STN-DBS in the med OFF/stim ON phase in both groups. The clinical evaluations of the effects of STN-DBS are listed in Table 1. Mean OFF phase UPDRS-III scores also significantly decreased from one day after surgery, respectively in both groups (Table 1). Other clinical scores including UPDRS-II scores, MOCA-C, ACE, FAB, VF-ACE are also listed in Table1.

The intermittent STN-DBS resulted in a significant improvement in MOCA-C (Fig 2.A), ACE (Fig 2.B), FAB (Fig 2.C) scores compared to the OFF state. [intermittent – MED OFF in MOCA-C, $t(4) = -3.539$, $p<0.05$; intermittent – MED OFF in ACE, $t(4) = -2.944$, $p<0.05$; intermittent – MED OFF in FAB, $t(4) = -4.811$, $p<0.01$].

The continuous STN-DBS resulted in no significant difference in MOCA-C, ACE, FAB scores compared to the OFF state [continuous – MED OFF in MOCA-C, $t(4) = -0.408$, n.s.; continuous – MED OFF in ACE, $t(4) = -0.459$, n.s.; continuous – MED OFF in FAB, $t(4) = -2.138$, n.s.]

Both intermittent and continuous STN DBS had no significant differences in VF-ACE scores compared to the OFF state (Fig 2.D) [intermittent – MED OFF in VF-ACE, $t(4) = -1.000$, n.s.; continuous – MED OFF in VF-ACE, $t(4) = -1.633$, n.s.]

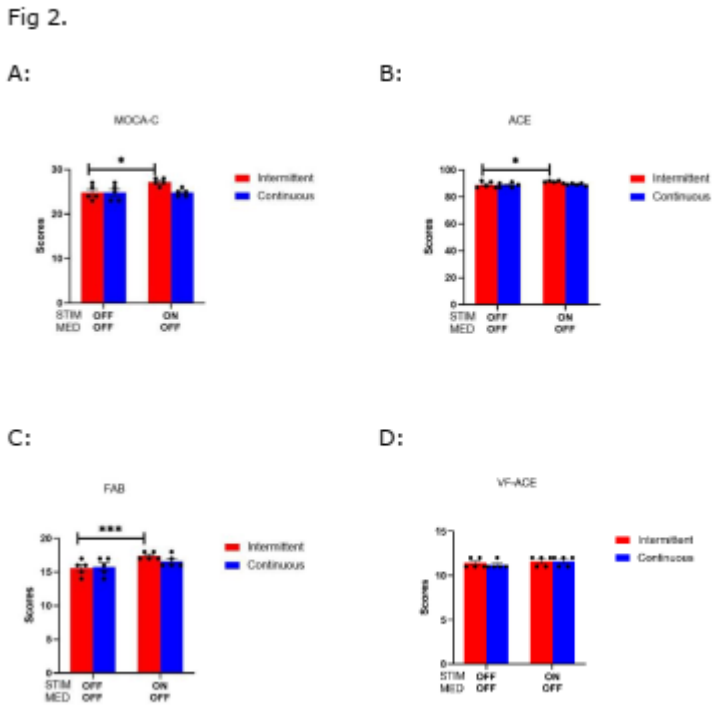


Fig 2. Intermittent STN-DBS showed a significant improvement in the Chinese version of Montreal Cognitive Assessment (MOCA-C) (Fig 2.A), Addenbrooke's Cognitive Examination (ACE) (Fig 2.B), frontal assessment battery (FAB) (Fig 2.C) scores compared to the OFF state [intermittent – MED OFF in MOCA-C, $t(4)=-3.539$, $p<0.05$; intermittent – MED OFF in ACE, $t(4)=-2.944$, $p<0.05$;intermittent – MED OFF in FAB, $t(4)=-4.811$, $p<0.01$]. Continuous STN-DBS showed a significant improvement in MOCA-C, ACE and FAB scores compared to the OFF state [continuous – MED OFF in MOCA-C, $t(4)=-0.408$, n.s.; continuous – MED OFF in ACE, $t(4)=-0.459$, n.s.; continuous – MED OFF in FAB, $t(4)=-2.138$, n.s.]. Both intermittent and continuous had no significant difference in VF-ACE scores compared to the OFF state (Fig 2.D) [intermittent – MED OFF in VF-ACE, $t(4)=-1.000$, n.s.; continuous – MED OFF in verbal fluency (VF)- ACE, $t(4)=-1.633$, n.s.].

