Anatomy and function of the fornix in the context of its potential as a therapeutic target

Suhan Senova,1 Anton Fomenko,2,3 Elise Gondard,3 Andres M Lozano2,3

ABSTRACT
The fornix is a white matter bundle located in the mesial aspect of the cerebral hemispheres, which connects various nodes of a limbic circuitry and is believed to play a key role in cognition and episodic memory recall. As the most prevalent cause of dementia, Alzheimer’s disease (AD) dramatically impairs the quality of life of patients and imposes a significant societal burden on the healthcare system. As an established treatment for movement disorders, deep brain stimulation (DBS) is currently being investigated in preclinical and clinical studies for treatment of memory impairment in AD by modulating fornix activity. Optimal target and stimulation parameters to potentially rescue memory deficits have yet to be determined. The aim of this review is to consolidate the structural and functional aspects of the fornix in the context of neuromodulation for memory deficits. We first present an anatomical and functional overview of the fibres and structures interconnected by the fornix. Recent evidence from preclinical models suggests that the fornix is subdivided into two distinct functional axes: a septohippocampal pathway and a subiculothalamic pathway. Each pathway’s target and origin structures are presented, followed by a discussion of their oscillatory dynamics and functional connectivity. Overall, neuromodulation of each pathway of the fornix is discussed in the context of evidence-based fornical DBS strategies. It is not yet known whether driving fornix activity can enhance cognition—optimal target and stimulation parameters to rescue memory deficits have yet to be determined.

INTRODUCTION
The fornix is a white matter bundle located in the mesial aspect of the cerebral hemispheres, which connects various nodes of a limbic circuitry and is believed to play a key role in cognition and episodic memory recall. While fornix lesions impair memory, it is not yet known whether driving fornix activity can enhance cognition. Stimulating a distributed fibre bundle such as the fornix could be a powerful and efficient method of targeting interconnected brain regions involved in cognitive processes. Importantly, brain-wide targets could be simultaneously modulated by stimulation of fornical fibres projecting from and to widely distributed regions. A detailed knowledge of the neuronal structures interconnected by the fornix bundle would be crucial to delineate the anti-dromic and orthodromic effects of neuromodulation. A comprehensive knowledge of the fornical anatomy could also serve to inform clinicians to cognitive subpathways and corresponding memory deficits that arise from neurodegenerative disease or lesions. Likewise, careful consideration of structures targeted by fornical projections could also inform the electrophysiologist of optimal deep brain stimulation (DBS) parameters to try to rescue particular types of memory deficits encountered in Alzheimer’s disease (AD), traumatic brain injuries, epilepsy or strokes.

Neuromodulation of the fornix by electrical DBS has recently been investigated as a treatment for memory impairment in patients with AD. In a phase I study, one-third of patients showed improvement or slowing in the rate of cognitive decline. In a recent phase II study, it was suggested that the subgroup of patients with AD over the age of 65 tended to experience a slower deterioration in memory, and a phase III trial is currently ongoing to study the neuropsychological effects of fornix DBS in this patient subgroup. Despite multiple clinical trials underway, optimal target and stimulation parameters to rescue memory deficits have yet to be determined, and the therapeutic benefit is modest.

Here, we review structural and functional aspects of the fornix in the context of neuromodulation for memory deficits. We suggest that the fornix should be subdivided into two distinct anatomico-functional axes: a fornical septohippocampal (SHP) pathway and a fornical subiculothalamic pathway. Each pathway’s target and origin structures will first be presented, followed by a discussion of their oscillatory dynamics and functional connectivity. In the context of evidence-based therapeutic fornical DBS strategies, neuromodulation of each axis will be discussed with supporting preclinical and clinical evidence.

Gross anatomy of the fornix
The fornix is a thin arched white matter bundle composed of myelinated association, projection and commissural fibres located in the mesial aspect of the cerebral hemispheres (figure 1). The human fornix contains approximately 1.2–2.7 million fibres in each hemisphere and fills a total volume of about 1000–1800 cubic millimetres. As a major hippocampal output structure, the fornix stretches longitudinally from the mesial temporal lobe to the diencephalon and basal forebrain. Medial to the floor of the temporal horn of the lateral ventricle, hippocampal fibres collect into a thin lamina known as the alveus. Fibres from the subiculum join the alveus as it courses postero-medially and bundles into the fimbria of the fornix.
As the fimbria enlarge in cross-sectional area by collecting additional fibres, they become known as the crura of the fornix. The crura arch supero-anteriorly under the splenium of the corpus callosum and project contralaterally via the thin triangular fornical commissure, also known as the *psalterium* or dorsal hippocampal commissure. The crura run paracentrally to form the fornicical body, which arches over the thalamus and under the septum pellucidum. Rostrally, the fornix body bifurcates into left and right columns that descend into the basal forebrain anterior to the interventricular foramina. The fornix columns divide at the anterior commissure—fibres travelling anteriorly form the pre-commissural fornix, while those curving posteriorly make up the post-commissural fornix. This division in structure reflects the two major fornicical functional pathways. Pre-commissural fibres house the septohippocampal pathway, also projecting to the forebrain. Post-commissural tracts originate from the subiculum and project to the thalamus, forming the direct subiculothalamic pathway, and the indirect subiculothalamic pathway which relays via the mammillary bodies (figure 2). Important cross-species differences exist between primate and rodent fornices, in part due to the distinct spatial configuration of their respective hippocampi. The rodent hippocampus is transposed more rostrodorsally, with its dorsal and ventral components hinged by a 90-degree flexure and envelopes the hippocampal formation as a sheet of fimbria and alveus fibres. Compared with primates, rodents have a more developed commissural system—their dorsal commissure spans almost the entire longitudinal axis of the fornix. In addition, a thin transverse lamina known as the ventral hippocampal commissure (VHC) is found in non-human primates and rodents just ventral to the columns at the level of the subfornical organ. The VHC carries decussating dentate gyrus fibres and is probably absent in humans, though histopathological evidence exists of decussating fibres in this region, which later studies were unable to reproduce. The dorsal fornix is the murine homologue to the human fornix body and its fibres arise from the temporal hippocampal pole and course along the undersurface of the corpus callosum, medial to the septal hippocampus. The thin dorsal fornix eventually disappears at the anterior commissure, where fibres of the rodent fimbria–fornix divide to reach their terminal nuclei.

**Fornicical Pathways and Memory Impairment**

*Lesions of the fornix*

In early literature, surgical lesioning of the anterior fornix for treatment of epilepsy was only rarely associated with subsequent memory deficits. More recently, cognitive deficits in episodic
memory are increasingly being reported in patients with injuries to the fornix (table 1).\textsuperscript{21–23} Bilateral lesions of the fornix anterior columns are associated with anterograde and retrograde amnesia.\textsuperscript{24,25} The fornix carries distinct functions depending on laterality; the left fornix primarily carries verbal memory information, while the right carries visuospatial memory information.\textsuperscript{26–28} In addition, the medial fornix carries fibres from the caudal hippocampus, which integrates object recognition within a spatial context such as in scene learning. The lateral aspect of the fornix is thought to carry projections from the more rostral hippocampus, which processes interoceptive signals for emotional and motivational learning and memory.\textsuperscript{29,30} After fornical lesions, a relative sparing of recognition is seen when compared with the loss of the recall of episodic information.\textsuperscript{23,27} Moreover, as nodes of the fornix, the mammillary bodies are classically considered to provide a relay for indirect hippocampal inputs to the anterior thalamic nuclei. In such models, the mammillary bodies do not have an independent role in memory. However, the medial aspect of the mammillary bodies’ contribution to memory is more dependent on afferents from the ventral tegmental nucleus of Gudden than from the hippocampal formation, while the lateral mammillary bodies, connected with the dorsal tegmental nucleus of Gudden, participate in generating head-direction signals.\textsuperscript{31}

### Early Alzheimer’s pathology and the fornix

The neuropathological hallmarks of AD, such as extracellular beta-amyloid and intracellular tau, can provide insight into the neuroanatomical progression of the disease and shed light into potential neuroanatomical hotspots for neurodegeneration-based therapy.\textsuperscript{32,33} The appearance of hyperphosphorylated cytoskeletal tau within the brain is found even before cognitive deficits are clinically apparent (ie, Braak and Braak AD stages 0 and 1), classically manifesting first in the entorhinal and transentorhinal regions.\textsuperscript{34,35} However, recent pathoanatomical studies in brains from cognitively intact individuals have found evidence of immunoreactive neuronal tau cytoskeletal pathology in subcortical nuclei interconnected by the fornix.\textsuperscript{34,35} Areas with presence of tau deposits in Braak stages 0 or 1 included the perifornical and lateral region of the hypothalamus, the dorso-medial, ventromedial, tuberomammillary and supramammillary nuclei, and subnuclei of the amygdala and thalamus known to be synaptically connected to the hippocampus via the fornical

<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>Fornical lesion location</th>
<th>Behavioural deficits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nilsson et al 1987</td>
<td>Rat</td>
<td>Post-commissural dorsal fornix</td>
<td>Spatial memory impairment</td>
</tr>
<tr>
<td>Aggleton et al\textsuperscript{23}</td>
<td>Rat</td>
<td>Post-commissural dorsal fornix</td>
<td>Spatial memory impairment but no recognition memory impairment</td>
</tr>
<tr>
<td>Waburton and Aggleton 1999</td>
<td>Rat</td>
<td>Post-commissural dorsal fornix</td>
<td>Spatial memory impairment but no recognition memory impairment</td>
</tr>
<tr>
<td>Howard et al 1989</td>
<td>Rat</td>
<td>Post-commissural dorsal fornix</td>
<td>Spatial memory impairment</td>
</tr>
<tr>
<td>Jettsch et al 1994</td>
<td>Rat</td>
<td>Post-commissural dorsal fornix</td>
<td>Spatial memory impairment</td>
</tr>
<tr>
<td>Fletcher et al 2006</td>
<td>Rat</td>
<td>Post-commissural dorsal fornix</td>
<td>Spatial memory impairment</td>
</tr>
<tr>
<td>Mala et al 2013</td>
<td>Rat</td>
<td>Post-commissural dorsal fornix</td>
<td>Spatial memory impairment</td>
</tr>
<tr>
<td>Ennaceur et al 1997</td>
<td>Rat</td>
<td>Post-commissural dorsal fornix</td>
<td>Spatial memory impairment but no recognition memory impairment</td>
</tr>
<tr>
<td>Waburton et al 2000</td>
<td>Rat</td>
<td>Post-commissural dorsal fornix</td>
<td>Spatial memory impairment but no recognition memory impairment</td>
</tr>
<tr>
<td>Phillips and LeDoux 1995</td>
<td>Rat</td>
<td>Post-commissural dorsal fornix</td>
<td>Contextual fear conditioning impairment</td>
</tr>
<tr>
<td>Maren and Fanselow 1997</td>
<td>Rat</td>
<td>Post-commissural dorsal fornix</td>
<td>Contextual fear conditioning impairment</td>
</tr>
<tr>
<td>Antoniadiis and McDonald 2006</td>
<td>Rat</td>
<td>Post-commissural dorsal fornix</td>
<td>Contextual fear conditioning impairment</td>
</tr>
<tr>
<td>Laurent-Demir and Jaffard 2000</td>
<td>Rat</td>
<td>Post-commissural dorsal fornix</td>
<td>No impairment in acoustic fear conditioning</td>
</tr>
<tr>
<td>Baldi et al 2013</td>
<td>Rat</td>
<td>Post-commissural dorsal fornix</td>
<td>No impairment in acoustic fear conditioning</td>
</tr>
<tr>
<td>Baldi et al 1998</td>
<td>Rat</td>
<td>Post-commissural dorsal fornix</td>
<td>Deficit in encoding but not retrieval in passive avoidance learning</td>
</tr>
<tr>
<td>Sziklas and Petrides 2002</td>
<td>Rat</td>
<td>Pre-commissural fornix</td>
<td>Spatial memory impairment but no impairment with a visual cue</td>
</tr>
<tr>
<td>Saunders et al 2005</td>
<td>Monkey</td>
<td>Fornix body</td>
<td>Impaired visual recognition</td>
</tr>
<tr>
<td>Wilson et al 2007</td>
<td>Monkey</td>
<td>Fornix body</td>
<td>Impairment in object discrimination</td>
</tr>
<tr>
<td>Buckley et al 2008</td>
<td>Monkey</td>
<td>Fornix body</td>
<td>Impairment of encoding but no recall of visuospatial memory</td>
</tr>
<tr>
<td>KwoK and Buckley 2010</td>
<td>Monkey</td>
<td>Fornix body</td>
<td>Impaired encoding of rapidly learnt visuospatial discrimination</td>
</tr>
<tr>
<td>Adamovich et al 2009</td>
<td>Human</td>
<td>Bilateral anterior columns of fornix</td>
<td>Retrograde and anterograde amnesia</td>
</tr>
<tr>
<td>Bawerja et al 2015</td>
<td>Human</td>
<td>Bilateral anterior columns of fornix</td>
<td>Retrograde and anterograde amnesia</td>
</tr>
<tr>
<td>Cameron et al 1981</td>
<td>Human</td>
<td>Left anterior column of fornix</td>
<td>Verbal memory deficit</td>
</tr>
<tr>
<td>Korenatsu et al 2010</td>
<td>Human</td>
<td>Left anterior column of fornix</td>
<td>Retrograde and anterograde amnesia</td>
</tr>
<tr>
<td>Vann et al 2008</td>
<td>Human</td>
<td>Anterior column of fornix</td>
<td>Anterograde amnesia but spared recognition memory</td>
</tr>
<tr>
<td>Gupta et al 2015</td>
<td>Human</td>
<td>Left anterior column and fornix body</td>
<td>Retrograde and anterograde amnesia</td>
</tr>
<tr>
<td>Hodges et al 1991</td>
<td>Human</td>
<td>Anterior genu of fornix</td>
<td>Anterograde amnesia</td>
</tr>
<tr>
<td>McMahon et al 1996</td>
<td>Human</td>
<td>Anterior genu of fornix</td>
<td>Anterograde amnesia</td>
</tr>
<tr>
<td>Murr et al 2012</td>
<td>Human</td>
<td>Anterior genu of fornix</td>
<td>Anterograde amnesia</td>
</tr>
<tr>
<td>Rizek et al 2013</td>
<td>Human</td>
<td>Anterior genu of fornix</td>
<td>Anterograde amnesia</td>
</tr>
<tr>
<td>Kauppila et al 2018</td>
<td>Human</td>
<td>Anterior genu of fornix</td>
<td>Anterograde verbal memory</td>
</tr>
<tr>
<td>Chen et al 2008</td>
<td>Human</td>
<td>Fornix body</td>
<td>Anterograde amnesia</td>
</tr>
<tr>
<td>Carota et al 2013</td>
<td>Human</td>
<td>Crura and body of the fornix</td>
<td>Anterograde amnesia</td>
</tr>
<tr>
<td>Tucker et al 1988</td>
<td>Human</td>
<td>Left fornix body</td>
<td>Anterograde amnesia</td>
</tr>
<tr>
<td>Yeo et al 2013</td>
<td>Human</td>
<td>Crura of the fornix</td>
<td>Anterograde amnesia</td>
</tr>
</tbody>
</table>
and entorhinal pathways.\textsuperscript{34} Since PET imaging studies point to the distribution of tau signal as a strong predictor of future local neurodegeneration and atrophy, neuromodulation of the fornix or its subnuclei may represent a logical strategy.\textsuperscript{33} Indeed, studies in animal and in vitro models of AD have shown that synaptic activation via chronic DBS reduces pathological tau and provided synaptic neuroprotection.\textsuperscript{37}

In addition to patterns of tau accumulation, another biomarker of AD correlating with disease severity includes alterations in cortical EEG dynamics.\textsuperscript{38} As a cortical dementia, hallmarks of underlying neuropathological changes in AD include decreases in lower cortical frequency bands (alpha and beta), and an increase in theta and delta rhythms.\textsuperscript{38,39} Reduced coherence of cortical alpha and beta bands is also seen in AD, suggesting losses in cortical synaptic function.\textsuperscript{38-40} Since the fornix is a deep structure connecting chiefly subcortical nuclei, at the time of this writing no DBS studies have studied cortical EEG changes in response to DBS, though it merits future study as a potentially useful outcome measure to monitor response to neuromodulation treatment.