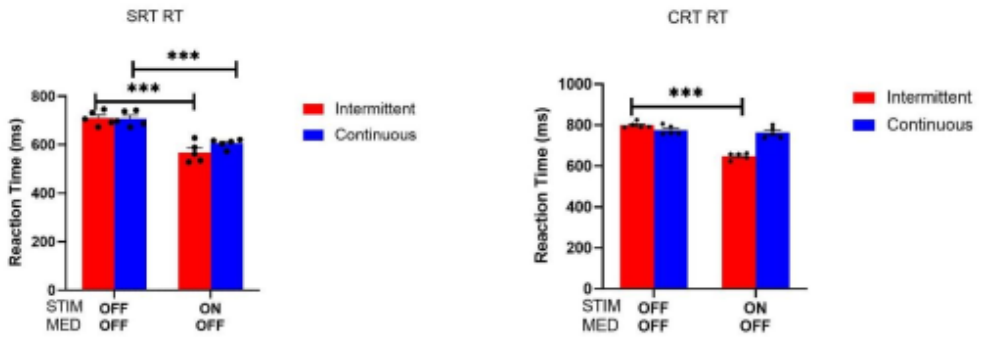
The effect of STN-DBS on reaction time tasks

Both intermittent and continuous STN-DBS groups improved the RT and MT performance in the SRT task. This finding suggests that STN-DBS contributes to motor preparation in response to simple command stimuli in SRT tasks because only one response can be selected, it is allowed to pre-program the response before the start of mandatory stimulation (Fig 3. A and B).

However, only the intermittent STN-DBS improved both the RT performance [t (4) =21.041, p<0.01] and MT performance [t (4) =5.605, p<0.01] in the CRT task. The continuous STN-DBS showed the RT performance [t (4) =1.283, ns] and MT performance [t (4) =2.204, ns] in the CRT task.

Fig 3.

A:



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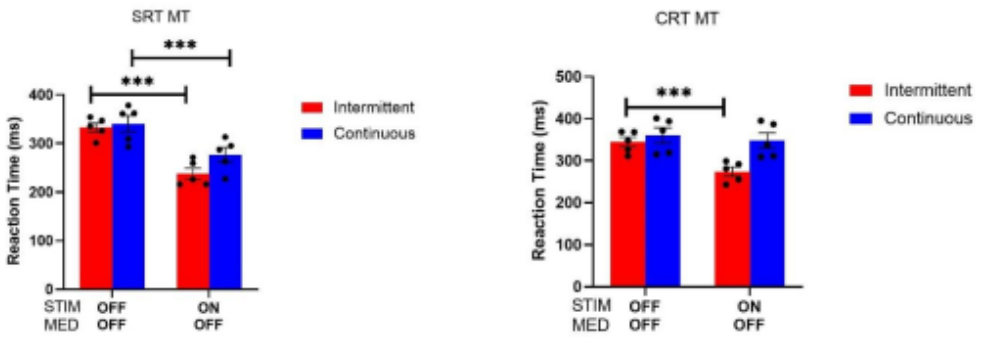

Fig. 3: The effects of intermittent and continuous stimulation on reaction time (A) and motor time (B) performance of PD patients performing in the simple reaction time (SRT) task and a complex reaction time (CRT) task. Only the intermittent STN DBS significantly improved both the RT performance [t(4)=21.041, p<0.01] and MT performance[t(4)=5.605, p<0.01] in the CRT task. The continuous STN DBS showed no difference in the RT performance [t(4)=1.283, ns] and MT performance [t(4)=2.204, ns] of the CRT task. All data represent mean ± SEM.

The effect of STN-DBS on CSF Neurotransmitters levels

Only in the intermittent group, the Ach levels were also significantly increased after 1 day of STN stimulation (t(4)=-3.200, $p < 0.05$, paired t-test). In the continuous group, the Ach levels were not significantly different after 1 day of STN stimulation (t (4) =0.343, n.s., paired t-test) (Fig.4). In both groups, Glu levels were not significantly different after 1 day of STN stimulation (group A: t (4) =0.206, n.s., paired t-test; group B: t (4) =-1.000, n.s., paired t-test) (Fig 4).

Fig.4

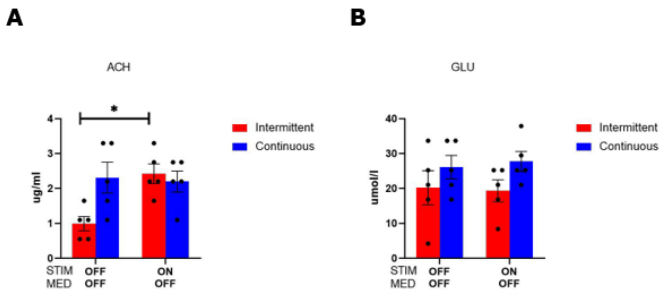

Fig 4. The effect of intermittent vs continuous STN-DBS on CSF neurotransmitter levels of Acetylcholine (Ach) and glutamate (Glu). A) the acetylcholine (Ach) levels were significantly increased after 1 day of STN-DBS only in the intermittent STN-DBS group (t(4)=-3.200, $p < 0.05$ vs continuous STN-DBS (t (4) =0.343, n.s. paired t-test)., paired t-test) (Fig.4). B), Glutamate (Glu) levels were not significantly difference after 1 day of STN-DBS in both groups (Intermittent : t (4) =0.206, n.s. vs continuous STN-DBS : t (4) =-1.000, n.s., paired t-test). Statistical significance was defined as a $p < 0.05$ and indicated as *p<0.05, ***p<0.01, and n.s. not significant.

Discussion

In the present study, intermittent STN-DBS has improved motor symptoms and cognitive function as well as increased CSF level of Ach. In particular, intermittent STN-DBS improved cognitive function in the MoCA-C, ACE, and FAB, and MT and RT of the SRT and CRT when compared to continuous STN-DBS. SRT and CRT tasks are commonly used in PD to reflect motor and cognitive function [33]. The performance of PD patients was slower in the CRT task compared to the SRT. Additionally, the present study also showed that the

concentration of Ach in the CSF increased significantly after intermittent STN-DBS and this could be related to the improvement in cognitive function.

There are several cognitive impairments associated with Parkinson's disease, including difficulty in attention, slowed mental processing, executive dysfunction, memory and recall problems, abnormal word retrieval and naming, and visual-spatial impairment [8]. Although RT performance in CRT tasks improved after continuous STN-DBS, the effect was less significant than intermittent DBS. The possible reason is that more complex cognitive processing is required in CRT tasks and the negative effects of continuous STN-DBS offset the RT performance [33]. Saint Cyr et al. also described the slowing down of cognitive processes after continuous STN DBS [34]. Continuous high-frequency stimulation is considered as an informational impairment, which is essentially the over-activation of the normal varied activity of the stimulated brain region [35]. However, the intermittent STN-DBS improved the RT performance both in the SRT task and the CRT task in this current study.

Some studies have shown that short high-frequency stimulation (HFS) of STN helps to increase the partial discharge frequency, and then it is inhibited with the extension of stimulation time [36]. Another study showed that STN-DBS would interfere with the ability of subjects to appropriately slow down their response when betting on their preferences, resulting in a reduction in response time [13, 37], and the intermittent DBS enhanced this [38]. In a basic neurophysiological study of using intermittent DBS instead of continuous DBS in patients with epilepsy, intermittent DBS can lead to the reduction of potential cumulative evoked potential lasting longer, suggesting the existence of homeostatic plasticity [39]. Another study showed that local depolarization block and activation of a local inhibitory

circuit may be the main reason to prevent somatic activation, and axon efferent flow can be activated by each intermittent stimulation pulse. Therefore, intermittent DBS could activate STN axons at each pulse transmission. [40].

Although it is not clear which neuronal components are the source of Ach release, the possible explanation for the results is that intermittent STN-DBS may activate the cholinergic system and induce Ach release. Several studies showed that the cholinergic neurons in the pedunculopontine tegmental nucleus (PPTg) project to STN [41, 42]. Furthermore, PPN-DBS shows a better clinical outcome when combined with STN DBS [43].

In this study, the intermittent DBS showed a higher Ach level of CSF than continuous DBS. The Ach may be one of the factors affecting cognitive function, such as learning process and memory [44-46]. There are complex fiber connections between subthalamic nucleus and striatum [47], and there are also functional connections between the marginal area of the striatum and hippocampus [48].Since the level hippocampal acetylcholine is related to cognitive function, anticholinesterase drugs are often used as an adjunct therapy to improve cognitive function in PD patients [49].