**Fornical connectivity and memory impairment**

Functional MRI studies are beginning to suggest that degeneration of the fornix bundle itself may precede hippocampal dysfunction and predict cognitive impairment better than structural measures such as hippocampal atrophy.\textsuperscript{41} Connectivity data from diffusion tensor imaging studies suggest that fornical measures correlate with episodic memory performance in various neuropathological conditions, as well as during brain development and ageing. On imaging, macrostructural and microstructural alterations of the fornix have been found to be robust predictors of episodic memory performance, independent of age and associated structural pathology.\textsuperscript{42} Specifically, in AD, atrophy of the fornix on structural MRI and reductions in fractional anisotropy have often been reported.\textsuperscript{43-45} Fornix atrophy may predict the onset of AD,\textsuperscript{46} even prior to clinical manifestations. Moreover, fornix fractional anisotropy reduction is correlated with cognitive decline in AD.\textsuperscript{46} While fornix lesions or degeneration are associated with memory impairment, it is not yet known whether driving fornix activity can enhance these functions.

**SEPTOHIPPOCAMPAL PATHWAY**

**Anatomy and spontaneous activity**

The projections from the medial septum (MS) to the hippocampus form the septohippocampal fornical pathway and are proposed to have important roles in cognition by modulating the activity of episodic memory circuits.\textsuperscript{47-49} As the terminal structure of this fornical pathway, the hippocampus is necessary for episodic memory, and is involved in the storage and recall of autobiographical events.\textsuperscript{50-51} Sensory cue inputs from the entorhinal cortical grid cells and memory-related internal brain activities govern the firing of hippocampal neurons.\textsuperscript{52,53}

Traditionally, the hippocampus has been thought to exhibit two dominant and behaviour-dependent local field potential (LFP) patterns: theta rhythm and large-amplitude irregular activity with sharp waves.\textsuperscript{54} Theta rhythm is a large-amplitude (1–2 mV) 4–10 Hz sinusoidal oscillation in the rat,\textsuperscript{55} with two defined subtypes: type 1 (7–10 Hz) is associated with voluntary movement and exploratory behaviour, whereas type 2 (4–6 Hz) is present during immobility, rapid eye movement (REM) sleep or urethane anaesthesia.\textsuperscript{59}

Theta oscillations are understood to be critical in hippocampal mnemonic and learning functions.\textsuperscript{58} The MS projections through the fornix are involved in hippocampal theta modulation.\textsuperscript{47,49,67,68} Rhythmically discharging cells of the MS in the diagonal band vertical nucleus fire synchronously with theta and may be involved in its pacing.\textsuperscript{58,67,69,70} In freely moving rats, MS neuron activity can be negatively (during sharp wave ripples) or positively (during theta waves) correlated with the activity of hippocampal neurons.\textsuperscript{71} The MS decreases the spiking of hippocampal pyramidal neurons and reduces their ability to fire in trains.\textsuperscript{47,49} Septohippocampal oscillatory regulation of neuronal activity also precisely synchronises postsynaptic potentials arriving at hippocampal pyramidal cells.\textsuperscript{72}

Conversely, disrupting or lesioning the MS eliminates the hippocampal theta rhythm. During MS inactivation by muscimol\textsuperscript{73} or lidocaine,\textsuperscript{74} grid cells recorded in the entorhinal cortex lose their spatial periodicity. Also, loss of theta via MS lesioning significantly alters performance on spatial\textsuperscript{49,77,81} as well as non-spatial tasks.\textsuperscript{49,80} However, after extinguishing hippocampal theta rhythm via pharmacological inactivation of the MS,\textsuperscript{77,81} location-specific firing of hippocampal place cells are maintained. Sensory cues seemingly guide hippocampal neural firing in rats, whereas MS inputs prevail over shorter timescales,\textsuperscript{81} and both support the formation of hippocampal spatial firing fields.

In rats, reciprocal hippocamposeptal projections exist to the cholinergic nuclei of the MS via fornical GABAergic neurons.\textsuperscript{74,75} The CA1 to MS fibres are sparse and project unilaterally, whereas those originating from CA2–3 project extensively and bilaterally.\textsuperscript{56} Topographically, the dorsal CA3 innervates the dorsal and medial parts of the MS; conversely, axons of the ventral CA3 reaches the lateral and ventral parts of the MS.\textsuperscript{86} The hippocamposeptal tract has been implicated in inhibiting\textsuperscript{77,81} and modulating\textsuperscript{77} theta generators in the septal region.\textsuperscript{85} For instance, in vitro rodent hippocampal preparations demonstrated the role of hippocamposeptal modulation in phasing the spiking of MS GABAergic neurons, while inhibiting acetylcholinergic and glutamatergic neurons in the same region.\textsuperscript{79}

**Electrical stimulation of the septohippocampal pathway**

Diverse stimulation parameters of the SHP have been explored. High-frequency (100 Hz) chronic or acute stimulation of the rodent SHP were found to induce hippocampal long-term potentiation and neurogenesis.\textsuperscript{80} Furthermore, upregulation of genes involved in synaptic function, cell survival and neurogenesis was observed in molecular expression studies after such SHP stimulations.\textsuperscript{91}

Because theta oscillations are critically involved in memory, SHP stimulation at theta frequencies has been thoroughly investigated in rodents (figure 1; table 2). Specifically, electrical stimulation of the MS in the 3–12 Hz range serves to experimentally mirror physiologic theta-like hippocampal LFP frequencies.\textsuperscript{95,96} Indeed, 5–7 Hz DBS exhibits electrophysiological characteristics similar to those of spontaneously occurring theta field activity,\textsuperscript{96} while higher stimulation frequencies produce hippocampal desynchronisation.\textsuperscript{97,98} Although CA1 pyramidal cells respond maximally to 6–8 Hz MS stimulation, electrical stimulation of medial septal nuclei does not produce typical physiological hippocampal theta-related activity.\textsuperscript{100} Nevertheless, restoring theta-like hippocampal activity by stimulation of the SHP was shown to rescue memory deficits in rats after MS inactivation.\textsuperscript{97} More precisely, irregular SHP stimulation resulted in little rhythmicity, while a fixed stimulatory frequency of 7.7 Hz triggered by...
supramammillary nucleus theta rhythmicity restored theta-like rhythmicity with abnormal waveforms. While both stimulation paradigms improved memory deficits, the latter was the most efficient. Thus, despite incomplete physiological reinstatement, promotion of synchronous low-frequency phasic firing rescues learning processes in rodent models. DBS of the SHP has also been shown to restore cognitive deficits associated with pathological brain states. For instance, in a rat model of traumatic brain injury, theta stimulation of the MS restored hippocampal theta oscillations and yielded improvements in object exploration when performed chronically, as well as improved spatial working memory when administered acutely before training.

Moreover, temporal co-ordination of theta and gamma rhythms is important for sequential memory retrieval, and facilitation of synaptic plasticity. After pharmacological inactivation of the MS, Shirvalkar and colleagues reported that acute theta burst stimulation (TBS) of the SHP within the fimbria–fornix of the MS, Shirvalkar and colleagues reported that acute theta spiking of hippocampal place cells, potentiates population theta oscillations and yielded improvements in object exploration when performed chronically, as well as improved spatial working memory when administered acutely before training. TBS is a distinct pattern of stimulation that has been investigated for its physiological relevance. Non-selective septal TBS resets hippocampal theta cell bursting during active behaviour while increasing theta synchronisation. This reset persists for 600–900 ms and enables dentate granule cell depolarisation at the time of sensory input arrival from the entorhinal cortex. This synchrony facilitates long-term potentiation, enabling synaptic plasticity, and ultimately enhancing the encoding of incoming information. TBS also entrains the spiking of hippocampal place cells, potentiates population spikes at CA1 and temporally regulates the place field spatial properties during active exploration.

### MS neuronal subpopulations

Septohippocampal projections encompass immunohistochemically and electrophysiologically distinct slow-firing and fast-firing neurons. These subpopulations can be further distinguished by their firing patterns and synaptic properties.

### Electrophysiology

GABAergic septohippocampal projection cells are crucial for hippocampal theta generation. For instance, the frequency of theta in urethane-anaesthetised rats depends on the activity of medial septal GABAergic cells. GABAergic neurons may pace the hippocampus in vivo via tonic, cluster or burst-firing patterns of discharge mediated by low-threshold spikes and prominent hyperpolarisation-activated cation (Ih) currents. Rhythmic bursting activity, observed at theta frequencies during wakefulness and REM sleep, is more pronounced in GABAergic and GABAergic neurons in the stratum radiatum of the CA1 and stratum lucidum of CA3. In primates, the MS projects to the hippocampus in a topographically oriented fashion: medial portions project via medial fornix fibres, and the lateral MSN project via the lateral fornix. To understand the physiology of the rodent SHP, the projection patterns for each MS neuronal subtype will be reviewed.

### MS GABAergic neurons

#### Connectivity

Medial septal GABAergic fibres terminate on vasoactive intestinal polypeptide–immunoreactive interneurons in strata pyramidale and lacunosum-moleculare of the CA1 and on calcineurin-immunoreactive and neuropeptide Y–immunoreactive GABAergic interneurons in the stratum radiatum of the CA1 and stratum lucidum of CA3. Inhibitory inputs terminate on neurons containing cholecystokinin, somatostatin and parvalbumin in the stratum oriens.

### Table 2: Stimulation of the post-commisural dorsal fornix in rodents and patients with epilepsy, along with main findings

<table>
<thead>
<tr>
<th>Study</th>
<th>Model</th>
<th>Stimulation pattern</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>William and Givens 2003</td>
<td>Rats</td>
<td>Acute single pulses</td>
<td>Hippocampal theta reset</td>
</tr>
<tr>
<td>McNaughton et al 2006</td>
<td>Rats, medial septum pharmacologically inactivated</td>
<td>Acute fixed theta</td>
<td>Increases theta power, improves initial performance in Morris Water Maze (MWM)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acute irregular theta</td>
<td>Does not increase theta power, nor improves performance in MWM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acute closed loop theta</td>
<td>Increases theta power, improves performance in MWM a sustained way</td>
</tr>
<tr>
<td>Shirvalkar et al 2010</td>
<td>Rats, medial septum pharmacologically inactivated</td>
<td>Acute theta</td>
<td>Does not improve theta–gamma coupling in hippocampus nor memory performance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acute 100 Hz</td>
<td>Does not improve theta–gamma coupling in hippocampus nor memory performance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acute TBS</td>
<td>Restores theta-gamma coupling in hippocampus and memory performance in radial water maze</td>
</tr>
<tr>
<td>Hao et al 2015</td>
<td>Mouse model of Rett syndrome</td>
<td>Chronic 100 Hz</td>
<td>Improves LTP and neurogenesis in hippocampus, and contextual fear memory</td>
</tr>
<tr>
<td>Miller et al 2015</td>
<td>Patients with epilepsy</td>
<td>Acute TBS</td>
<td>Improvement in visuospatial memory</td>
</tr>
</tbody>
</table>

LTP, long-term potentiation; MWM, Morris water maze; TBS, theta burst stimulation.
wake cycle. Rhythmic bursting activity at theta frequencies is tightly coupled to hippocampal theta waves: glutamic acid decarboxylase (GAD)–positive neurons are distributed in almost equivalent proportions between T type (burst-firing at trough of hippocampus theta) and P type (peak of hippocampus theta) whereas the parvalbumin/GAD+ are largely T type.136,154,155,158 Furthermore, the hyperpolarisation-activated and cyclic nucleotide-gated non-selective cation channel (HCN) of some MS GABAergic neurons has been suggested to play a pacemaker role for theta oscillations134,135 by disinhibiting the hippocampal pyramidal cells via rhythmic inputs to hippocampal GABAergic interneurons.127,159 Activity changes within parvalbumin and/or HCN neurons precede changes in hippocampal interneurons and theta rhythm.158

Behaviour
An important component of hippocampal network dynamics and plasticity during learning160 is the modulation of CA1 interneuron activity by the septohippocampal GABAergic pathway during salient sensation and locomotion. Furthermore, Cav3.1 T-type Ca2+ channels are highly expressed in the septohippocampal GABAergic projection neurons161 and are critically involved in controlling object exploration through modulating hippocampal theta type 2 theta rhythm.162 Specifically, optogenetic activation of this pathway in mice selectively enhances novel object exploration and type 2 theta rhythm, whereas inhibition of the same pathway decreases both exploration and the rhythm.162

MS cholinergic neurons
Connectivity
Medial septal cholinergic terminals project to all regions of the hippocampus,163,164 especially the stratum oriens of the CA1 and CA3 subfields.165–167 These terminals synapse with pyramidal cell dendrites168 and cell bodies and dendrites of GABA-containing and somatostatin-containing interneurons147,166,169,170 and dentate granule cells.171 As the majority of axon terminals are diffusely organised172 and do not associate with distinct postsynaptic sites,165,166,168,173 cholinergic transmission in the hippocampus is likely primarily mediated by volume transmission. The cholinergic projections to the hippocampus may therefore tonically maintain an extracellular ambient level of acetylcholine,174 leading to long-lasting effects.173,175

Acetylcholine release within hippocampal circuits results in the activation of both metabotropic muscarinic (mACHRs) and ionotrophic nicotinic (nACHRs) acetylcholine (ACh) receptors. Nicotinic receptors are expressed in dentate granule cells, pyramidal cells and interneurons both presynaptically and postsynaptically.176–180 interneuron axons terminating on excitatory and inhibitory neurons177–181 and at inhibitory synapses contacting pyramidal neurons.182 Muscarinic receptors are expressed in soma and dendrites of pyramidal neurons and granule cells,183 with a small fraction expressed on axons and terminals.182 These receptors are also found in interneurones184,185 and in fibres surrounding pyramidal cells. The highest density of expression is found presynaptically in GABAergic terminals projecting onto the perisomatic region of pyramidal cells,186–189 and postsynaptically in dendrites and cell bodies of interneurons in the stratum oriens and alveus of CA1,190 or in glutamatergic terminals.197

Electrophysiology
As a consequence of their presynaptic and postsynaptic location, muscarinic receptors can have diverse impacts on hippocampal neuronal activity, influencing the net effect of ACh. Choline acetyltransferase (ChAT)–positive neurons have a long duration spike136 and fire at a lower frequency136,191 during the inactive (3.4±0.3 Hz) compared with active behavioural state (4.7±0.3 Hz).115 ACh has an excitatory effect on GABAAergic and glutamatergic neurons within the MS.141,192,193 Cation flux through nACHRs mediates fast excitatory synaptic responses.194,195,196 Fast membrane depolarisation triggers activation of voltage-gated Ca2+ channels, second messenger systems involving cAMP196,197 and release from intracellular stores.202,203 Moreover, nAChRs may modulate pre-existing oscillatory states204,205 by enhancing a slow calcium-dependent potassium conductance that reduces the firing of stratum oriens interneurons.206 In contrast to the fast response produced by activation of nAChRs, mACHR-mediated transmission is slow, owing to their dependence on G-protein-coupled signalling mechanisms.207 Moreover, M1/M3 mACHR activation sharpens interneurons’ firing precision to theta frequency input, leading interneurons to amplify theta oscillations.208

ACh can suppress or enhance presynaptic neurotransmitter release in the hippocampus.178,179,182,184,185,209–215 ACh can facilitate and induce hippocampal long-term potentiation (LTP) or depression (LTD).128,199,216–225 The precise mechanism and direction of modulation may depend on ACh concentration, the timing of its release, exposure time, and the temporal sequence of nAChR and mACHR activation in relation to ongoing neuronal activity.199,226–228

Cholinergic MS neurons can be selectively activated by optogenetics.115 The evoked hippocampal response involves direct activation of ChAT projections together with indirect activation of non-ChAT septal neurons. Hippocampal neurons respond with an initial inhibition followed by rebound potentiation, inhibition and biphasic response, including potentiation and subsequent inhibition. Optogenetic septal ChAT stimulation exerts frequency-dependent and behaviour-dependent effect on hippocampal formation. The spiking of hippocampal neurons is significantly increased by 50 Hz but not 10 Hz septal stimulation and spiking increase is higher for inactive behavioural state than for active behavioural state.