Ach, and Glu are the main neurotransmitters that play key roles in cognitive function. [50, 51]. Since Bartus et al first published that acetylcholine plays an important role in cognitive functions such as learning and memory [52, 53], a large number of experimental studies have shown that Ach is related to memory function, especially short-term memory and working memory[54]. Glutamate, as the main excitatory neurotransmitter, plays an important role in learning, memory and cognition by regulating synaptic plasticity and neural circuit function [55, 56].

In conclusion, this is one of the first studies investigating intermittent STN-DBS as a potential treatment for the cognitive impairments in PD patients. Our results show that intermittent STN-DBS is more effective in terms of cognitive function when compared to continuous DBS. Furthermore, the increase in CSF Ach levels may be linked to the cognitive effects of intermittent STN-DBS.

Limitations

There are some limitations in our study design. We did not have a sham group. Another limitation is that the test is conducted in Chinese, which may cause unintentional bias.

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Chapter 6

Discussion and Conclusion

Discussion

Neuromodulation significantly improves our understanding of the role of circuits in brain function and the subsequent role of neural circuit disorders in neurological and mental diseases. Deep brain stimulation is an effective treatment for motor symptoms of PD and seems to be a promising potential therapy for the treatment of cognitive and memory impairment in AD. DBS of fornix or NBM seems to be a safe and well tolerated treatment without new neurological deficits or side effects caused by permanent stimulation.

I have provided the up-to-date research on deep brain stimulation techniques and the effectson memory neuromodulation and cognition (Chapter 2). The most important published studies dealing with deep brain stimulation in AD have been reviewed from basic research to clinical studies in AD patients. Researchers have demonstrated that non-invasive stimulation methods like repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) and invasive stimulation methods like deep brain stimulation could enhance performances on memory and cognitive impairment in AD. However, the large differences between non-invasive stimulation parameters and the risk of bias affect most studies to varying degrees[1]. Clinical studies have shown that the targeting of critical nodes in the memory circuit such as the fornix and NBM might be relevant for memory neuromodulation [2-4]. Therefore, I conducted a systematic search for studies that reported clinical and preclinical outcomes of deep brain stimulation within the fornix (Chapter3). I identified 12 studies (7 clinical, 5 preclinical) that examined the effectsof fornix stimulation in Alzheimer disease (n=8), traumatic brain injury (n=2), Rett syndrome (n=1), and temporal lobe epilepsy (n=1). Overall, fornix stimulation can lead to decreased rates of cognitive decline (in humans), enhanced memory (in humans and animals), visuo-spatial memorization (in humans and animals), and improving verbal recollection (in humans). While the exact mechanisms of action are not completely understood, studies suggest fornix DBS to be

involved with increased functional connectivity and neurotransmitter levels, as well as enhanced neuroplasticity. NBM DBS has proven to be safe and could promote cognition in Alzheimer's patients [5-9], although optimal stimulation parameters and exact/precise mechanisms of action are yet to be elucidated. Thus, I investigated the role of different stimulation parameters of NBM DBS in a rat model of experimental dementia (Chapter 4).In this study, I found that NBM DBS with optimal stimulation parameters (20 Hz, biphasic pulse shape, 100 μA amplitude and 280 μs pulse width at the duration intermittent with 20s on, 40s off)has the potential to improve memory functions, which has no side-effects on anxiety levels and general motor activity. Additionally, there is evidence for stimulation-induced promotion of adult neurogenesis in NBM DBS animals when compared tosham. Next, I investigated whether NBM DBS could have long-term effects on neural plasticity. Synapses are considered to be the main pathological target of AD and other forms of dementia, and synaptic loss is considered to be the best association between AD and memory impairment [10, 11]. Synaptophysin is an important membrane protein located in the synaptic vesicles, which played an important role in the release of neurotransmitters[12]. The density of immunoreactive presynaptic boutons in CA1 and CA3 regions of hippocampus in the NBM DBS group was significantly increased when compared to sham. NBM DBS might induce long-term potentiation related mechanisms.