Although cholinergic neurons are not pacemakers for hippocampal theta oscillations, they are involved in the generation and modulation of some of their attributes.59,62,67,74,148–150,229,230 However, the long post-hyperpolarisation period, small Ih and slow firing rates characteristic of MS cholinergic neurons limit their capacity to pace theta-related rhythmically bursting activity.126,128,135,126,231 Moreover, selective lesioning of MS cholinergic neurons reduces the number of rhythmically bursting neurons in the MS but does not eradicate hippocampal theta.131 Cholinergic MS neurons have been shown to display very slow, theta-unrelated firing in vivo, suggesting that these neurons might not act as pacemakers but rather generate theta activity via the tonic excitation of MS GABAAergic and glutamatergic neurons136,141,192 and hippocampal interneurons and principal cells.166 Although in vivo coupling between phasic ACh release and theta oscillations has been shown, theta initiation was found not to require ACh.191 Cholinergic neurons firing was also seen to follow theta oscillations, making them pro-arousal and not pacemaker neurons for hippocampal theta oscillations.191

Cholinergic neurons also modulate the amplitude of theta oscillations. Microdialysis of ACh release132 and selective lesions of septohippocampal cholinergic neurons233 showed that cholinergic neurons selectively modulate the amplitude of theta oscillations, and not its frequency.136,234 Optogenetic activation of MS cholinergic neurons affects hippocampal oscillations in the


Neurosurgery

J Neurol Neurosurg Psychiatry on December 26, 2022 by guest. Protected by copyright.
theta range in a behaviour-dependent and frequency-dependent manner. Theta and gamma both significantly increase for inactive compared with active rodents and for low-frequency stimulation (8–10 Hz). The overall tone of ACh is lower under urethane anaesthesia than in the awake animal and the faster theta seen during movement is largely non-cholinergically mediated, whereas the cholinergic input provides a background of sensory input-related drive to the hippocampus during movement. Another robust effect of optogenetic activation of MS cholinergic neurons in both anaesthetised and behaving mice is the suppression of slow oscillations in the supratheta frequency band.

Cholinergic neurons have been found to play a role in hippocampal theta and synaptic plasticity. For instance, the local field synchronisation expressed a preference for a low-frequency stimulation protocol, whereas the hippocampal neuronal response showed significant increase after 50 Hz but not after 10 Hz optogenetic septal stimulation. While slow-spiking septal cholinergic neurons are linked to the amplitude of theta rhythm by tonically depolarising pyramidal cells and basket interneurons, the fast-spiking septal GABAergic cells are linked to the frequency of theta rhythm by periodically hyperpolarising hippocampal basket cells and rhythmically disinhibiting the pyramidal cells.

**Behaviour**

Optogenetic cholinergic stimulation of the MS does not exert a consistent effect on locomotion velocity and motor behaviour. In fact, inhibition from different classes of interneurons create gamma oscillations within each theta cycle, and the modulated efficacy of excitatory inputs at different theta phases can selectively influence the timing of pyramidal cell firing. Therefore, promotion of co-ordinated firing and rhythmic activity by ACh release may provide an increase in the baseline excitability of neurons. This results in enhanced neural responses to glutamate and promotes neural interactions facilitating memory formation. Within this system, synaptic input that arrives during the positive phase of theta induces LTP while input that occurs during the negative phase induces LTD or depotentiation. In addition, cholinergic receptor activation enhances LTP induction during exploration and theta entrained hippocampal place cell activity. Therefore, high cholinergic tone during phases of exploration, in synergy with optimally timed theta, enhances plasticity.

**MS glutamatergic neurons**

**Connectivity**

Some glutamatergic neurons within the MS provide functional excitatory input to local cholinergic and GABAAergic neurons. Others send direct projections to a restricted number of pyramidal cells and interneurons in the hippocampus. Electrophysiology

Medial septal glutamatergic neurons expressing type 2 vesicular glutamate transporters (VGluT2) are likely involved in hippocampal theta generation. They display a heterogeneous firing pattern, including fast, slow, bursts, and cluster-firing (8–14 Hz, half of glutamatergic neurons) properties in slice. Glutamatergic neurons also have intrinsic firing properties that may play an important role in pacing the hippocampus in vivo: they can discharge in recurrent clusters of action potentials, interspersed with intrinsically generated subthreshold membrane potential oscillations.

**Septohippocampal pathway summary**

Overall, MS burst-firing GABAAergic neurons are important in generating, maintaining and pacing hippocampal theta activity by modulating GABAergic hippocampal interneurons and, indirectly, pyramidal cells. In turn, slow-firing cholinergic cells modulate theta amplitude. Septal glutamatergic neurons provide dense connections within the septum and comparatively sparse projections across the hippocampus: their rhythmic activation can powerfully drive hippocampal rhythms through local septal interactions rather than through direct projections to the hippocampus.

Furthermore, spontaneous activity of MS neurons can be influenced by different inputs from the locus coeruleus, raphe...
nuclei and hypothalamus.\(^{254,255}\) Therefore, in addition to acting as one of several extrinsic rhythm generators that work in concert to amplify and regulate intrinsic theta generators within the hippocampus, the MS may relay and pace theta rhythm by integrating inputs from neighbouring brain regions.\(^{256}\) Taken together, a neurophysiologically-inspired septal DBS protocol should combine at minimum a low-frequency cholinergic-like and high-frequency GABAergic-like stimuli.

**SUBICULOTHALAMIC PATHWAY**

Subicular projections to the anterior thalamic nuclei (ATN) and the mammillary bodies (MB) connect distant nodes of the circuit of Papez and propagate the theta rhythm generated by the MS. To understand the role of these projections, anatomy, physiology and functions of these nodes will be explored.

**Subiculum**

The subiculum constitutes the major output structure of the hippocampal formation.\(^{257}\) As the final relay in a polysynaptic loop between the entorhinal cortex (EC) and the hippocampus, it integrates and distributes processed spatial and mnemonic information to cortical and subcortical brain regions.\(^{258}\) Pyramidal neurons, which form the chief output of the subiculum, are divided into two main groups based on their firing mode: regular-spiking and bursting neurons (figure 3).\(^{259–263}\) Regular-spiking neurons fire a single action potential with 60–160 ms interspike intervals, whereas bursting neurons emit 2–5 action potentials at high frequency (2–5 ms interspike intervals) with decreasing successive spike amplitudes\(^{260,262,263}\) followed by a 20–30 ms refractory period with subsequent return to spiking. Bursting neurons are better suited to discriminate the content of high-frequency input, such as that occurring during gamma oscillations, than regular-spiking neurons.\(^{262}\) Interneurons are also found in the subiculum, as fast spiking units with an inter-spike interval of 7–10 ms and small spike width (<0.2 ms). There are more bursting than non-bursting neurons in the subiculum and both groups are distributed in an organised fashion along the proximal–distal axis, with more regular-spiking neurons close to CA1, and more bursting neurons close to the presubiculum.\(^{260,262}\) Moreover, there are distinct output targets in different portions of subiculum.\(^{257,258,264–268}\) The subiculum can be divided in four regions following the dorso-ventral and the proximo-distal axes, each of which serves as the origin of different parallel efferent projections with very few collateralisation.\(^{265,269–271}\) Bursting and regular spiking cells mainly target respectively the presubiculum and the EC.\(^{272}\) Neurons projecting to the nucleus accumbens are located in the proximal subiculum and consist mostly of regular-spiking neurons (~80%) whereas neurons projecting to the ventromedial hypothalamus are located in the distal subiculum consisting mostly of bursting neurons (~80%), and neurons projecting to thalamus are located in the middle portion of subiculum with a bursting probability of 50%.\(^{273}\) Differences in the distribution and projection of regular-spiking and bursting neurons suggest that different types of information are conveyed from the subiculum to its various targets. Furthermore, the subiculum is capable of intrinsically generating two major memory relevant network rhythms: SWR\(^{274}\) and gamma oscillations.\(^{275}\) Gamma activity arises after tetanic stimulation of the subiculum or the hippocampal CA1 region.\(^{276,277}\) SWR might mediate memory consolidation and gamma oscillations the encoding of new information. A relatively small proportion of subicular recordings are phase-locked to theta. Nevertheless, similar to the hippocampus, subicular theta is characterised by theta oscillations dominating exploratory behaviours, while SWR occur mainly during alert, still and quiet behaviours.

Several studies have investigated subicular functional connectivity and plasticity.\(^{278}\) In 2000, Gigg and colleagues showed that stimulation of CA1 produced excitation–inhibition sequences in bursting and non-bursting subicular principal cells and interneurons.\(^{278}\) The predominant subicular response to EC stimulation was weak inhibition, suggesting that EC bypasses the hippocampus, modulating the output of the subiculum and thus hippocampal–cortical interaction. Finally, a small depolarising response is observed when CA3 is stimulated and there is no response to dentate gyrus stimulation.\(^{261}\) Commins et al found in vivo paired pulse facilitation (interstimulus interval of 50 ms) as well as LTP by high-frequency stimulation and TBS at the CA1–subiculum synapse.\(^{279–281}\) One hertz low-frequency CA1 stimulation induced frequency-dependent LTD in bursting neurons and LTP in regular spiking subicular neurons, and this bidirectional plasticity relied on the co-activation of muscarinic ACh receptors.\(^{282}\) Finally, EC–subiculum synapses respond to low frequency–induced LTD\(^{283}\) and high frequency–induced LTP.\(^{284}\)

In parallel, several studies have reported that subicular neurons show spatially selective firing.\(^{20,285–287}\) Subicular neurons can be divided into three general classes: neurons coding head direction, neurons the firing rate of which reflects position but is modulated by head direction and neurons encoding place.\(^{280}\) The main output of CA1 is subiculum, but subicular place fields appear to be of lower resolution than those of CA1.\(^{287}\) Interestingly, the CA1 and subiculum have been found to operate in a complementary fashion to encode information in a spatial delayed-non-match-to-sample task.\(^{289}\) Subicular neural responses in this task were generally related to shorter delays (15 s or less); conversely, CA1 neural activity was related to long-delay (>15 s) trial-specific information. Finally, the subiculum receives a direct projection from the perirhinal cortex, where neurons are responsive to the novelty or familiarity of objects encountered in...
Maniculohypothalamic tract

The lateral and medial MB are connected with different subregions of the hypothalamus. Dense hippocampal projections from the lateral MB innervate the medial MB, whereas the medial MB projects to the lateral MB, particularly through the posterior subicular complex. The lateral MB has reciprocal connections with the ventral and posterior thalamic nuclei, the lateral MB projects bilaterally to the anterior medial and anterior ventral thalamic nuclei, and the medial MB projects to the anterior thalamic nuclei. Both the lateral and medial MB have direct connections with the subiculum, the hippocampus, the parahippocampal gyrus, and the posterior cingulate cortex. Dense hippocampal projections from the lateral MB terminate in the medial MB, which receives a large number of these inputs. Dense hippocampal projections from the lateral MB also terminate in the mediodorsal nucleus of Gudden. Both the lateral and medial MB have reciprocal connections with the ventral and posterior thalamic nuclei, the lateral MB projects bilaterally to the anterior medial and anterior ventral thalamic nuclei, and the medial MB projects to the anterior thalamic nuclei. Both the lateral and medial MB have direct connections with the subiculum, the hippocampus, the parahippocampal gyrus, and the posterior cingulate cortex. Dense hippocampal projections from the lateral MB terminate in the medial MB, which receives a large number of these inputs. Dense hippocampal projections from the lateral MB also terminate in the mediodorsal nucleus of Gudden.

Table 3: Stimulation of the pre-commissural fornix in rodents, along with main findings

<table>
<thead>
<tr>
<th>Projections</th>
<th>Study</th>
<th>Model</th>
<th>Stimulation pattern</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Mamad 2015</td>
<td>Rats</td>
<td>Theta burst</td>
<td>Resets the spiking of hippocampal theta cells, increases theta synchronisation, entrains the spiking of hippocampal place cells</td>
</tr>
<tr>
<td>Cholinergic neurons and/or projections</td>
<td>Van der Casteele et al 2014</td>
<td>Mice, with or without urethane anaesthesia</td>
<td>Sine wave, 1–12 Hz</td>
<td>Enhance theta rhythm, suppress peri-theta frequency bands</td>
</tr>
<tr>
<td></td>
<td>Dannenberg et al 2015</td>
<td>Mice, under urethane anaesthesia</td>
<td>Square pulses, 5–40 Hz</td>
<td>Increase firing of hippocampal inhibitory interneurons and decrease firing of principal cells</td>
</tr>
<tr>
<td></td>
<td>Mamad et al 2015</td>
<td>Rats, awake</td>
<td>Square pulses, 8–30 Hz</td>
<td>The most potent effect on hippocampal theta amplitude was observed after 8–10 Hz stimulation and in a non-active behavioural state</td>
</tr>
<tr>
<td>GABAergic neurons and/or projections</td>
<td>Gangadharan et al 2016</td>
<td>Mice, freely moving</td>
<td>Square pulses, 10 or 20 Hz</td>
<td>Enhance type 2 theta rhythm, object exploration and not open-field exploration behaviour</td>
</tr>
<tr>
<td></td>
<td>Fuhrmann et al 2015</td>
<td>Mice, freely moving</td>
<td>3–12 Hz</td>
<td>Enable initiation of locomotion and theta oscillations as well as the active regulation of locomotion speed</td>
</tr>
<tr>
<td></td>
<td>Robinson et al 2016</td>
<td>Mice, freely moving</td>
<td>4–12 Hz</td>
<td>MS glutamatergic neurons synchronise hippocampal theta rhythms whereas activation of their projections to the hippocampus through fornix stimulation has no effect on theta rhythms</td>
</tr>
</tbody>
</table>

MS, medial septum.
also innervated by the supramammillary nuclei, the tuberomammillary nucleus and the septal region.31

Inputs to MB from both the hippocampal formation and the prefrontal cortex are excitatory, but the projections from the tegmental nuclei are inhibitory.311 MB efferents to both anterior thalamic and tegmental nuclei are excitatory.311,312 Neurochemically, the efferents from MB to the ATN use glutamate, aspartate and enkephalin.312

Head-direction neurons are found in the lateral MB, but not the medial MB of the rat. Head-direction signals in the lateral MB precede the signal in the anterior thalamus indicating that the lateral mammillary signal helps to drive the thalamic signal.305,313 Moreover, medial MB neurons fire rhythmically in phase with hippocampal theta,314 whereas few such cells exist in the lateral MB. Septal inactivation eliminates theta activity in the MB but not in the adjacent supramammillary nucleus.48 suggesting that MB is part of a descending system driven from the septum/hippocampus, whereas the supramammillary nucleus is a part of an ascending system generating theta.315 Thus, the MB are a key relay of hippocampal theta rhythm to the ATN and distal circuits.