Next, I evaluated the different effects of intermittent (20s on, 40s off) and continuous subthalamic nucleus deep brain stimulation (STN DBS) on cognitive function in patients with PD (Chapter 5). Most attention is focused on the motor performance in PD patients. However, the cognitive function related to PD also deserves attention. As a technically feasible and informative index, Reaction Time (RT) evaluation is widely used in neurophysiology and cognitive processing speed of the brain [13-15]. The most commonly used RT tasks include simple RT (SRT) and choice RT (CRT). Both continuous or intermittent DBS could improve

motor symptoms. However, only intermittent STN DBS led to a significant improvement of performance in the SRT and CRT tasks.

Although the data presented in this thesis are meaningful to some extent, some limitations need to be considered.In our experimental design of the preclinical and clinical studies, we employed only short periods of stimulation. However, sustained chronic stimulation is used in the clinical settings. Further research, is needed to assess the effects of long-term stimulation. A further limitation is related to the pharmacological model of dementia, which does not fully satisfy face, construct, predictive and aetiological validity of Alzheimer's Disease. The perfect model would account for aetiology, symptomatology, treatment and physiological basis. Animal models in general do not meet all of these criteria, but nevertheless, the scopolamine model may serve a pivotal role in predicting clinical outcomes of treatment strategies using DBS and investigating their mechanisms of action.

To date, the mechanisms of action of DBS in memory disorders are not fully understood. Recently, it has been reported that DBS could activate the electrical effects of local and neural networks and regulate their oscillation activities[16, 17]. Studies showed that the stimulation of entorhinal cortex, fornix and medial septal nucleus could increase theta oscillations in the hippocampus and enhance spatial memory in humans and rodents[18-21].In future studies, Iintend to apply DBSin the NBM in order to evaluate the hippocampal theta-frequency (4–12 Hz) electroencephalographic (EEG) activity induced by acute DBS.

Conclusion

Performing DBS of the fornix or in the NBM seems to be a safe and well tolerated therapy without causing new neurological deficits or permanent stimulation-inducedside effects. In my experimental work, I identified that NBM DBS with optimal stimulation parameters, has the potential to improve memory function in conditions characterized by memory impairment.

Additionally, there seems to be a causal relationship between stimulation-induced promotion of adult neurogenesis and spatial memory enhancement.

The intermittent stimulation model of DBS also seems to be an effective and safe treatment for cognitive function in PD patients, albeit in a different brain target, such as the subthalamic nucleus. Ongoing clinical trials will need to demonstrate that DBS can lead to a reduction in the clinical significance of disease progression so that DBS can be used as an established treatment option for patients with dementia.

The PhD trajectory has taught me a lot, including not only the latest research in the world of DBS technology, but also DBS as a tool to improve memory and cognitive function, as well as the effects and corresponding mechanisms of different stimulation parameters. In my future work, study and life, I will continue to engage in DBS related research, maintain close contact and cooperation with the Department of Neuroscience of Maastricht University, and deeply practice the knowledge and research learned during my PhD.

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Valorization Addendum

Dementia is a cognitive impairment or cognitive decline that affects an individual's independent life, which will interfere with professional, family or social functions. A new case of dementia occurs every four seconds around the world [1]. According to statistics, the prevalence of dementia among people over 65 years old is as high as 7% around the world. The higher prevalence of dementia in developed countries (8-10%) may be due to longer life expectancy [2]. In today's society, dementia and its most common cause is AD, which has an imminent impact on the public health sector. According to the latest estimate, the global prevalence of dementia will increase to 115.4 million dementia patients by 2050 [2, 3]. It is difficult for any country in the world to bear the inherent cost of the burden of this disease. The most common early symptom is memory loss. As the disease progresses, symptoms may include language problems, disorientation, lack of motivation, difficulty speaking and writing and behavioral problems [4]. According to records, the global cost of dementia treatment in 2010 was US $818 billion. It is estimated that by 2030, this figure will reach $2 trillion [5]. Because AD patients have serious cognitive impairment, they need nursing and other care, which will affect family members emotionally and economically, and increase the cost of treatment and care [6, 7].

Given the limited efficacy of drugs associated with Alzheimer's disease and the occasional significant side effects associated with use, there is increasing interest in the use of non drug treatments, such as deep brain stimulation (DBS). In 1984, Turnbull et al. first used DBS of nucleus basalis of Meynert (NBM) for the treatment of AD patient. Even though there was no improvement in memory or cognition, researchers found some cortical glucose metabolic activity and limited arrest of deterioration [8]. After that, Hamani et al.found memory enhancement when using fornix DBS to treat obesity in 2008 [9]. Based on that study, a Phase I trial of DBS in the fornix of 6 patients with early AD was investigated. Bilateral stimulation of the fornix proved to be feasible and safe, having no serious adverse events [10]. Two patients experienced autobiographical experiential phenomena during surgery. Moreover, after 12-month DBS treatment, the patients exhibited improved memory and cognitive function, increased glucose metabolism [11]. And they also found enlarged bilateral hippocampal volume and slowing of mean hippocampal atrophy [12]. However, the sample size of six patients is small and the hippocampal enlargement was only found in two patients which may represent a chance finding. The stimulation parameters applied to AD patients may not be disease-specific. The mechanism of action and long-term efficacy evaluation of DBS need to be further studied.

Therefore, this thesis aims to study the most favorable effects of the target structure and stimulation parameters of DBS in an experimental model of dementia. In addition, this thesis describes the potential mechanism of DBS in memory recovery. To further study the mechanism of the process of DBS in the nucleus basalis of Meynert (NBM) inneurochemical changes hippocampus, synaptic plasticity and neurophysiology.

In view of the epidemiology and socio-economic impact of AD mentioned above, we divide the contribution of this paper into the following three main targets.
Consistent with this, by studying the experimental model of DBS in dementia, we may have found that this treatment scheme is not only suitable for patients with dementia, but also for patients with other central nervous system diseases with cognitive impairment. For example, obsessive-compulsive disorder, depression and addiction often suffer from cognitive impairment. Therefore, this paper provides preliminary evidence to help clarify how DBS may improve cognitive function in dementia and other neurological and mental diseases.

The second target group includes the public, society and the state. According to records, the global cost of dementia treatment in 2010 was US $818 billion. It is estimated that by 2030, this figure will reach $2 trillion [5]. The direct and indirect economic burden caused by AD has a significant impact on society and the country. Dementia seriously affects every health system in the world. A large number of resources and funds are used for dementia patients and their caregivers. There is evidence that the economic burden of middle-income countries is just beginning to appear [13-15]. DBS aims not only to improve the quality of life and physical health of AD patients, but also to reduce the related economic and social impact.

The third target group includes doctors and scientists in relevant disciplines in medicine and science. Neurologists and neurosurgeons are interested in the most favorable effect of specific brain targets of DBS on memory recovery. The scientific community and neuromodulation companies may benefit from the findings of this thesis and find future clinical and experimental animal models of DBS in the treatment of dementia. For example, this thesis found that intermittent stimulation may be an effective stimulation mode for DBS to improve memory, which coincides with the latest progress of adaptive DBS system in the treatment of epilepsy.

The main findings of this thesis are the preclinical treatment upgrading of DBS in the experimental dementia rat model and its possible mechanism. The results of this thesis provide evidence that intermittent DBS targeting NBM shows better memory performance in dementia experimental rat model, and can reverse the memory defect caused by scopolamine and increase the formation of new cells in hippocampal dentate gyrus.

Most DBS studies of mental diseases are first carried out in humans. Due to the similarity between clinical data and animal research results, the animal model in this paper is of great value for finding new DBS targets and memory recovery settings. These preclinical studies on memory recovery may bring a new direction for DBS in the treatment of dementia patients, and have a good prospect for patients with cognitive impairment diseases. Intermittent NBM DBS with optimal stimulation parameters has the potential to improve memory function, and has no side effects on anxiety level and general motor activities. In addition, there were significant differences in stimulation induced adult neurogenesis between NBM DBS group and sham operation group. And intermittent NBM DBS may induce long-term potentiation related mechanisms. NBM DBS activates the hippocampus and regulates the expression of neurotrophic factors and synaptic plasticity markers, which play a key role in memory processing. In addition, this paper also found that intermittent STN DBS seems to be an effective and safe treatment for cognitive impairment in PD patients. In this paper, we outline various behavioral and plasticity changes after electrical stimulation. These are safe and effective neuromodulation techniques with high selectivity and specificity, which provide ideas for improving the efficacy and reducing side effects in the transformation model.

Due to the relevance of the project to patients, society and the scientific community, the knowledge and new insights generated will be shared with relevant organizations, medical and scientific communities. Relevant research results have been or will be published in peer-reviewed international journals and presented at national and international conferences.

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Curriculum Vitae

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