The MB likely contribute to memory via processes at least in part independent from their hippocampal inputs, such as afferents from the limbic mesencephalon.31 For instance, the pathway responsible for maintaining head direction in rats originates in the dorsal tegmental nucleus of Gudden and projects to the lateral MB, terminating in the anterodorsal thalamic nuclei. Conversely, the regulation of theta rhythm and the optimisation of synaptic plasticity originates in the ventral tegmental nucleus of Gudden, projects to the medial MB and terminates in the anteroventral thalamic nuclei.

### Subiculothalamic projections

Anterior thalamic functions rely on direct hippocampal inputs through the direct pathway, as well as indirect information via the indirect pathway.316 The subicular cells projecting to the MB also project to the EC, while the direct subiculothalamic cells do not.291 Since these distinct cell populations may not mediate the same functions, knowing the electrophysiological properties of their synapse with ATN neurons, especially their plasticity and latent periods, is crucial in order to modulate them appropriately with DBS.

Electrical stimulation of the dorsal fornix (figure 2) evokes distinct electrophysiological responses due to stimulation of direct and indirect subiculothalamic cells. In particular, a triphasic response is seen in ATN neurons consisting of a small negative wave followed by a small positive wave and then a long negative wave, with latent periods of 1.5 to 4 ms.317 After a first shock applied to the MB, a period of decreased responsiveness follows the orthodromic activation of AT cells, with a peak at 40 ms and lasting 70–80 ms. MB stimuli delivered at low frequencies ≤1 Hz or ≥2 Hz evoke respectively a 220 ms long trisphasic or monophasic inhibitory postsynaptic potentials in most ATN neurons of preclinical models.291 The hippocampo-mammillary axon terminals were stimulated by Laxton et al at 3 Hz in six patients with AD: the peak of the first evoked response had a 38–52 ms latency and was localised to the hippocampal and parahippocampal gyri, likely corresponding to antidromic activation. At longer latencies (102–256 ms), significant activation of the posterior cingulate gyrus and precuneus area of the parietal lobe was seen suggesting previous activation of the ATN to low frequencies.

Prolonged high-frequency stimulation applied to either the dorsal fornix or the mammillothalamic tract (MTT) did not result in long-lasting thalamic theta activity in rats (table 5). Moreover, 15 min–1 Hz low-frequency stimulation of the dorsal fornix induced augmentation of thalamic low-theta and high-theta over delta ratios for about 120 min in parallel with depressing thalamic synaptic responses whereas the same protocol applied to MTT failed to evoke significant oscillatory changes.301,304 Synaptic depression has been proposed as a dynamic gain control mechanism in cortical information processing318 and hippocampal theta may modify thalamic responsiveness to stimuli coming from the tegmental area via the MB. Differences in basal synaptic transmission, short-term and long-term synaptic plasticity were found between the hippocampo-thalamic and mammillothalamic tracts. A brain-derived neurotrophic factor-dependent augmentation of synaptic transmission was observed only at mammillothalamic synapses. Paired-pulse stimulation, however, induced facilitation in both pathways. The amplitude of the thalamic activity was readily potentiated after high-frequency stimulation of the mammillothalamic tract but not of the dorsal fornix. Low-frequency stimulation of the mammillothalamic tract induced potentiation.301,305 Seemingly, the two major inputs to the ATN have opposing or complementary actions.

<table>
<thead>
<tr>
<th>Study</th>
<th>Model</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamani et al 2010</td>
<td>1 obese patient</td>
<td>Acute 130 Hz DBS induced old memories recall</td>
</tr>
<tr>
<td>Laxton et al 2010</td>
<td>6 Patients with AD</td>
<td>Clinical trial phase I: fornical DBS was safe and drove neural activity in the memory circuit, including the entorhinal and hippocampal areas, and activated the brain’s default mode network</td>
</tr>
<tr>
<td>Smith et al 2012</td>
<td>6 Patients with AD</td>
<td>Increased connectivity after 1 year of DBS is observed. The persistent cortical metabolic increases after 1 year of DBS were associated with better clinical outcomes</td>
</tr>
<tr>
<td>Sankar et al 2015</td>
<td>Patients with AD</td>
<td>In addition to modulating neural circuit activity, fornical DBS influenced the natural course of brain atrophy in a neurodegenerative disease</td>
</tr>
<tr>
<td>Lozano et al 2016</td>
<td>Patients with AD</td>
<td>Clinical trial phase II: no significant differences in the primary cognitive outcomes in the ‘on’ vs ‘off’ stimulation group at 12 months, but in patients &gt;65 years old was associated with a trend towards both benefit on clinical outcomes</td>
</tr>
<tr>
<td>Heschem et al 2015a</td>
<td>Rats</td>
<td>1 hour of 100 Hz DBS increased c-Fos in CA1 and CA3 and led to ACh increase in hippocampus peaking 20 min after stimulus onset, and no change of glutamate</td>
</tr>
<tr>
<td>Zhang et al 2015</td>
<td>Rats with hippocampal AP 1–42</td>
<td>24-hour-long DBS facilitated hippocampus-dependent spatial memory 4 weeks later</td>
</tr>
<tr>
<td>Heschem et al 2016</td>
<td>Rats</td>
<td>Acute 100 Hz DBS improved performance in Morris Water Maze test</td>
</tr>
<tr>
<td>Heschem et al 2013</td>
<td>Rats, IP scopolamine</td>
<td>Fornical DBS reversed the memory impairing effects of scopolamine. DBS efficacy was not sensitive to the frequency of stimulation, but rather to current levels</td>
</tr>
<tr>
<td>Gondard et al 2015</td>
<td>Rats</td>
<td>Fornical DBS triggers hippocampal activity and rapidly modulates the expression of neurotrophic factors and markers of synaptic plasticity known to play key roles in memory processing</td>
</tr>
</tbody>
</table>

ACh, acetylcholine; AD, Alzheimer’s disease; AP 1-42, amyloid peptide 1-42; DBS, deep brain stimulation; IP, intraperitoneal.
Following clinical trials of human fornix stimulation, Hescham showed in 2016 that 1-hour stimulation at 100 Hz of the hippocampo-mammillary axon terminals induced a selective activation of cells in the CA1 and CA3 subfields of the rodent dorsal hippocampus. In addition, they observed a substantial increase in the extracellular hippocampal ACh, which peaked 20 min after stimulus onset, whereas hippocampal glutamate levels did not change compared with baseline. In a rat model of scopolamine-induced dementia, acute bilateral DBS of the hippocampo-mammillary axon terminals reversed the memory impairing effects of scopolamine in the object location task. Both 10 Hz and 100 Hz stimulations were found to be efficient, but a higher current density threshold was needed at 10 Hz.

**Human fornical subiculo-mammillary stimulation**

Several trials have documented the clinical effects of chronic fornical subiculo-mammillary axon terminals for DBS (Table 4). In a patient with morbid obesity, bilateral DBS to the hypothalamus, which is closely associated with the ventral post-commissural fornix, elicited recall of autobiographical memories. Subsequently, an open-label phase I trial of fornix DBS was initiated: patients with mild-to-moderate AD were implanted with electrodes 2 mm anterior to the columns of the ventral post-commissural fornix. Patients received high-frequency DBS for 12 months, and PET studies a year later revealed increased corticoglucose metabolism that were correlated with improved cognitive measures in two orthogonal networks: a frontal-temporal-parietal-strahtal-thalamic network and a frontal-temporal-parietal-occipital-hippocampal network. The finding of increased glucose metabolism is a striking contrast to the longitudinal metabolic decline generally seen in patients with AD. Moreover, clinical evaluation of the AD Assessment Scale cognitive subscale (ADAS-Cog) and the Mini Mental State Examination (MMSE) suggested possible slowing in the rate of progressive cognitive decline in certain patients with AD. In the aforementioned cortical regions, higher baseline metabolism prior to DBS and increased metabolism after 1 year of DBS were correlated with better outcomes in global cognition, memory and quality of life. A single case report showed evidence of stabilisation of MMSE and ADAS-Cog scores at 1-year follow-up and subjective improvement. Although a subsequent crossover randomised phase II clinical trial did not yield cognitive benefits in all patients with AD, those over the age of 65 showed a slower decline. A multicentre phase III trial is now underway to assess which AD patient subgroup will benefit most from fornical DBS.

### Subiculothalamic pathway summary

Overall, a characteristic feature of the anterior thalamic neurons is their ability to fire rhythmically in the theta range. These 5–12 Hz oscillations in the anterior ventral nucleus of the thalamus receive descending inputs from the subiculum and ascending inputs from the medial MB via the fornical subiculo-thalamic pathway. Theta rhythm is thought to play a critical role in the mnemonic functions of the limbic system and has been shown to play a role in the mnemonic functions of the limbic system.

---

**Table 5** Stimulation of the dorsal fornix or the mammillothalamic tract in rodent models

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Species</th>
<th>Stimulation frequency (Hz)</th>
<th>Dorsal fornix</th>
<th>Mammillothalamic tract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalamic theta oscillations</td>
<td>Rat</td>
<td>1</td>
<td>Increase of theta power (Tsanov et al 2011c)</td>
<td>No increase of theta power (Tsanov et al 2011c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>No increase of theta power (Tsanov et al 2011c)</td>
<td>No increase of theta power (Tsanov et al 2011c)</td>
</tr>
<tr>
<td>Thalamic synaptic plasticity</td>
<td>Rat</td>
<td>1</td>
<td>LTD of FP slope and amplitude (Tsanov et al 2011d)</td>
<td>LTP of FP slope and amplitude (Tsanov et al 2011d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>LTP of FP slope, no effect on FP amplitude (Tsanov et al 2011d)</td>
<td>LTP of FP slope and amplitude (Tsanov et al 2011d)</td>
</tr>
<tr>
<td>Biochemistry</td>
<td>Rat scopolamine IP (0.1 mg/kg)</td>
<td>10</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>–</td>
<td>c-Fos increase in infralimbic and prelimbic cortices (Hescham et al 2015b)</td>
</tr>
<tr>
<td>Memory</td>
<td>Rat scopolamine IP (0.1 mg/kg)</td>
<td>10</td>
<td>–</td>
<td>No effect on object location task (Hescham et al 2015b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>–</td>
<td>No effect on object location task (Hescham et al 2015b)</td>
</tr>
</tbody>
</table>

FP, field potential; IP, intraperitoneal; LTD, long-term depression; LTP, long-term potentiation.

**Table 6** Presence of theta and/or gamma oscillations within the various nodes of the Papez circuit interconnected by the fornix, and effects of electrical stimulation of these nodes

<table>
<thead>
<tr>
<th>Target</th>
<th>Theta oscillations</th>
<th>Gamma oscillations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial septum</td>
<td>Projections to hippocampus</td>
<td>–</td>
</tr>
<tr>
<td>- Cholinergic</td>
<td>Enhance hippocampal theta rhythm</td>
<td>–</td>
</tr>
<tr>
<td>Medial septum</td>
<td>Projections to hippocampus</td>
<td>–</td>
</tr>
<tr>
<td>- GABAergic</td>
<td>Enhance hippocampal type 2 theta rhythm</td>
<td>–</td>
</tr>
<tr>
<td>Medial septum</td>
<td>Projections to hippocampus do not influence hippocampal theta</td>
<td>–</td>
</tr>
<tr>
<td>- Glutamatergic</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hippocampus (CA1, CA3, DG)</td>
<td>Intrinsic theta oscillations</td>
<td>Intrinsic gamma oscillations</td>
</tr>
<tr>
<td>Entorhinal cortex</td>
<td>Intrinsic theta oscillations</td>
<td>Gamma oscillations; drives hippocampal gamma oscillations at the trough of hippocampal theta at the level of the hippocampal fissure (Senova et al 2018)</td>
</tr>
<tr>
<td>Subiculum</td>
<td>Occasional theta oscillations; projections enhance thalamic theta power</td>
<td>Intrinsic gamma oscillations</td>
</tr>
<tr>
<td>Anterior thalamus (AD, AV, AM)</td>
<td>Intrinsic theta oscillations</td>
<td>–</td>
</tr>
<tr>
<td>Mammillary bodies</td>
<td>Theta oscillations; projections to the anterior thalamus do not influence thalamic theta</td>
<td>–</td>
</tr>
</tbody>
</table>

oscillatory patterns in the theta range may enable synaptic plasticity. Furthermore, inactivation of the MS and thus of the fornix septohippocampal pathway abolishes theta discharge in both the hippocampus and MB, two of the major regions providing inputs to anterior thalamus. Hence, the ATN appear to be part of a descending system driven from the MS via the fornix septohippocampal pathway, and theta oscillations in the anterior thalamus might complement hippocampal–diencephalic memory processing after propagation through the fornical subicularthalamic pathway.

Overall summary and future directions
Understanding the complex interconnections within the circuit of Papez mediated by the fornix sheds light on memory function in healthy and disease states. This framework is also relevant in the context of the design of future clinical DBS strategies. The fornical septohippocampal axis generates brain oscillations that are central to memory processes, such as theta and gamma oscillations, as well as theta–gamma cross-frequency coupling (table 6). The fornical subicularthalamic pathway relays these rhythms across the nodes of the circuit of Papez, mediating diverse functional aspects of memory. The topography of projections and interplay of intrinsic rhythms give rise to synaptic plasticity and memory consolidation. Given the complex neurophysiology and connectivity of subnuclei within the circuit of Papez, future neuromodulation devices should strive to deliver individualised therapy in various nodes of the circuit of Papez in response to real-time electrophysiological data. Biotechnological innovations in the field of neuromodulation are needed to optimise sensing and delivery algorithms, as well as power management strategies in future DBS devices. Prospective randomised and double-blinded human trials are underway to evaluate the true potential of DBS to rescue memory deficits in patients with neurodegenerative, vascular or traumatic lesions of the circuit of Papez. Please see online supplementary file 1 for references 61 to 308.

Contributors SS and AF are joint first authors and contributed equally to the manuscript. SS conceived the manuscript and wrote the first draft. AF created figures, wrote the anatomy and pathology chapters and revised the manuscript. EG contributed the imaging and clinical trials chapter. AL oversaw revisions and approved the final manuscript.

Funding This review was made possible by the R.R. Tasker Chair in Functional Neurosurgery, University Health Network (AHL) and the University of Manitoba Clinician Investigator Program (AF).

Competing interests AHL is a consultant to Medtronic, St Jude, Boston Scientific, Functional Neuromodulation and Insightec.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC) 4.0 license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iDs
Anton Fomenko http://orcid.org/0000-0003-4131-6784
André M Lozano http://orcid.org/0000-0001-8257-3694

REFERENCES


SUPPLEMENTAL REFERENCES


131. Manns ID, Mainville L, Jones BE. Evidence for glutamate, in addition to acetylcholine and GABA, neurotransmitter synthesis in basal forebrain neurons projecting to the entorhinal cortex. *Neuroscience*. 2001;107:249–263.

133. Gritti I, Henny P, Galloni F, Mainville L, Mariotti M, Jones BE. Stereological estimates of the basal forebrain cell population in the rat, including neurons containing choline acetyltransferase, glutamic acid decarboxylase or phosphate-activated glutaminase and colocalizing vesicular glutamate transporters. *Neuroscience*. 2006;143:1051–1064.


181. Bell KA, Shim H, Chen C-K, McQuiston AR. Nicotinic excitatory postsynaptic potentials in hippocampal CA1 interneurons are predominantly mediated by nicotinic receptors that contain a4 and b2 subunits. *Neuropharmacology.* 2011;61:1379–1388.


286. Sharp PE. Subicular place cells expand or contract their spatial firing pattern to fit the size of the environment in an open field but not in the presence of barriers: Comparison with hippocampal place cells. *Behav Neurosci*. 1999;113:643–662.


